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Long-term land use in Amazon influence the dynamic of microbial communities in soil and rhizosphere

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ABSTRACT

Brazil has become the world leader in soy production, leading to an increase in the conversion of the Amazon rainforest into cropland. These actions had consequences for Forest's biodiversity, including the soil. In this sense, a better understanding of how long-term land use affects soil microbial communities, and their functions is urgent. This study aimed to evaluate the long-term land-use effects over bacterial and archaeal communities in soil and soybean rhizosphere in the Amazon region. For this, mesocosms experiments were carried out with Amazon soils with a history of 2-, 8-, and 20-years of agricultural use. We then assessed the bacterial and archaeal communities based on the 16S rRNA sequencing and real-time PCR. Our results showed a distinct bacterial community structure in soils with 20-years of land use. For both, bulk soil and soybean rhizosphere with 20-years of use, there was an increase in the abundance of Gemmatimonadetes, Chloroflexi, Firmicutes, and Planctomycetes. Interestingly, the niche occupancy analysis revealed an increase of specialist microbes in these soils. Also, these soils with 20-years of use showed a more complex network for both bulk and rhizosphere samples, highlighting the importance of Actinobacteria and Chloroflexi phyla to soil network structure. Our analysis also revealed an increased abundance of total bacteria, N-fixers, and ammonia-oxidizers bacteria in rhizosphere soil with 20-years of use. In addition, based on the potential functional analysis, nitrification processes increased in those soils. However, we noticed a homogenization in the abundance of the genes between rhizosphere and bulk soil with 20-years of use. In general, the differences were associated with changes in soil chemical characteristics such as pH, Ca^{2+}, Mg^{2+}, and organic matter, which are a consequence of liming and no-till practices over time. Our findings demonstrate that long-term agriculture in Amazon soils affects microbial community composition and functions, bringing new insights to better understand anthropogenic actions over the soil microbiome.

1. Introduction

The Amazon biome has the world's biggest tropical rainforest, rich in animal and plant diversity (da Silva et al., 2005; Heckenberger et al., 2007). In addition, the region provides ecosystem services, such as carbon sequestration, controlling atmospheric temperature, and precipitation (Junk et al., 2011; Davidson et al., 2012; Paula et al., 2014; Meyer et al., 2017). However, this ecosystem is under threat from anthropogenic activities, mainly due to its conversion to pastures and agricultural fields (reviewed by Domingues et al., 2020). For instance, in the last decades, the soybean (Glycine max (L.) Merr.) production has gained increasing importance in the Brazilian Amazon region, taking place through cropland intensification into pristine forests (Lathuillière et al., 2017). The expansion of soybean cultivation areas to the Amazon region was possible due to long-term selection programs of soybean breeding for low-latitude regions (Almeida et al., 1999) and the isolation and inoculation of N-fixing strains capable of supplying N to soybean (Hungria et al., 2020). Brazil is currently the world's largest soybean

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producer and is responsible for producing 132.9 million tons in the crop year 2020/2021. On the other hand, the cultivated area increased by 4.1% in the same year (IBGE, 2021), indicating the deforestation of native areas for this crop expansion.

Several previous studies have shown that forest-to-agriculture conversion affects soil biodiversity and ecological functioning in the Amazon region (Paula et al., 2014; Mendes et al., 2015; Pedrinho et al., 2019; Meyer et al., 2020), including the N cycle (Merloti et al., 2019; Pedrinho et al., 2020). These authors concluded that the soil management, low diversity of plants, and exudates released by roots were the main factors responsible for changing the chemical, physical, and biological properties of soils. Consequently, changes in the microbial communities' structure, composition, and potential functions that inhabited these soils were reported. Besides, a loss of soil microbial diversity and biotic homogenization due to the forest-to-pasture and agriculture conversion were found in the Amazon region (Rodrigues et al., 2013; Mueller et al., 2014; Goss-Souza et al., 2020).

The soil microorganisms play essential roles in the ecological and metabolic process and are crucial organisms in biogeochemical cycles (Rosado et al., 2019). They regulate complex N cycle processes such as N-fixation, nitrification, and denitrification (Zilio et al., 2020). Furthermore, microbes are partners in symbiotic relationships with plants, supplying the nutrients necessary for plant productivity (Jacoby et al., 2017). The rhizosphere can be widely defined as a region of the soil in direct contact or adjacent to the plant roots, being influenced by its exudates (Philippot et al., 2013a, 2013b). The primary source of microbial diversity in the rhizosphere is the bulk soil, except for some endophytes that come from crop seeds (Sugiyama et al., 2014) and, in the case of soybean lands, seed pre-inoculation with N-fixing bacteria (reviewed by Meena et al., 2018). Therefore, this region is a place that harbors several interactions between microbes and plants. (Pathan et al., 2019). Thus, assessing the soil microbial diversity, co-occurrence, and potential functions is essential to elucidate the interactions between bulk and rhizosphere organisms, thus better understanding how these communities respond to long-term land use.

In the long term, soil and crop management showed interesting results over the soybean microbiome. Bolajii et al. (2021) highlighted microbial community resilience that did not change their diversity and composition at soybean rhizosphere and bulk soil after 2 years of intercropping. On the other hand, Liu et al. (2020) found that 13 years of soybean cultivation and intercropping changed the soil microbial abundance and structure. The authors indicated the possibility of developing disease-suppressive soils with long-term soybean crops by increasing the abundance of benefic bacteria and decreasing potential pathogens. The selection of a beneficial microbiome for soybean was also found by Yuan et al. (2021). The authors noticed a reduction of the nematodes cysts and increasing beneficial soybean bacteria after 36 years of soybean and intercropping. In this sense, studies focusing on land use and soil microbial communities can help create sustainable crop practices, increase the beneficial soil microbiome for plants, improve crop yield (Neupane et al., 2021), and avoid deforestation by agriculture (Erb et al., 2016).

Some studies highlighted the importance the of soil soybean microbiome on Amazon region. For example, based on microbial networks of co-occurrence in Amazonian soybean fields, Mendes et al. (2014) identified that the soybean microbial rhizosphere network was less complex than bulk soil. Moreover, Goss-Souza et al. (2019) found that Amazon forest-to-agricultural conversion alters the soybean rhizosphere microbiome composition while the functional potential remains stable. However, information regarding the bulk and rhizosphere diversity, niche occupancy, and co-occurrence networks linking to the N-cycle in Amazonian forest-to-agricultural chronosequence lands are still missing. In this sense, this study aims to reveal the consequences of long-term land use on microbial communities in the soybean rhizosphere and bulk soil from the Amazon region. We used 16S rRNA amplicon sequencing to access the bacterial and archaeal microbial communities. Also, their abundance in soil was evaluated through marker-genes quantification with Real-time PCR. In addition, bioinformatics tools and statistical analysis were performed to evaluate their correlation with soil chemical characteristics, microbial network co-occurrence, and potential functions. Here, we hypothesize that long-term land use affects the microbial communities' structure, composition, interactions, and potential functions related to the N cycle, mainly in the oldest soils, being these changes a consequence of soil chemical characteristics that were changed over time. Then, the following questions were made to answer this hypothesis: (i) What changes the long-term land use carry out on bacterial and archaeal abundance, structure, composition, and diversity? (ii) How does the long-term land use modify the microbial network co-occurrence and niche occupancy? (iii) Which changes long-term land use performs on marker-genes abundance and potential functions both related to N cycle? Lastly, (iv) which soil chemical characteristics are mainly responsible for the found changes?

2. Material and methods

2.1. Land-use history and soil sampling

The sampled areas were located in the Amazon region, in cropping lands with different years of agricultural use. Bulk soil samples were collected in October 2016, in agricultural fields, located in the municipality of Belterra, Pará State, Brazil. The climate of the region is classified as Am (Köppen classification), tropical monsoon, with an average annual air temperature of 26 °C and precipitation of 2150 mm (average of the past 30 years). In order to assess the dynamic of soil microbial community (i.e., Bacteria and Archaea), we selected a chronosequence of agricultural fields spanning 2- (2°44′22.6″S, 54°55′50.2″W- 165 m altitude), 8- (2°41′52.1″S, 54°55′46.8″W- 163 m altitude), and 20-years of cultivation (2°40′58.1″S, 54°55′46.5″W- 164 m altitude) in a no-till system, with successive crop rotation. All sites were initially a native forest and later were converted to agricultural fields through a process of selective logging of valuable timber, followed by slash-and-burn deforestation of the remaining vegetation. Afterward, common rice (*Oryza sativa*) was cultivated for at least one season, to reduce Al toxicity and prepare the soil for further cropping. The agricultural fields have been cultivated in a no-till cropping system, with successive crop rotation, including millet (*Pennisetum glaucum*), soybean (*Glycine max*), and maize (*Zea mays*). All agricultural fields have been received the application of liming, fertilizers (N–P–K as base fertilizer and topdressing urea, depending on the crop), and pesticides according to the recommendation of local agronomists and based on soil fertility analysis. The soil type of all selected areas was classified as Oxisol (Typic Haplustox) based on the Soil Classification System.

At the time of sampling, fields were cultivated with soybean plants at the initial blossoming phase. At each site, we first removed the litter layer and then collected soil samples from the 0–20 cm profile and stored them in sterilized plastic bags. A total of 35 soil samples (~3 kg of soil per sample) were collected in each field in a zigzag pattern across an area of 10 ha^{-1}. Approximately, 105 kg of soil was collected in each field and then transported to the Cell and Molecular Biology Laboratory at the Center for Nuclear Energy in Agriculture (CENA/USP, Piracicaba, Brazil) for the preparation of the mesocosm experiment.

2.2. Mesocosm experiment

In order to control environmental variables and compare only the differences among soils with different time of use, we have conducted mesocosm experiments. The soil samples collected at the agricultural fields were used in the mesocosm experiment, where soybean plants were grown in a greenhouse at CENA/USP, Piracicaba, Sao Paulo State, Brazil. The experiment was performed in the greenhouse to reduce the influence of environmental factors on the growth of soybean plants, such as the variation in temperature and water content. To simulate field

conditions, the same cultivar used in the Amazonian fields was used in the mesocosm, i.e., soybean *Glycine max* (L.) Merril. (Monsoy 6410 IPRO- Bt RR2). The soybean seeds were inoculated with *Bradyrhizobium japonicum* strain Semia 5079 (commercial product) in a concentration of 10^9 viable cells per kg of seed, to ensure biological nitrogen fixation (Bizarro et al., 2011).

The mesocosms consisted of plastic pots (20 cm height × 16 cm diameter) with a 4 cm pebble layer at the bottom (for drainage). Three soybean seeds were sown per pot, which was filled with approximately 5 kg of soil. The experiment was performed with nine pots with no plant, to assess the bulk soil effect, and nine pots with soybean plants, to assess the rhizosphere effect, totaling 18 pots (3 agricultural fields × 2 soil compartments × 3 replicates). The greenhouse was kept at 28/19 °C (day/night) with a 12-h photoperiod. Soil moisture was maintained at 60% of field capacity (simulating field conditions). Soybean plants were collected after 45 days of plant growth, which corresponds to the R1 stage (50% flowering plants). The soybean plants were carefully removed from the pots and transported on ice to the laboratory. Afterward, the roots were shaken to remove the loose soil and the remaining soil attached to the roots (defined as rhizosphere soil) was collected using sterile brushes. Soil samples from the pots without plant, were collected and considered as bulk soil.

2.3. Soil chemical analysis

The chemical analysis was carried out in accordance with the guidelines laid out in the Manual of Soil Analysis Methods (Embrapa, 1997). Soil samples were air-dried before being sieved via a 2 mm sieve. In a 1:2.5 soil/water suspension, the pH of the soil was determined. The Kjeldahl method was used to calculate total nitrogen, NH_4^+, and NO_3^- (Bremner, 1960). The dichromate/titrimetric method was used to determine organic matter (OM) (Nelson, Sommers, 1974). KCl 1M was used to extract exchangeable Ca^{2+}, Mg^{2+}, and Al^{3+}. Calcium (Ca^{2+}) and Mg^{2+} were quantified by atomic absorption spectrometry and Al^{3+} by acid-base titration. Potassium (K^+) and P were extracted using ion-exchange resin and measured by flame photometry and calorimetry, respectively. Potential acidity (H + Al) was determined in SMP buffer solution. Some of the results allowed the calculation of other parameters such as exchangeable bases (E.B.; the sum of Ca^{2+}, Mg^{2+}, and K^+), cation exchange capacity (C.E.C.; the sum of Ca^{2+}, Mg^{2+}, and K^+, Al^{3+}, and H^+), base saturation (V%; the percentage relation between E.B. and C.E.C.), and Al saturation (m%; the percentage relation between Al^{3+} and C.E.C.).

2.4. DNA extraction

The PowerSoil DNA Isolation Kit (MoBio Laboratories, Carlsbad, CA, USA) was used to extract total soil DNA, which was done according to the extraction procedure for tropical soils (Venturini et al., 2020). After that, DNA quality was validated using a 1% sodium boric acid agarose gel electrophoresis (Brody and Kern, 2004), and DNA concentration was quantified using NanoDrop 1000 spectrophotometry (Thermo Scientific, Wilmington, DE, EUA).

2.5. Phylogenetic and functional marker genes quantification (Real-time PCR)

The size of the microbial community and the abundance of genes related to the N cycle were quantified through StepOnePlus Real-Time PCR System with 96-well plates (Applied Biosystems, Foster City, CA, USA). The primers used to quantify the size of Bacterial and Archaeal communities (both based on the 16S rRNA gene), N-fixers (based on the *nif*H gene), ammonia oxidizers (both based on the *amo*A gene from Bacteria and Archaea), and denitrifiers (based on *nir*K and *nos*Z genes) are described in Supplementary Table 1. For each marker gene, a PCR was performed to amplify its quantity using specific strains. Then, and based on the known amount for each targeted gene, standard curves were performed through serial dilutions (with 10° to 10^{10} gene copies). Information regarding strains used for standard curves, primers, and reaction conditions for amplifying the genes is described in Supplementary Table 1. Only standard curves with R^2 greater than 0.98 were considered for further analysis. The results were processed using the StepOnePlus Real-Time software version 2.2.2 (Applied Biosystems, Foster City, CA, USA) and exported to Excel (Microsoft) for the calculation of the number of gene copies per gram of soil to further statistical analysis.

2.6. 16S rRNA amplicon sequencing and bioinformatics analyses

For taxonomical profiling, the amplicon sequencing targeting the V3–V4 region of the 16S rRNA genes of Bacteria (primers **341F**-CCTACGGGNGGCWGCAG and **805R**-GACTACHVGGGTATCTAATCC) and Archaea (primers **Arch915f**-AGGAATTGGCGGGGGAGCAC and **1386R**-GCGGTGTGTGCAAGGAGC) were performed at the Center for Functional Genomic Research (ESALQ/USP), located in the municipally of Piracicaba, Sao Paulo State, Brazil. In total, 18 DNA samples' libraries (3 agricultural fields x 2 soil compartments x 3 replicates) were prepared using the Miseq Reagent Kit v3 (Illumina, San Diego, CA, USA), following the manufacturer's instructions for the Illumina MiSeq platform (2 × 250 bp paired-end). Primers and reaction conditions used for amplification are described in Supplementary Table 1.

The bacterial and archaeal 16S rRNA sequences generated were processed using QIIME 2 version 2017.11 (Caporaso et al., 2010). The steps for processing the sequences were previously described by Merloti et al. (2019). Briefly, the sequences were demultiplexed and low quality and chimera sequences were removed using DADA2 (Callahan et al., 2017). Then, the samples were rarefied to 50,000 and 88,000 sequences for Bacteria and Archaea, respectively, following the number of the lowest sample. The taxonomic identification was performed using the SILVA database (v.128) with 99% of similarity (Quast et al., 2012). The taxonomic matrices generated for Bacteria and Archaea were used for further statistical analyses. Sequences were submitted to the NCBI Sequence Read Archive under the identification PRJNA764025.

The potential functional profile was assessed through the FAPROTAX database (version 1.1) (Louca et al., 2016). The software has a database with functions of microorganisms cultivated and annotated. The program correlated our compositional matrix from Bacteria and Archaea (based on 16S rRNA sequencing, at the genus level) with its database and mapped the potential functions verified in a putative functional table. The results were transformed in relative abundance and analyzed statistically.

2.7. Statistical analysis

All statistical analyses were performed using the following design: 3 agricultural fields representing the no-till cropping chronosequence (2-, 8- and 20- years), 2 soil compartments (bulk soil and rhizosphere) and 3 replicates. Before the analyses, Shapiro-Wilk and Levene tests were performed to check the data distribution and homoscedasticity, respectively. Soil chemical properties and marker-genes abundance (from Real-time PCR analysis) were compared by analysis of variance, followed by Tukey post hoc tests for pairwise comparisons ($P < 0.05$). Non-metric multidimensional scaling (NMDS) was used to assess the microbial community structure. For this, we utilized the bacterial and archaeal taxonomic matrices at the genus level. NMDS plots were generated using "vegan" (Oksanen et al., 2013) and "ggplot2" packages (Wickham, 2011) in the R platform (RStudio Team, 2020). In addition, we used the permutational multivariate analysis of variance (PERMANOVA) to test whether the clustering of community structures was due to selected sample categories (chronosequence and soil compartment) or random (Anderson, 2001). The PAST software v.3.0 (Hammer et al., 2001) was used to calculate the Taxa_S (richness) and Simpson

(taxonomic diversity) indexes based on the bacterial and archaeal taxonomic matrices at the genera level. After that, the results were compared by the Tukey test for pairwise comparisons ($P < 0.05$).

The Statistical Analysis of Metagenomic Profiles (STAMP) software (Parks et al., 2014) was used to determine statistical differences in microbial taxonomic composition and their potential functions. The bacterial and archaeal compositional matrices (at phyla and order levels) and the putative functional table (created by the FAPROTAX) were used as input. P-values were calculated using the two-sided Tukey-Kramer test, followed by Benjamini-Hochberg false discovery rate (Benjamini and Hochberg, 1995). Spearman's rank correlation coefficients were calculated to explore the relationship between soil chemical properties with microbial groups and soil chemical properties with marker-genes abundance using the 'corrplot' package (Wei, 2017) in R, and a correction was performed using Benjamini–Hochberg false discovery rate (Benjamini and Hochberg, 1995). Only correlations with P-value higher than 0.05 were plotted.

The multinomial species classification method (CLAM) was used to verify niche occupancy, using the "vegan" package and the function "clamtest" in R. (RStudio Team, 2020). This method compares the abundance of the microbial community in two different treatments and categorizes the microorganisms into different groups, such as generalists (a group that is equally distributed in both treatments), specialists (a group that is more abundant in one treatment than the other), and rare (Chazdon et al., 2011). Finally, network analysis was used to assess the intricacy of connections among microbial groups in different stages of the chronosequence and soil compartments. The Python module "SparCC" was used to perform non-random co-occurrence studies (Friedman and Alm 2012). The genus-level bacterial and archaeal composition matrices were used for analysis. SparCC correlations between microbial taxa were calculated for each network, and only strong (SparCC >0.9 or - 0.9) and highly significant ($P < 0.01$) relationships were chosen. The interactive platform Gephi was used to calculate network visualization and property measurements (Bastian et al., 2009).

3. Results

3.1. Soil chemical characterization

The soil chemical properties are presented in Supplementary Table 2. In general, bulk soil samples of agricultural fields with 2- and 8-years of land use presented higher amounts of inorganic N (*i.e.,* NH_4^+ and NO_3^-) in comparison to the field with 20-years ($P < 0.05$). On the other hand, we observed an increase in the amount of total N (*i.e.,* inorganic + organic forms) in the agricultural field with 20-years in comparison to newer sites ($P < 0.05$).

When soybeans were grown on control conditions ("mesocosm") in these different soils, we observed great changes in the chemical properties of the rhizospheric soil. In general, the rhizosphere of soybean plants grown on the soil with 2-years of use presented a higher amount of NH_4^+ in comparison to the rhizosphere of plants grown on fields with 8- and 20-years of land use ($P < 0.05$). Moreover, we observed that the rhizosphere of soybeans grown on soil from the agricultural field with 20-years presented higher pH, OM, Ca^{2+}, EB, CEC, and V% in comparison to the other sites ($P < 0.05$). Lastly, when comparisons were performed between the bulk soil and rhizosphere of each long-term land use, we observed that soybean plants were able to increase greatly the soil pH in the rhizosphere region when compared to the bulk soil ($P < 0.05$). For all other chemical properties, bulk soil presented higher values in comparison to the rhizosphere.

3.2. Microbial community structure and diversity

The non-metric multidimensional scaling (NMDS) was performed to visualize whether microbial community structure presented distinct patterns across the chronosequence stages and soil compartments. For bacterial community structure, we observed that samples were clustered according to the long-term land use, with clear segregation of the agricultural field with 20-years from the fields with 2- and 8-years (Fig. 1A; stress value 0.1056, $P < 0.05$). Moreover, we observed that, in general, rhizosphere samples were more similar to each other than to bulk soil samples. Lastly, we did not observe differences in the archaeal community structure based on NMDS and ANOSIM analysis (Fig. 1B; stress value 0.1791, $P > 0.05$).

The richness and diversity (Simpson index) of Bacteria and Archaea did not differ among the bulk soil samples from the different agricultural fields (Fig. 2, $P > 0.05$). In the rhizosphere, our results demonstrated that bacterial diversity decreased from fields with 2- to 20- years of history ($P < 0.05$). Conversely, archaeal diversity in the rhizosphere of soybean plants grown on agricultural fields with 20- years was higher than those grown on fields with 2- and 8- years ($P < 0.05$). We did not observe differences in the richness of Bacteria and Archaea in the rhizosphere soil ($P > 0.05$). Also, a soil microbial diversity and richness comparison between bulk soils and rhizosphere in each year was performed. The statistical difference is represented by low-case letters and P-values with an asterisk (*) in Fig. 2. It was noticed that only the Archaea diversity changed. In soils with 2- and 8- years, the Simpson index decreased at rhizosphere compared to bulk soil (both comparisons with $P < 0.01$).

3.3. Microbial community composition

The amplicon sequencing for the 16S rRNA gene of Bacteria and Archaea generated approximately 2,700,000 reads after quality trimming and rarefaction (Supplementary Table 3). The general microbial community was composed of 25 phyla with 23 belonging to Bacteria (Fig. 3A) and 2 to Archaea (Fig. 3B). Proteobacteria (23% of all sequences), Actinobacteria (23%), Acidobacteria (14%), Chloroflexi (12%), Planctomycetes (6%), Firmicutes (4%), Verrucomicrobia (4%), Gemmatimonadetes (2%), Bacteroidetes (2%), Candidate division TM7 (1%), and Nitrospirae (1%) were the most abundant bacterial phyla for all soil samples and together represent a total of 92% of the bacterial community. Furthermore, we observed that Thaumarchaeota (98%) and Euryarchaeota (1%) phyla were the most abundant archaeal phyla for all soil samples and together they represented 99% of the archaeal community.

The microbial composition of bulk soil and rhizosphere were altered across the chronosequence stages and soil compartments. In the bulk soil, we observed an over-representation of the phyla Gemmatimonadetes, Chloroflexi, and Firmicutes in the agricultural field with 20- years in comparison to the other sites ($P < 0.01$, Supplementary Figure 2). On the other hand, the abundance of the phyla Bacteroidetes, Actinobacteria, and Nitrospirae decreased in the agricultural field with 20-years when compared to the other agricultural fields ($P < 0.01$, Supplementary Figure 2). Interestingly, we did not observed differences in the abundance of archaeal phyla among the agricultural fields ($P < 0.05$, Supplementary Figures 3 and 4).

In the rhizosphere, we noticed that soybeans grown on the agricultural field with 20-years presented an increase in the abundance of Planctomycetales, Chloroflexi, and Gemmatimonadetes compared to the plants grown in the other fields ($P < 0.01$, Supplementary Figure 1). On the other hand, the abundance of Verrucomicrobia and Cyanobacteria decreased in the rhizosphere of soybeans grown on the agricultural field with 20-years compared to those grown on fields with 2- and 8- years of history ($P < 0.01$). Also, the abundance of Proteobacteria decreased in rhizosphere soils with 8- and 20- years compared to 2- years rhizosphere soil ($P < 0.01$).

Lastly, we compared the microbial composition between bulk soil and soybean rhizosphere of each land-use time (Fig. 3). In general, we observed that the rhizosphere of soybean with 2-years presented a higher abundance of the phyla Candidate division TM7, SHA.109, and SM2F11 when compared to bulk soil ($P < 0.01$). For the agricultural

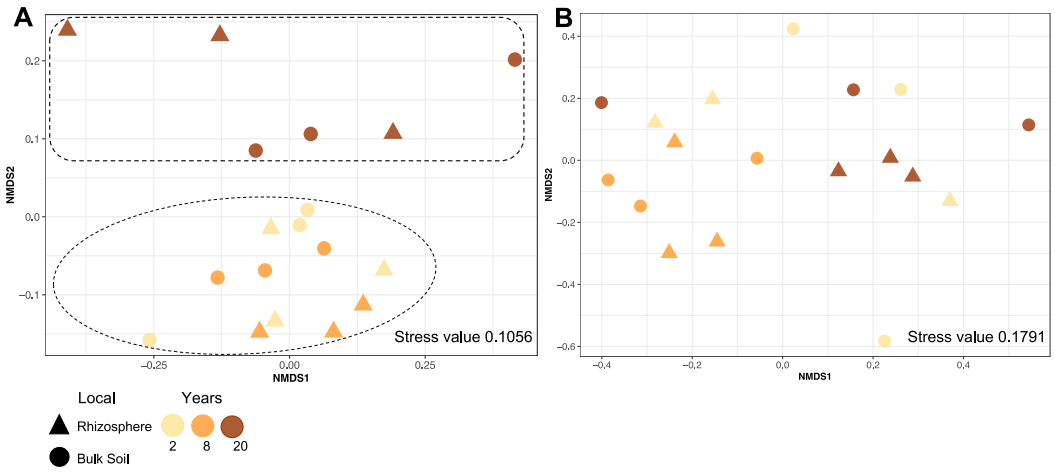

Fig. 1. Non-metric multidimensional scaling analyses (NMDS) from bulk soil and rhizosphere samples of soybean plants grown on soils from agricultural fields with 2-, 8-, and 20- years of history. Taxonomic analysis of Bacteria (A) and Archaea (B) using relative abundance based on the 16S rRNA gene at genera level and Bray-Curtis distance. The dashed lines indicate significant clusters by ANOSIM analysis ($P < 0.05$).

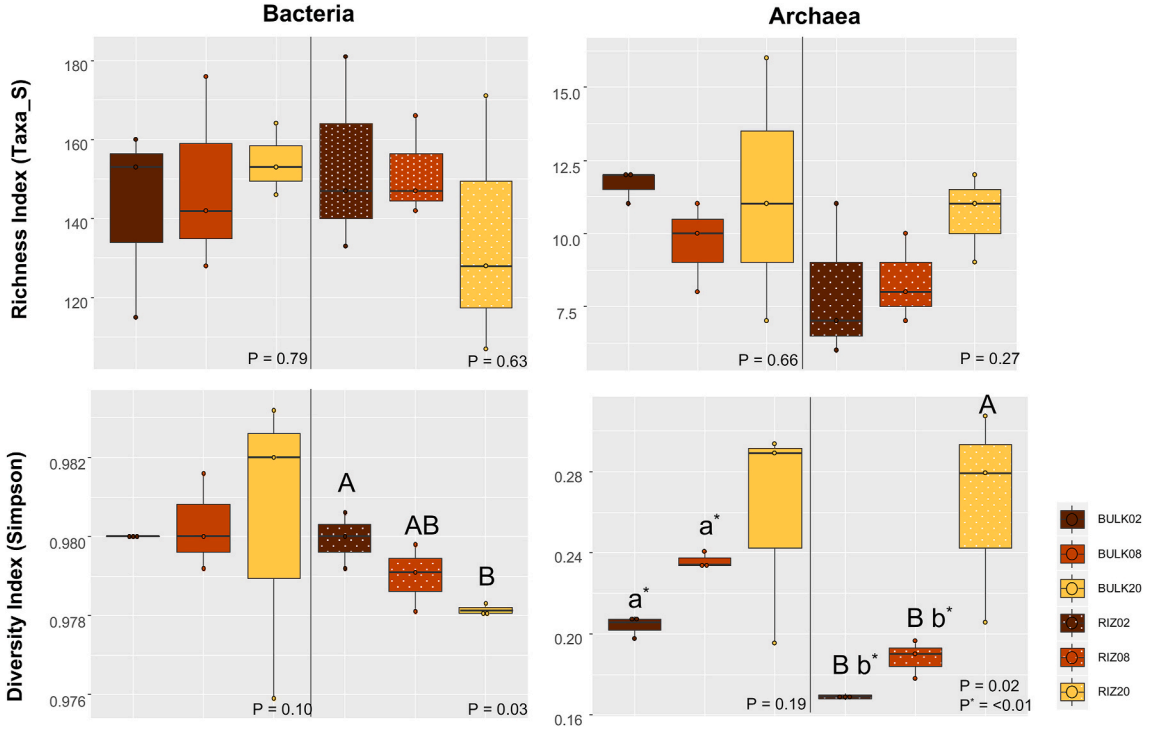

Fig. 2. Richness and Simpson diversity indices of bacterial and archaeal communities in the bulk soil and rhizosphere of soybean plants grown on soil from agricultural fields with 2-, 8-, and 20- years of history. Capital-case letters indicate significant differences between bulk soils and between rhizospheres, based on Tukey test ($P < 0.05$). Low-case letters indicate significant differences between bulk soil and rhizosphere in each year, based on Tukey test ($P < 0.05$).

field with 8-years, we observed that soybean rhizosphere presented a higher abundance of the phyla Candidate division TM7 and Armatimonadetes when compared to bulk soil ($P < 0.05$). Conversely, for this same field, we observed a higher abundance of the phyla Planctomycetes and Chloroflexi in the bulk soil in comparison to the soybean rhizosphere ($P < 0.03$). For the agricultural field with 20-years, we did not observe an increase of any microbial group in the soybean rhizosphere ($P > 0.05$). Moreover, no differences in the abundance of Archaea phyla were observed when compared the bulk and rhizosphere soils of each agricultural field ($P > 0.05$).

3.4. Niche occupancy

Niche occupancy of bulk soil and rhizosphere were also altered across the chronosequence stages and soil compartments. In the bulk soil, we observed that samples from the agricultural field with 20-years of history presented a higher presence of microbial specialists in comparison to the other fields (Fig. 4A). When comparing bulk soil samples from the agricultural fields with 2- and 20-years, we observed that 48.3% of Bacteria and 44.4% of Archaea groups were classified as generalists (mutually present in both sites), while 31.7% of Bacteria and 38.9% of Archaea were classified as specialists and were exclusively presented in the agricultural field with 20-years (Fig. 4A). Similar results

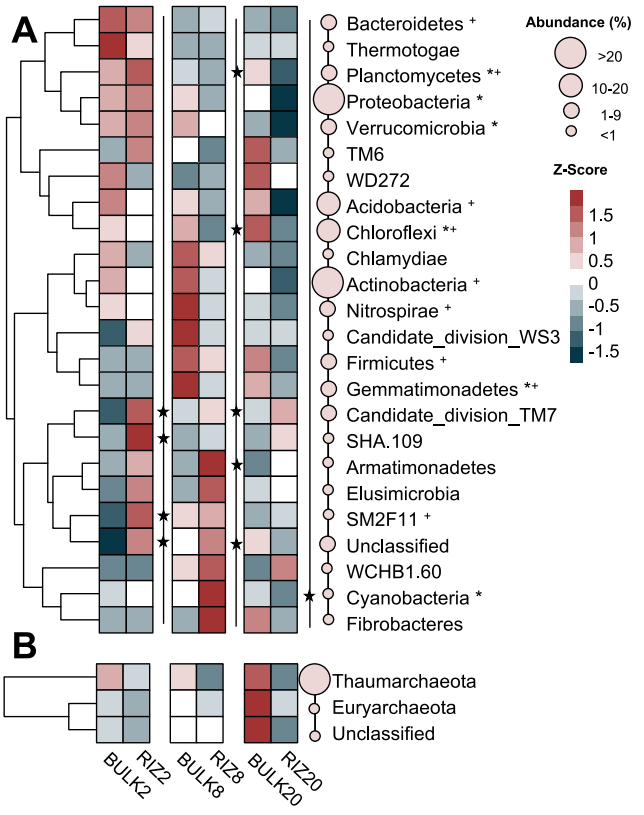

Fig. 3. Heat maps showing the relative abundance of *Bacteria* (A) and *Archaea* (B) at phyla level (based on *16S rRNA* gene) from samples of bulk soil and rhizosphere of soybean plants grown on the soils from agricultural fields with 2-, 8-, and 20- years of history. Phyla with star sign (★) represent the significant difference between bulk soil and soybean rhizosphere of each agricultural use by post-hoc Tukey-Kramer test ($P < 0.05$). Phyla with an asterisk sign (*) represents the significant difference among soybean rhizospheres by post-hoc Tukey-Kramer. Phyla with a plus sign ($^{+}$) represent the significant difference among bulk soils by post-hoc Tukey-Kramer test.

were observed when compared bulk soil samples from the agricultural fields with 8- and 20- years (Fig. 4C). Bulk soil showed 30.9 and 51.4% of specialists for Bacteria and Archaea respectively, while specialists were 56.2 for Bacteria and 37.5% for Archaea.

We did not observe an increase in the presence of Bacteria specialists in the rhizosphere of soybeans grown on the soil from the agricultural field with 20-years of history in comparison to those grown on fields with 2- and 8-years (Fig. 4B). On the other hand, for the Archaea groups, when comparing the rhizosphere of soybeans grown on the soil from the fields with 2- and 20-years, we observed that 64.3% were classified as generalists, while 21.4% were classified as specialists and were exclusively presented in the rhizosphere of soybeans grown on the agricultural field with 20-years of history (Fig. 4B). In addition, the Archaea specialists and generalists were 42.9% and 28.6%, respectively, for the comparison between soil rhizosphere with 8- and 20- years (Fig. 4B).

Lastly, we compared the niche occupancy between bulk soil and soybean rhizosphere of each land-use time (Fig. 4C). In general, the Bacteria generalists were kept steady among all the agricultural fields in the chronosequence, ranging from 50.6 to 52.0% (Fig. 4C). On the other hand, we observed a gradual increase in the presence of Bacteria specialists in the bulk soil samples from the fields with 2- to 20-years, and consequently, a reduction of this same group in the soybean rhizosphere. For the Archaea groups, we observed a great increase in the population of generalists in the field with 20-years (80.0%) when compared to the field with 2-years (50.0%) (Fig. 4C). Interestingly, Archaea specialists in the bulk soil and soybean rhizosphere were greatly affected by long-term land use. In general, we observed a decrease of Archaea specialists in both soil compartments throughout the years. In especial, we highlight a huge reduction of specialists in the rhizosphere of soybeans grown on the field with 20-years, where members of this group almost disappear.

3.5. Co-occurrence network analysis

The network analysis demonstrated a clear difference between bulk soil and rhizosphere samples (Fig. 5 and Supplementary Table 4). In general, we observed that the number of nodes and edges (*i.e.,* positive and negative correlations among microbial groups) in the bulk soil were higher than in the rhizosphere. Bulk soil samples from the agricultural field with 20-years (number of nodes = 151, edges = 906, average degree = 11.93) exhibited more connections than the fields with 2- (number of nodes = 113, edges = 429, average degree = 6.28) and 8-years of history (number of nodes = 128, edges = 402, average degree = 3.98) (Supplementary Table 4). The same pattern was observed for the rhizosphere. In general, the rhizosphere of soybeans grown in the soil from the field with 20-years (number of nodes = 125, edges = 549, average degree = 8.75) exhibited a more connected network when compared to the rhizosphere of soybeans grown on the fields with 2- (number of nodes = 117, edges = 368, average degree = 6.33) and 8-years (number of nodes = 100, edges = 348, average degree = 6.96) (Supplementary Table 4).

The number of positive correlations among microbial groups in the bulk soil and rhizosphere increased greatly from 2- to 20-years. The number of negative connections in bulk soil increased slightly from 2- to 20-years, while in the rhizosphere remained stable throughout the years. Looking into the networks, the microbial groups (represented by colored nodes) that presented a great number of significant correlations (blue lines = positive; red lines = negative) in the bulk soil were: α-Proteobacteria, Chloroflexi, and Planctomycetes in the agricultural field with 2-years; Planctomycetes, Euryarchaeota, and Chloroflexi in the field with 8-years; and Actinobacteria, Chloroflexi, and α-Proteobacteria in the field with 20-years history (Supplementary Table 5). However, for rhizosphere the groups were: Candidate TM7, Chloroflexi, and Actinobacteria in the field with 2-years; Acidobacteria, Actinobacteria, Gemmatimonadetes, and Candidate TM7 in the field with 8-years; and Actinobacteria, Bacteroidetes, and Chloroflexi in the field with 20-years (Supplementary Table 5).

3.6. Phylogenetic and functional marker genes and functional profile of the soil microbial community

The abundance of marker genes was carried out to measure the size of Bacteria and Archaea communities in the soil (Fig. 6 and Supplementary Table 6). For that, the quantification of the 16S rRNA gene from Bacteria and Archaea was performed. In addition, to evaluate the soil microbial communities related to N cycle, functional marker genes were quantified. It was measured the community sizes of N-fixers (represented by the *nif*H gene), ammonia-oxidizers (*amo*A gene from Bacteria and Archaea), and denitrifiers (*nir*K and *nos*Z genes).

In the comparison between rhizospheres (lower-case letters in Fig. 6), we observed that the abundance of 16S rRNA genes of Bacteria and Archaea (Fig. 6 A and B) increased in soil with 20-years when compared to soils with 2- and 8-years (both with $P < 0.01$). The abundance of *amo*A from Bacteria (AOB) gene (Fig. 6 C) was higher in the rhizosphere with 8-years in comparison with 2- and 20-years ($P < 0.01$). The *amo*A from Archaea (AOA) and *nos*Z genes (Fig. 6 D and F) decreased their abundance in soils with 20-years when compared to soils with 2- and 8-years (both $P < 0.01$). In addition, the *nif*H gene (Fig. 6 G) showed a lower abundance in the soil with 8-years when compared to those with 2- and 20-years ($P < 0.01$).

In the comparison between bulk soils (upper-case letters in the graph), we observed that the abundance of 16S rRNA of Bacteria and

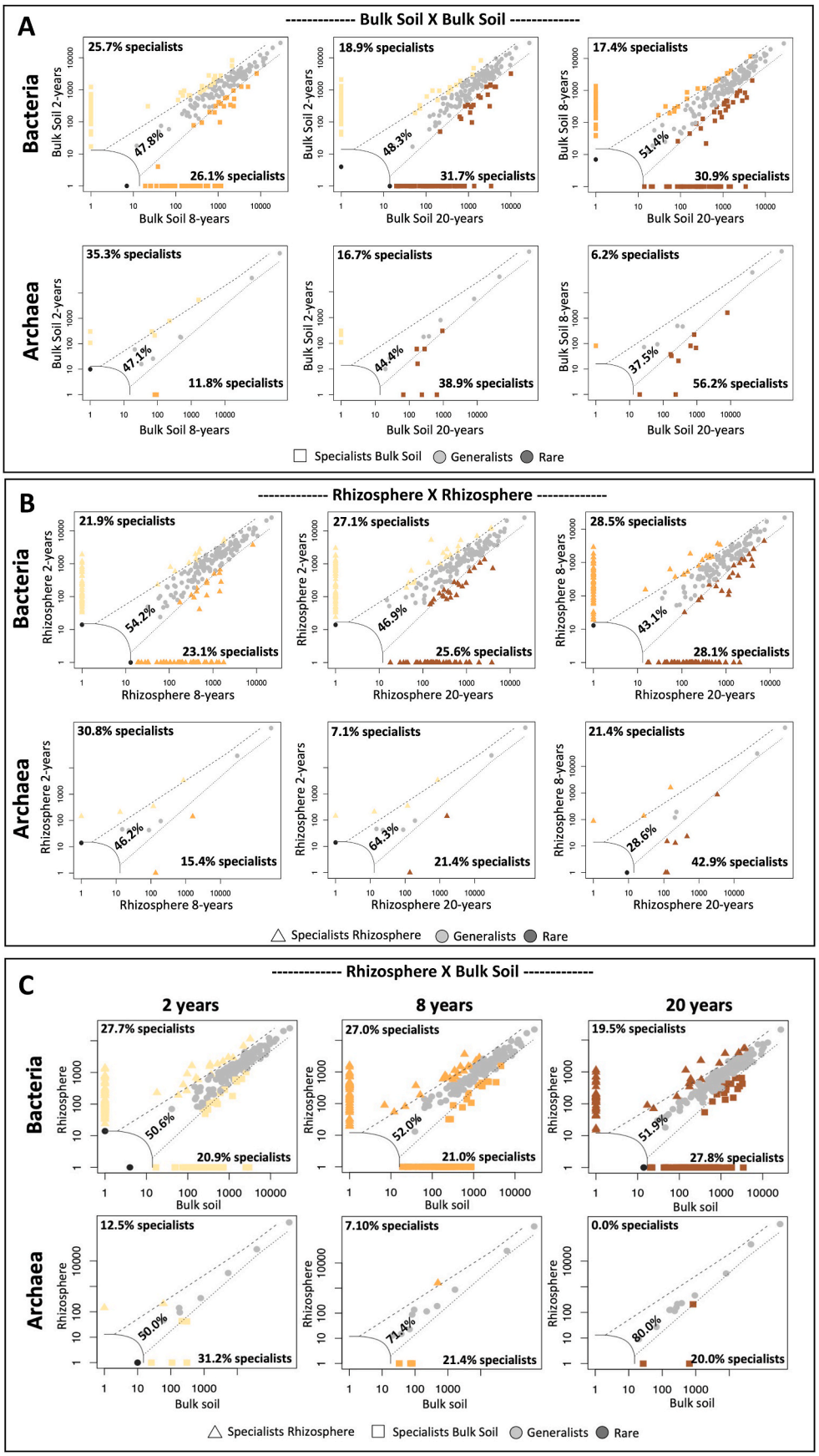

Fig. 4. Multinomial species classification method (CLAM) for the niche occupancy test for Bacteria and Archaea domain. The proportion of generalists, specialists, and rare is displayed in the graphs. The comparison was made between bulk soils (**A**), between rhizospheres (**B**), and in each year between bulk soil and rhizosphere (**C**).

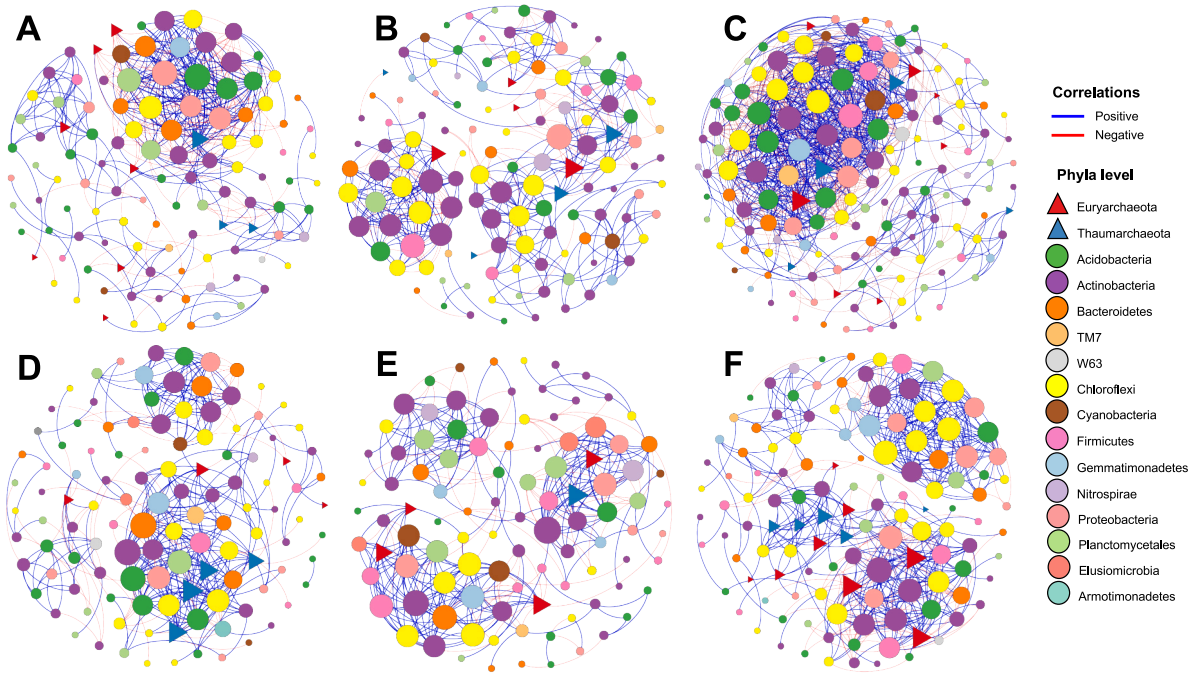

Fig. 5. Network co-occurrence analysis of microbial communities based on 16S rRNA sequencing of Bacteria and Archaea from bulk soil samples of agricultural fields with 2- (A), 8- (B), and 20- years (C) and rhizosphere of soybean plants grown on the soils from agricultural fields with 2- (D), 8- (E) and 20- years (F) of history. A connection stands for SparCC correlation with magnitude >0.9 (positive correlation – blue edges) or < −0.9 (negative correlation – red edges) and statistically significant ($P < 0.01$). Each node represents the genera level, and the node size is proportional to the number of connections (degree). Each node was colored at the phylum level.

Archaea and AOA genes decreased in soils with 20-years when compared to those with 2- and 8-years ($P < 0.05$). In addition, the *nos*Z gene showed a lower abundance in soils with 8-years in comparison to soils with 2- and 20-years ($P < 0.01$).

Then, when comparisons were performed between bulk soil and rhizosphere of each year (asterisk sign in the graph), we found that soils with 2-years of land use presented 5 genes with significant differences. There was a decrease in the abundance of 16S rRNA from Bacteria and Archaea, AOB, and *nos*Z genes in the rhizosphere while the abundance of the AOA gene increased in the rhizosphere ($P < 0.05$). Also, in soils with 8-years 6 genes with significant differences were found. The abundance of 16S rRNA from Bacteria and Archaea, *nir*K, and *nif*H genes decreased in the rhizosphere. On the other hand, the abundance of AOA and *nos*Z genes were higher in the bulk soils. Finally, in the soils with 20-years of land use, only the *nir*K gene increased in the soybean rhizosphere.

The Spearman rank correlation between the abundance of marker genes and soil chemical properties are presented in Supplementary Figure 5B. We found 12 positive correlations between 16S rRNA from Bacteria, with 16S rRNA from Archaea and AOB genes, and with the soil properties NH_4^+, NO_3^-, pH, OM, S, K^+, Ca^{2+}, Mg^{2+}, SB, and CEC. Also, the gene 16S rRNA from Bacteria presented one negative correlations with the AOA gene. The 16S rRNA gene from Archaea showed two positive correlations with 16S rRNA from Bacteria and AOA genes. Regarding the markers gene for ammonia-oxidizers, two positive correlations for the AOA gene were found, including 16S from Archaea and *nos*Z genes. On the other hand, the gene showed 11 negative correlations with soil properties, including NH_4^+, NO_3^-, OM, S, K^+, Ca^{2+}, Mg^{2+}, S.B., CEC, and V%. The AOB gene showed 4 positive correlations, including correlations with 16S rRNA from Bacteria, S, K^+, and H_+Al. Furthermore, the gene presented only one negative correlation with soil pH. The N-fixers marker gene *nif*H presented one positive correlation with the AOA gene and three negative correlations with soil properties, including NH_4^+, NO_3^- and K^+. The denitrification marker gene, *nos*Z, presented two positive correlations, including 16S rRNA from Archaea and AOA genes. No correlation was found for the *nir*K gene.

Lastly, the functional profile of Bacteria and Archaea (based on 16S rRNA) was predicted using the FAPROTAX database (Supplementary Figure 6). We observed that aerobic ammonia oxidation and nitrification were the only functions related to N cycle that differed among the treatments ($P < 0.05$). In general, we found an increase in the number of sequences affiliated to both functions in the rhizosphere and bulk soil of land use with 20-years ($P < 0.05$). Also, when the comparison was performed between bulk soil and rhizosphere of each year, only the aerobic ammonia oxidation showed differences in the soil with 20-years of land use, the results pointed that the process increased in the rhizosphere compared to bulk soil ($P < 0.05$). No difference was found for the Archaea functional profile.

4. Discussion

4.1. Effect of Amazon long-term soil use on microbial communities

In general, the results demonstrated that long-term land use altered soil chemical properties and the microbial community dynamics. The slash-and-burn technique, used by farmers to initially clean the land, negatively affected soil carbon and nitrogen by accelerating the SOM mineralization process (Melo et al., 2017; Pedrinho et al., 2020). In recently deforested areas (i.e., agricultural field with 2-years), this practice led to a momentary increase of the inorganic N pool (i.e., NH_4^+ and NO_3^-) (Piccolo et al., 1994) and helped to raise soil pH, exchangeable bases, and base saturation (Navarrete et al., 2015). Initially, all these conditions stimulated the cash crop (i.e., soybean and corn) to grow and establish itself in this new agricultural field. It is worth mentioning here that, if the correct soil management practices are not applied (i.e., no-till, crop rotation, liming, and fertilization), after a few years of exploitation there is a significant decline in soil nutrient

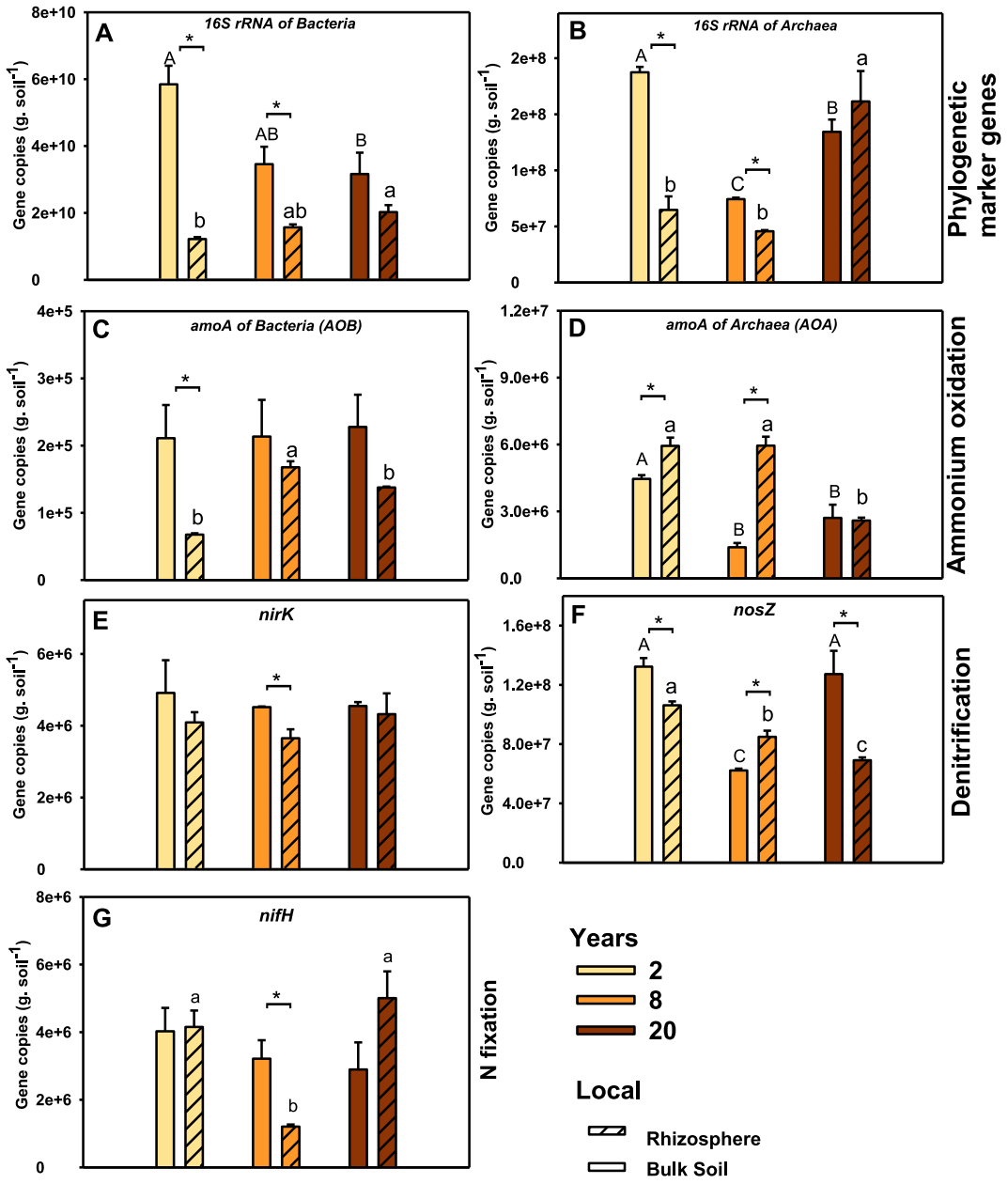

Fig. 6. Abundance of the marker genes 16S rRNA of *Bacteria* (A) and *Archaea* (B), *amo*A of *Bacteria* (C), and *Archaea* (D), *nirK* (E), *nosZ,* (F), and *nifH* (E) from samples of bulk soil and soybean rhizosphere of agriculture areas with 2-, 8- and 20 years of land-use. Error bars represent the standard deviation of three independent replicates. Upper-case letters refer to significant differences between bulk soils (Tukey test, $P < 0.05$). Lower-case letters indicate significant differences between rhizospheres (Tukey test, $P < 0.05$). Asterisks indicate the significant difference between bulk soil and rhizosphere in each year (Tukey test, $P < 0.05$).

availability and biological activity (McGrath et al., 2001; Durrer et al., 2021). On the other hand, when the right soil management practices are used over time, there is a gradual enrichment of microbial abundance (Bossolani et al., 2021) and soil fertility, especially the organic C and N (Mbuthia et al., 2015), such as observed in the agricultural field with 20-years.

Our results also demonstrated that soybean plants were able to explore differently the soil nutrients across the agricultural fields. Apparently, soil management practices used over time in the agricultural field with 20-years (including liming and fertilizer application) provided an accumulation of soil nutrients in the rhizosphere of soybeans. In general, we observed that soil pH, Ca^{2+}, Mg^{2+}, EB, and CEC increased in the rhizosphere of soybeans grown in this soil. Interestingly, all of these soil chemical properties can be linked to the liming practice, which is commonly used in agricultural fields in Brazil to increase the soil pH and make soils more suitable for crop production (Lammel et al., 2015; Mendes et al., 2015). The rhizosphere of soybeans grown on the agricultural field with 20-years also presented high amounts of OM compared to the field with 2-years, which can be related to the no-till system that incorporates crop residues into the soil and contributes to the increase of C and OM over time (Durigan et al., 2017). Lastly, comparing bulk soil with rhizosphere, we observed that soil pH increased in the rhizosphere in all land-use times. The ability of different plant species (including cereal plants and vegetables) to increase soil pH in the rhizosphere region has been previously reported by several authors (Fan et al., 2018; Kuzyakov and Razavi, 2019; Wan et al., 2020). According to these studies, depending on the initial soil pH, plants can release H^+ and OH^- by the roots in order to maintain the balance between cations and anions and mobilize nutrients on the root surface. In this sense, all these mentioned conditions create microhabitats in the

rhizosphere that are occupied by different groups of microorganisms, which help in plant nutrition, growth, and protection (Mendes et al., 2014).

Besides the changes in the soil chemical properties, our results demonstrated that long-term land use affected the bacterial community structure. In general, bulk soil and rhizosphere samples from the agricultural field with 20-years of history were clustered together and were distinct from samples of agricultural fields with 2- and 8-years. Similar results were observed by Goss-Souza et al. (2019) and Mendes et al. (2014), which studied the effect of long-term land use on soybean rhizosphere microbial communities. According to them, this segregation can be related to the long-term soil management practices (i.e., no-till and crop rotation) used by farmers in this agricultural region, which, over time, helps to build up soil fertility and improve the physical, chemical, and consequently, biological properties.

4.2. Soil microbial communities assembling

Regarding the richness and microbial diversity, we did not observe significant differences among the bulk soil samples from the different agricultural fields. On the other hand, soybean plants were able to alter the bacterial and archaeal diversity in the rhizosphere region. In general, the bacterial diversity in the rhizosphere of soybean plants grown on the agricultural field with 20-years decreased, while the archaeal diversity increased for this same field. According to Mendes et al. (2014) and Chapelle et al. (2016), the microbial community of the rhizosphere is part of a complex food web that utilizes the organic substances released by the plant roots. These organic substances, in general, simple carbon compounds (i.e., small sugars, amino acids, organic acids, and enzymes), are the main driving forces in the regulation of microbial diversity and activity in the rhizosphere region. Furthermore, we highlight that bulk soil, the adjacent root-free soil, is the main source of microbial species in the rhizosphere (Mendes et al., 2014; Bakker et al., 2015). Thus, the long-term forest-to-agriculture conversion can alter the microbial community in the bulk soil, and consequently, have an impact on the assembly and composition of the rhizosphere community (Goss-Souza et al., 2019). Furthermore, the differences found in the rhizosphere may be related to the soil memory effect (Li et al., 2019; Longley et al., 2020). The same long-term land use could homogenize bulk soil communities and consequently, decrease the diversity in the rhizosphere. We noticed in our study that the Bacteria diversity index was higher than Archaea. The bacterial diversity is usually higher than archaea in almost all environments, but this could be related to the uneven representation of archaea in the databanks (Louca et al., 2019).

In general, we observed that the microbial composition of bulk soil and soybean rhizosphere was influenced by long-term land use. For both, the agricultural field with 20-years of land use presented the most distinct microbial composition when compared to the other fields. In the bulk soil, we observed that the agricultural field with 20-years presented an increase in the abundance of Gemmatimonadetes, Chloroflexi, and Firmicutes. Gemmatimonadetes has been identified as a cosmopolitan phylum, with high abundances in different land-use systems including agriculture, pasture, forest, and contaminated soils (Debruyn et al., 2011). Previous studies have identified positive correlations of different members of this phylum with soil C and N (Debruyn et al., 2011), which can help us to explain the increased abundance of Gemmatimonadetes in the long-term cropping system (Goss-Souza et al. 2017, 2019). Different studies have also demonstrated that Chloroflexi and Firmicutes members have a preference for environments where C and N are highly available (Rodrigues et al., 2013). Furthermore, these microorganisms are known to be highly adaptable to stressing conditions including desiccation and high-temperature variation, which are striking features of Amazon tropical soils (Fullerton and Moyer 2016; Battistuzzi and Hedges 2009; Goss-Souza et al., 2019). Conversely, we observed a decrease in the abundance of the phyla Bacteroidetes, Actinobacteria, and Nitrospirae in the agricultural field with 20-years of history. Bacteroidetes are among the most represented phyla in studied soils, and typically represent around 5% of the total soil microbial community (Larsbrink and McKee, 2020; Fierer et al., 2012). Members of this phylum are specialized in the degradation of complex organic compounds (i.e., cellulose, hemicelluloses, and pectins) typically found in large quantities in the aboveground litter of forest soils (Wolińska et al., 2017). The leaves, stems, and other newly dead plant tissue in the upper horizon of forest soils represent an important micro-habitat for Bacteroidetes, where they can thrive and become increasingly dominant as the decomposition process of litter material progresses (Brabcová et al., 2016). Actinobacteria have also been noticed in forest soils and early successional stages of forest-to-pasture (da C Jesus et al., 2009) and forest-to-agriculture conversion (Merloti et al., 2019), with lower percentages being found after a long period of conversion, mainly due to increases in soil pH (Kuramae et al., 2012). Interestingly, in our study, we observed a negative correlation of Actinobacteria phylum with Ca^{2+} and CEC, both soil properties linked to soil pH. Thus, long-term land use changed the preferential environmental conditions of these organisms (forest soils), which may explain the decrease of these groups in soils with 20 years of use. Lastly, the Nitrospirae phylum has important members (i.e., Nitrobacter and Nitrospira) acting in the nitrification process in tropical and subtropical soils (Mendes et al., 2015; Palomo et al., 2018). Previous studies have associated inorganic N fertilization with a high abundance of Nitrospirae in agricultural fields (Attard et al., 2010; Goss-Souza et al., 2017). However, we did not observe that in our study, probably because the amount of inorganic N pool (*i.e.*, NH_4^+ and NO_3^-) in the agricultural field with 20-years was very low compared to the field with 2-years, which may help us to explain the lower abundance of this phylum.

In the rhizosphere, we observed that the agricultural field with 20-years presented an increase in the abundance of the phyla Planctomycetes, Chloroflexi, and Gemmatimonadetes. Previous studies performed in the Amazon region by Goss-Souza et al. (2019) and Mendes et al. (2014) also found an increase in the abundance of Planctomycetes in the rhizosphere of soybean plants grown in a long-term cropping system. Members of this phylum occur mainly in soils with pH ranging from 5.5 to 6.0 (Goss-Souza et al., 2019). According to Cabello et al. (2009), Planctomycetes are also involved in different N transformation processes, including the anammox (*i.e.*, oxidation of NH_4^+ to N_2O using NO_2^- as an electron acceptor), which occurs under anaerobic conditions (Cabello et al., 2009). Thus, the rhizosphere seems to have the perfect conditions for Planctomycetes members, since in this region there is high carbon and low O_2 concentration, which may favor the growth of anoxic microorganisms. Interestingly, Chloroflexi and Gemmatimonadetes presented a high abundance in the bulk soil as previously mentioned and also in the rhizosphere of soybeans grown on the agricultural field with 20-years. Here, we argue that the composition of the microbial community and its activity in the rhizosphere has a large influence from the surrounding soil community (Mendes et al., 2014; Bakker et al., 2015). In this sense, soybean plants grown on the agricultural field with 20-years were able to take advantage and select a large portion of these microorganisms, which have a preference for environments where C and N are highly available (Debruyn et al., 2011; Rodrigues et al., 2013) and in many cases can help in plant growth and protection (Mendes et al., 2018).

On the other hand, we observed a decrease in the abundance of the phyla Proteobacteria, Cyanobacteria, and Verrucomicrobia in the rhizosphere of soybean plants grown on the agricultural field with 20-years of history. Proteobacteria has been described as a dominant group in tropical forest soils, with a significant decrease after the land-use change (da C Jesus et al., 2009; Navarrete et al., 2015; Goss-Souza et al., 2017). Furthermore, Proteobacteria members possess a versatile metabolism and are important to the global carbon, nitrogen, and sulfur cycling, functions which are usually abundant in forest soils (Pedrinho et al., 2019; Mendes et al., 2015). Cyanobacteria members also possess a versatile metabolism and are important to the C and N cycling in both

marine and terrestrial environments (Schirmacher et al., 2020). In our study, the phylum Cyanobacteria correlated negatively with pH, SB, and V%, suggesting that this group has a preference for acid soils (typically found in tropical forest soils), as previously mentioned by Zhang et al. (2017). We highlight that Verrucomicrobia is an abundant phylum in many different terrestrial ecosystems, including in tropical forest soils (Bergmann et al., 2011; Fierer et al., 2013). Verrucomicrobia members have potential roles in methane oxidation and degradation of complex C compounds (Martinez-Garcia et al., 2012). According to Nie et al. (2018), in general, Verrucomicrobia are oligotrophic and members of this phylum exhibited a decrease in abundance in fields with high N availability. In this sense, the conversion of forest for agriculture associated with land use over time may have modified the optimal development environment of these groups and caused their decrease in soils with 20 years of use.

When comparison was performed between the bulk soil and rhizosphere according to land-use time, we observed that long-term land use influenced the selection of microbial groups by the soybean plants. In general, we observed an increase of phyla Candidate division TM7, SHA.109, SM2F11, and Armatimonadetes in the rhizosphere of soybean plants grown on soils from the agricultural fields with 2- and 8-years of history. Interestingly, all these phyla together represented less than 1% of the general bacterial community. They are considered candidate phyla, which means bacterial groups without culture-based representatives and with very few known functions. Moreover, Candidate division TM7 and Armatimonadetes have been previously described as part of the "rare biosphere", and it is believed that they can exhibit unique ecology roles in comparison to the most abundant phyla in the soil (Lynch et al., 2015).

4.3. Soil microbial communities dynamics and interactions

Then, we assessed the dynamics of the microbial groups comparing the niche occupancy across the different chronosequence stages and soil compartments. In general, our results demonstrated that soil management practices used over time (*i.e.*, no-till, crop rotation, liming, and fertilization) created favorable conditions for microbial specialists in the agricultural field with 20-years of land use. According to Monard et al. (2016), microbial specialists possess a narrow range of niches and a greater fitness when they meet their optimal conditions. Microbial specialists are also remarkably responsive to environmental disturbances, including changes in vegetation cover and soil physicochemical properties (Pandit et al., 2009; Pedrinho et al., 2020). For that reason, we believe that soil management practices used in this field over time changed soil chemical and biological properties and may have created very specific conditions that favored the growth and diversity of microbial specialists in both compartments, bulk soil and rhizosphere. Interestingly, we observed that Planctomycetaceae (Planctomycetes phylum), two uncultured Ktedonobacteria (Chroloflexi phylum), and Nitrosomonadaceae (Proteobacteria phylum) were among the top 10 most abundant microbial specialists in the agricultural field with 20-years. Interestingly, all these phyla have members involved in different N transformation processes, including annamox (Van Niftrik et al., 2009) and ammonia oxidation (Segawa et al., 2014; Barton et al., 2014).

Further, we used network analysis to better disentangle the dynamics of the microbial community across the different chronosequence stages and soil compartments. In general, we observed an increase in the number of correlations among microbial groups in the agricultural field with 20-years when compared to fields 2- and 8- years. This result may be linked to the environmental role performed by some microbial groups in this area. Previous studies suggested that a decrease in soil microbial diversity could lead to microbial homogenization and losses of soil functions (Rodrigues et al., 2013; Philippot et al., 2013a). Therefore, we believe that the increase in the number of correlations and complexity among microbial groups in the agricultural field with 20-years could be related to the increase of cooperative and trophic interactions in these soils (Morriën et al., 2017; Khan et al., 2019). This evidence is reinforced by the higher number of positive correlations over negatives observed in the networks from this field. Furthermore, we highlight a higher network complexity in bulk soil compared to the rhizosphere. According to Mendes et al. (2014), the adjacent root-free soil (bulk soil) is the main source of microbial species and diversity in the rhizosphere. For that reason, the microbial community in the rhizosphere is considered a subset of the bulk soil community with less diversity (Philippot et al., 2013b). Considering the degree (*i.e.*, the number of links that a node has to other nodes) and betweenness centrality (*i.e.*, the number of times a node plays a role as a connector along the shortest path between two other nodes), we observed a prevalence of the phyla Chloroflexi and Actinobacteria in the networks of the agricultural field with 20-years of history. Interestingly, among the top 10 most abundant specialists on soil with 20-years, it was found 3 members belonging to Actinobacteria phylum (with the last classification described as Solirubrobacterales, Acidothermus, and Geodermatophilaceae) and the other two to Chloroflexi (both uncultured Ktedonobacteria). These groups have members characterized as resistant to oxidative stress (Normand et al., 2014), responsible for plant biomass degradation (Berry et al., 2014), and mediating geochemical recycling (Hu et al., 2019). It is worth mentioning that both Actinobacteria and Chloroflexi phyla are characterized by having many members responsible for producing secondary metabolites (Yabe et al., 2017; Barka et al., 2016), which may favor the interaction with other microbial communities, and thus, making them essential parts of the microbial network.

4.4. Soil microbial communities' potential functions

The microbial communities' size was measured through Real-Time qPCR. We used phylogenetic primers to quantify total Bacteria and Archaea across the different chronosequence stages and soil compartments. Also, to access the microbial community functions related to N cycle, we quantified functional marker genes of N-fixation (*nif*H), nitrification (*amo*A from Bacteria and Archaea), and denitrification (*nir*K and *nos*Z). In general, our results pointed to an increase in the abundance of total Bacteria in rhizosphere soils with 20-years of land use. Interestingly, the 16S rRNA of Bacteria was positively correlated to many soil nutrients including the soil pH. It is worth mentioning that copiotrophic environments can favor the abundance of bacteria (Wang et al., 2021; Halter et al., 2020). The neutral soil pH and great nutrient availability can create optimal development conditions for many groups of bacteria (Merloti et al., 2019). Thus, the plant exudation and the soil amendment over the years may have favored the growth of bacteria in these soils. The N-fixers (represented by the *nif*H gene) also increased in the rhizosphere soil with 20-years. This gene was found in many Amazonian agricultural soils (Mirza et al., 2014; Pedrinho et al., 2020). A positive correlation with many soil nutrients was found and indicated the preference of N-fixers to copiotroph and non-acid environments (Mirza et al., 2014; Merloti et al., 2019). Interestingly, our results pointed that this gene correlated negatively with nitrate and ammonium. Both soil properties decreased in soils with 20-years. In this way, the absence of available N may have increased the abundance of this community. According to Li et al. (2019) N-fixers are usually inhibited by the high available of N in agricultural soils. However, when these elements are scarce, they can increase their abundance and activity to supplement the plant's needs for this nutrient (Ciampitti et al., 2018; Li et al., 2019). Considering the ammonia-oxidizing bacteria (represented by *amo*A from bacteria), there was an increase in the rhizosphere of soybean plants grown in the soil with 20-years. On the other hand, the ammonia-oxidizing archaea (represented by *amo*A from archaea) decreased in the same soils. The nitrogen made available by the N-fixing communities, together with the amendments performed over the years, may have contributed to the greater abundance of this group of microorganisms. This explanation is reinforced by the positive correlations

between these group of bacteria and several soil nutrients observed in our study. It is worth mentioning that bacteria and archaea compete for the same substrate that they extract energy (ammonium) (French et al., 2021). In addition, unlike bacteria, ammonia-oxidizing archaea usually prefer more acidic environments and less availability of nutrients (Hu et al., 2014). In our study, this gene showed many negative correlations with soil nutrients. Thus, the described environmental characteristics that favored ammonifying bacteria in the soil may have inhibited the oxidizing ammonia archaea in the same environment, explaining our results. Our study suggests the predominance of bacteria in the oxidation of ammonia. This suggestion is reinforced by the analysis of functional prediction, which found a higher percentage of aerobic ammonia oxidation and nitrification processes in rhizosphere soils with 20-years of land use.

Finally, considering the comparison between rhizosphere and bulk soil in each year, we can emphasize that the long-term land use has changed the size of the total microbial communities and those genes related to the N cycle. We noticed that long-term land use turned the microbial abundance more similar between bulk and rhizosphere. In soil with 2-years, five genes showed statistical differences between bulk soil and rhizosphere, while in soils with 8-years, there were six genes with the same differences. However, in the soil with 20-years of land use, only one gene showed a statistical difference between rhizosphere and bulk soil. These results pointed that the long-term land use also caused a homogenization in the abundance of microbial communities, as seen in the composition and niche occupancy analysis.

5. Conclusion

In conclusion, our results pointed that long-term land use influenced the soil bacterial and archaeal communities and their potential functions related to N cycle in both bulk soil and rhizosphere. Microbial communities' structure from bulk and rhizosphere of soybean plants grown in the soil with 20-years of land use were different from the more recent soils (2- and 8-years). The bacterial diversity index decreased in the rhizosphere with 20-years while the archaeal diversity increased. We noticed that Gemmatimonadetes, Chloroflexi, Firmicutes, and Planctomycetes were the main phyla that increased in soils with 20-years of land use. The microbial community's interactions were also different in the oldest agricultural soil, with more correlations between microbial groups and a great importance of Actinobacteria and Chloroflexi phyla for network co-occurrence structure. The N cycle functions were also influenced by the long-term land use, mainly in the rhizosphere. N-fixers and bacteria ammonia-oxidizers abundance increased in the rhizosphere soil with 20-years. The potential functions also pointed that the nitrification process increased in the same soils. We correlated these results with the agricultural practices used over the years, such as liming and no-till, that influenced soils chemical characteristics such as pH, Ca_2^+, Mg_2^+, and OM. It is worth mentioning that the size of bacterial communities became similar between bulk soil and rhizosphere along the time of land use. Our finds suggest the importance of future research to understand how long-term land use influences soil microbial interactions and their potential functions in bulk soil and rhizosphere, bringing new information for more sustainable use of the soil for agricultural practices. Also, future studies of the dynamics of plant-associated microbial communities within agriculture should consider assess the microbiome assembly in different plant compartments and developmental stages (Moroenyane et al., 2021), which would help researchers to manipulate plant microbiomes for beneficial services.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.rhisph.2022.100482.

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