Investigating the effects of Brachiaria (Syn. Urochloa) varieties on soil properties and microbiome

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Abstract

Background and Aims

The *Brachiaria* sp. (synonymous with *Urochloa*) is one of Brazil's main grass species used in livestock production and has become the focus of breeding genetic programs to enhance its resistance to drought, flooding, and pests, as well as improving its palatability to animals. However, there is a limited understanding of how genetic breeding can affect the soil microbiome and its potential functions. Thus, this study aimed to investigate the impact of four different *Brachiaria* varieties on the soil prokaryotic and fungal communities, particularly emphasizing their potential functions related to the N-cycle.

Methods

We combined molecular techniques, such as quantitative PCR and amplicon sequencing, to target prokaryotic and fungi communities and traditional soil and plant chemical analyses.

Results

Our findings revealed that all varieties improved soil porosity, P content, and organic carbon. Soil acidity, nutrient availability, and porosity were the main drivers of the microbial communities. The *Nitrososphaeraceae*, *Gaiellales*, *Conexibacter*, *Sphingomonas*, *Hydnophlebia meloi*, *Conocybe*, and *Cladosporium* were the main taxa associated with the dissimilarities between the *Brachiaria* varieties and the Control. In addition, the presence of the plants increased potential microbial functions such as Chemoheterotroph, Aerobic-Chemoheterotroph, and Pathotroph-Saprotroph groups. The study also identified the ability of each variety to recruit nitrogen-fixing and bacterial and archaeal ammonia-oxidizing communities.

Conclusion

Our findings suggest that selecting an efficient *Brachiaria* variety could positively impact soil quality, improving agricultural systems and increasing food production.

Introduction

The *Brachiaria* sp. (synonymous with *Urochloa*) is one of the main grass species used in livestock production lands in South America (Pizarro et al. 1996) and Brazil (Dias-Filho, 2016). The C4 plant has aggressive root growth and significant biomass production and can grow in soils with low fertility (Dias-Filho, 2016). Those characteristics make this species suitable for tropical climates and farms with low technology and investment. However, the lack of grassland maintenance and investments also created a land degradation scenario, and nowadays, more than 60% of the Brazilian grasslands have some level of

degradation (Project MapBiomas, 2022). In this sense, over the past 40–45 years, this grassland species started to be the target of breeding genetic programs to meet different goals (Pizarro et al. 1996). As a result, different varieties have emerged as resistant to various stressors, including drought, fire, leafhoppers, and low-fertility soils. Additionally, these varieties offer a high nutritional value and are easily digestible by the cattle, making them an attractive option for livestock production (Euclides et al. 2016). Thus, grasslands in Brazil have transitioned from being solely a subsistence crop with low use of technology and investment by farmers to being managed with more advanced and conservation-oriented agricultural practices (Dias-Filho, 2016; (Baptistella et al. 2020).

Brachiaria has recently become a popular choice for intercropping and crop rotation systems due to the growing demand for sustainable agriculture and the implementation of conservationist practice (Kluthcouski et al., 2000). Using *Brachiaria* in these systems benefits soil characteristics such as soil organic matter, porosity, protection against erosion, and suppression of weeds (Almeida and Rosolem 2016; Eri et al. 2020; Bieluczyk et al. 2020; Silva et al. 2021). Together, those characteristics can improve crop yield production in consortium or rotation with *Brachiaria* (Brandan et al. 2017; Crusciol et al. 2021). However, despite the potential benefits of using *Brachiaria* in intercropping and crop rotation systems, little is known about how the genetic breeding of these plants impacts the selection of the soil microbiome.

Recent studies have shown that *Brachiaria* has a complex relationship with the soil microbiome, involving competitive advantages in nutrient acquisition and mutualistic associations with arbuscular mycorrhizal fungi (AMF). For instance, some *Brachiaria* varieties can inhibit soil nitrifiers in a process known as biological nitrification inhibition (Subbarao et al. 2009). The inhibition gives and competitive advantage to this plant in the competition for the N available in the soil against microbes, which can also potentially decrease the N losses through the denitrification process (Subbarao et al. 2009; Momesso et al. 2022). Additionally, the association with AMF can enhance phosphorus uptake by the plant (Nakamura et al. 2020). However, little now about how these plants can impact the overall soil microbiome composition and their potential functions.

In this study, we aimed to investigate the impact of different *Brachiaria* varieties on prokaryotic (archaeal and bacterial) and fungal communities and their potential functions, focusing on the N-cycle. By combining molecular techniques such as quantitative PCR (qPCR) and amplicon sequencing with traditional soil and plant chemical analyses, we sought to validate our hypothesis that (1) *Brachiaria* varieties can modify the soil microbiome and (2) recruit microorganisms that help in its nutrition and development. To achieve this, we addressed the following questions: (i) Do the *Bracharia* varieties cause changes in soil physical-chemical characteristics, and which factors are affected? (ii) What changes do the *Brachiaria* varieties induce in the microbial communities? (iii) If so, which soil characteristics are associated with these changes? (iv) How do the *Brachiaria* varieties modify the microbial potential functions and marker genes related to N-cycle in the soil?

Material And Methods

Field characteristics and experiment design

The field experiment was carried out at the Experimental Farm Station from State Sao Paulo State University (UNESP) in Botucatu, southern São Paulo State, Brazil (22° 83' 3" S, 48° 42' 64" W, elevation 765 m). The field area was used for agriculture for the last ten years, and the land-use history is presented in Supplementary Table 1. The region's soil type was classified as sandy clay loam kaolinitic and thermic Typic Haplorthox (USDA, 2022). The region's climate is Cwa type, according to the Köppen–Geiger climate classification system The region's long-term (1956–2020) annual average temperature is 20.7°C (maximum of 26.1°C and minimum of 15.3°C). The annual rainfall average is 1360 mm. The soil texture from the field experiment was classified as medium texture (309 g kg^{-1} of clay; Supplementary Table 2).

The experiment was initiated in January 2019. The previous crop grown in the area was soybean with no-till management. After the soybean harvest, the soil was prepared by adding P and K in the seeding line based on the soil fertility analysis and following fertilization recommendations for the São Paulo State (Souza et al., 2007; Cantarella et al., 1997). The following varieties of *Brachiaria* were sown in a density of 5 kg ha^{-1} of viable seeds: (1) *Brachiaria brizantha* cv. Marandu (treatment BM); (2) *Brachiaria ruziziensis* (treatment BR); (3) *Brachiaria* spp. cv. Ipyporã - BRS RB331 (treatment BI and a hybrid of *Brachiaria ruziziensis* × *Brachiaria brizantha*); (4) *Brachiaria brizantha* cv. BRS Paiaguás (treatment BP). In addition to the varieties, a control treatment (Ctrl) was added, representing the soil without the plant. The BM and BI are recent varieties available in the Brazilian seed market (2017), while the BR and BP are considered the most used varieties in Brazil. More information about the chosen *Brachiaria* varieties' characteristics is available in Supplementary Table 3.

The experimental design was a randomized block with four replications. A total of 20 plots were carried out ((4 varieties of *Brachiaria* + 1 control) × 4 replicates). Each plot had 15 m × 20 m and a space of 2 m between each plot (details are available in Supplementary Fig. 1). Fifteen months after the sowing (mature forage grasses established in the field), plants and soil were sampled (April 2020).

Soil sampling

Five points were sampled in each plot (1 central point and 4 in each corner) in the 0–10 cm layer (details in Supplementary Fig. 1). After that, the samples were mixed to form one composite sample per plot. Approximately 600 g of soil were sampled to perform soil chemical and texture characteristics. Undisturbed samples were carried out using an auger and metal rings (100 cm^3). After removal, the samples were rolled up and immobilized on cling film, delicately stored in cardboard boxes, and transported to the laboratory for analysis of density and porosity. Lastly, 50 mg was sampled in falcon tubes, immediately frozen in nitrogen (N) liquid, and stored in a -20°C freezer for further DNA extraction.

Plant sampling, biomass, and N content

A total of 30 random *Brachiaria* leaves (only the central third parts) in each plot were sampled for N quantification, following the methodology of Malavolta et al. (1997). In addition, SPAD index readings estimated the N Leaf content using a hand-held chlorophyll meter model SPAD-502 Plus (Konica Minolta).

Within each plot, the *Brachiaria* roots were sampled thrice using an auger and metal rings (100 cm^3) while the shoot plants' biomass was collected in an area of 1 × 1 m. After that, the *Brachiaria*'*s* shoot biomass and roots were dried in the oven with forced ventilation at 60°C for three days. After that, the samples were weighed and converted to kg ha^{-1}.

Soil chemical and physical analysis

The soil macro- and micronutrients were measured based on the methodology proposed by(Cantarella et al. 1998) for Brazilian tropical soils. Details about each method were described by (Bossolani et al. 2020). The analyses were based on homogenized soil using a 2 mm sieve, air-dried, and weighed according to the need for each analysis (gravimetric method). Briefly, soil pH and the soil organic carbon (SOC) were measured using 0.01M $CaCl_2$ and Walkley–Black method, respectively. P-phosphate (P), potassium (K), calcium (Ca^{2+}), and magnesium (Mg^{2+}) were extracted by anion exchange resin and determined by colorimetric method. S-Sulfate was extracted by 0.01 M calcium phosphate solution and quantified by the turbidimetric method using $BaSO_4$. Manganese (Mn), zinc (Zn), copper (Cu), and iron (Fe) were extracted using DTPA and determined by atomic absorption spectrometry. Aluminum (Al) was extracted using KCl and measured by titration. The Potential acidity (H + Al) was determined by the Shoemaker-McLean-Pratt method. Ammonium (NH_4^+) and nitrate (NO_3^-) were extracted using KCL and quantified by the calorimetric method. Based on the results, the sum of bases (SB) was calculated by the sum of the cations K, Ca^{2+}, and Mg^{2+}; cation exchange capacity (CEC) by the sum of H_+Al and cations; base saturation ($V_\%$) was determined by SB/CEC and Al saturation ($m_\%$) by Al/CEC. Finally, the sum of NH_4^+ with NO_3^- resulted in the N-Inorganic, and the difference between N-Total and N-Inorganic calculated the N-Organic.

The soil porosity analysis was based on(Klute and Dirksen 1986) and (Smith and Mullins 2001) methodologies. First, undeformed soil samples were saturated with water for 48 hours. After that, samples were weighed and taken to Richard's pressure chamber on porous plates under − 0.006 MPa tension until they stabilized. Then, samples were weighed and dried in a forced-air oven at 105°C for 60 hours. The total porosity was calculated by the difference between the weights of the water-saturated and dried samples. The macro-porosity was obtained by the difference between the water content of the water-saturated samples from Richard's pressure chamber. The micro-porosity was based on the difference between the total porosity and macro-porosity.

Soil DNA extraction

The total DNA from soil samples was extracted using the Power Soil DNA Isolation Kit (Qiagen, Hilden, German) based on 0.25 g and following the manufacturer's instructions. Briefly, the soil sample was homogenized and the microbial cells were lysed by mechanical and chemical methods. After that, the total genomic DNA was captured on a silica membrane in a spin column format, washed, and eluted from the membrane to obtain the extract. In addition, the DNA concentration was measured using the Qubit

fluorometer (Invitrogen, Carlsbad, USA) according to the manufacturer's protocol. Finally, the DNA quality was checked through 1% sodium boric acid agarose gel electrophoresis analysis (Brody and Kern 2004).

PCR in Real-Time (qPCR) of marker-genes

The size of the soil microbial communities was measured through StepOnePlus™ Real-Time PCR System (qPCR) with 96-well plates (Applied Biosystems, Foster City, CA, USA). The qPCR was carried out for the bacteria (based on *16S rRNA* from Bacteria), archaea (also based on *16S rRNA* gene), and fungi communities (based on *ITS* gene). Also, the quantification of microbes related to the N-cycle was performed, including the N-fixers (based on the *nif*H gene) and the bacterial ammonia-oxidizers (AOB, based on the *amo*A gene from Bacteria) and archaeal ammonia-oxidizers (AOA, based on the *amo*A gene from Archaea). First, a PCR was performed using DNA extracted from strains that harbor the gene of interest for each gene. After the PCR product was quantified, standard curves were created after serial dilutions. The 16S rRNA gene quantification (from bacteria and archaea) was based on 10µL that contained: 5µL of qPCR SYBR Green Master Mix, 1µL of each primer (5pmol), 1.5µL of ultrapure water, 1µL template DNA, and 0.5µL bovine serum albumin (BSA; 10 mg ml^{-1}). For the ITS gene, the qPCR analysis was based on 25µl that contained: 12.5µl qPCR SYBR Green Master Mix, 1.25µl of each primer (10pmol), 2.5µL BSA (10 mg ml-1), and 1µL template DNA. The analyses of melting curves were performed from 68 to 95°C, and all standard curves had R2 greater than 0.98. The results were analyzed using the StepOnePlus™ Real-Time software version 2.2.2 (Applied Biosystems, Foster City, CA, USA). Strains used to construct the standard curves, primers, and reaction conditions for the amplification of the genes are described in Supplementary Table 4.

DNA Sequencing and Bioinformatic analysis

The amplicon sequencing analyzes were performed for the 16S rRNA and ITS genes performed at the Center for Functional Genomic Research (ESALQ/USP), located in the municipally of Piracicaba, São Paulo state, Brazil. In addition, a PCR was performed for each barcode. The primers and reaction conditions used for amplification are described in Supplementary Table 4. In total, 20 libraries for each barcode were prepared using the MiSeq Reagent Kit v3 (Illumina, San Diego, CA, USA), following the manufacturer's instructions for the Illumina MiSeq platform (2 × 150 bp paired-end).

The sequences for both prokaryotic (based on *16S rRNA*) and fungal communities (based on the *ITS*) were processed using the DADA2 pipeline (Callahan 2017) in the R environment (R Core Team, 2021). First, the primers from the demultiplexed data were removed. After, the data were trimmed and filtered to remove low-quality sequences and, the denoising inference step was performed (based on the learn error step). After that, the forward and reverse sequences were merged, and the chimeric sequences were removed based on the "consensus" method. Next, the taxonomic inference was performed using the SILVA database (v. 138; (Quast et al. 2012) to the *16S rRNA* data and the UNITE (v. 8.2; (Kõljalg et al. 2013)) to the *ITS*. A compositional matrix was generated for each amplicon. Finally, the prokaryotic matrix was rarefied to 27585 sequences per sample, and the fungal matrix to 22687 sequences per sample

(Supplementary Fig. 2 and Supplementary Table 5). Sequences were submitted to the NCBI Sequence Read Archive under the identification BioProject ID PRJNA947540.

Statistical analysis

The statistical analysis was performed using the varieties of *Brachiaria* (4 varieties + Control) as a factor and with 4 biological replicates, totaling 20 samples. The effect of the blocks was only considered for statistical analysis. The data were analyzed using the R platform (R Core Team, 2021), graphs were created with the “ggplot2” package (v. 3.4.0; (Wickham 2016), and statistical analysis was performed with the packages “vegan” (v.2.6-4; (Oksanen et al. 2013) and “agricolae” (v. 1.3-5;(De Mendiburu and Simon 2015). Distance-based redundancy analysis (db-RDA) was performed to check the soil prokaryotic and fungal community structure and its correlation with the soil characteristics. For the analysis, the ASVs compositional table from the 16S rRNA and ITS sequencing were used as biological matrices and transformed in the Bray-Curtis distance. After that, the soil characteristics were used as an explanatory matrix. The significance of each soil characteristic in the distribution of the biological data was tested by the PERMANOVA test (based on 999 permutations). The significance of the obtained clusters was calculated by the ADONIS permutational test followed by the pairwise Adonis test (both based on 999 permutations and adjusted by Bonferroni correction). The soil microbial diversity (Shannon-Index), richness (Taxa S index), and Simper dissimilarity (in %) were calculated using the “vegan” package. The gene abundance obtained by the qPCR analysis, the soil microbial diversity, and richness were represented as a box-plot. The Simper results were based on the comparison between the Control and the varieties of *Brachiaria*, exported to Excel (Microsoft) and plotted as Bar-Plots. The statistical differences between groups for the soil physical-chemical and plant characteristics, microbial diversity, richness, and gene abundance datasets were based on the One-Way ANOVA test, followed by the LSD test to compare groups ($P < 0.05$) with p-value adjusted by Bonferroni correction. A functional prediction annotation was performed to access the potential functions displayed by the soil microbes. The prokaryotic community matrix at the genus level was used as input in the FAPROTAX database (v. 1.2.4;(Louca et al. 2016), which maps prokaryotic taxa to putative functions using information based on functional annotations of cultivated representatives. For fungi and nematodes, their compositional matrices at the specie level were used as input in the FUNGuild database (v.1.1; Nguyen et al. 2016). It annotated these soil communities in trophic modes, and only confidence scores of ‘Probable’ and ‘Highly Probable’ were used. The group comparison for microbial composition and potential functions datasets was based on the One-Way ANOVA, followed by the post-hoc Tukey-Kramer test ($P < 0.05$) and after Benjamin-Hochberg FDR correction. To test the correlation between gene abundance, soil, and plant characteristics, a Spearman rank analysis was carried out and represented as a correlogram using the “corrplot” package (v. 0.92; Wei and Simko, 2021).

Results

The soil and plant characteristics

The soil physics and chemical characteristics are represented in Table 1. Our results showed that the varieties of *Brachiaria* changed the soil pH and nutrient availability. The BR presented the lowest soil pH compared to the other treatments, including the Control. Consequently, the availability of soil nutrients dependent on the pH also changed. In general, soil K, Ca^{2+}, Mg^{2+}, SB, $V_{\%}$, and S under the influence of the BR variety presented the lowest values compared to the Control and the other varieties. On the other hand, the BR showed the highest potential acidity (H + Al). The varieties changed the SOC concentration and P_{resin} availability in the soil. Soils cultivated with *Brachiaria* varieties presented more SOC than the Control; in addition, BI showed the highest SOC value. Also, the varieties presented more abundance of P compared to the Control. Interestingly, soil N also changed according to the varieties. The BR variety presented the lowest value for N-total and N-Organic, whereas the varieties BP and BI presented the highest ones. The varieties showed the lowest N-Inorganic values compared to the Control. For NH_4^+, the Control and the varieties BM and BI showed the lowest values, while the varieties BR and BP presented the highest ones. However, the Control showed the highest values for NO_3^- compared to the varieties. Lastly, the soil-physical characteristics also changed according to the presence of the varieties of *Brachiaria*. The varieties BR and BI decreased the soil density compared to the other treatments. However, the presence of plants increased the soil Total-Porosity and Macro-Porosity, compared to the Control. Regarding the N content in the plants, the N-Leaf concentration and SPAD index were 30% lower in the BR variety compared to the BI. However, the Shoot-Biomass was 30% higher in the BI variety compared to the average of the other varieties. Lastly, the BI variety showed a Root-Biomass 61% higher than the BP variety.

Microbial community structure and diversity

The db-RDA analysis was performed to investigate the prokaryotic and fungal community structure and their correlation to the soil characteristics, represented in Fig. 1. The results indicated that the varieties of *Brachiaria* performed as a driver for the soil microbial community structure and that soil characteristics played an important role in these changes. For the prokaryotic communities, the analysis indicated 57.5% of the data inertia was explained by the varieties of *Brachiaria* (Supplementary Table 6) and 43% considering the sum of axis 1 and 2, as represented in Fig. 1A. The samples clustered according to the varieties, and the multivariate Adonis test statically validated these segregations (Supplementary Table 7 and dashed lines represented in Fig. 1A). Regarding the soil characteristics, the results pointed out that almost all soil chemical characteristics (except K, S, and Micro-Porosity) contributed significantly to the found segregations of the samples (based on the PERMANOVA test; 999 permutations; $P < 0.05$). Similar results were found for the fungal communities (Fig. 1B). The data inertia explained by the varieties was 63% (Supplementary Table 6) and 46.8% considering the sum of the axis 1 and 2. Also, the varieties significantly segregate the samples (Supplementary Table 7 and dashed lines represented in Fig. 1B). The only soil characteristics that did not correlate significantly with the samples were K, S, soil density, and Total-Porosity (based on the PERMANOVA test; 999 permutations; $P < 0.05$). The prokaryotic diversity was lower in the BI variety compared to the other treatments (Supplementary Fig. 3A, $P = 0.02$). However, the fungal diversity was higher in the BI and BP varieties compared to the BR (Supplementary Fig. 3B, $P <$

0.01). Also, even though we did not observe statistical differences in the microbial richness (Supplementary Fig. 3C and 3D, P > 0.05), the values in the soils under the influence of varieties of *Brachiaria* were higher compared to the Control.

Microbial community composition and dissimilarity

The *16S rRNA* and ITS amplicon sequencing generated 1 005 440 sequences after the quality control steps and rarefaction (Supplementary Table 5). The *16S rRNA* sequences were classified into 34 phyla, including 3 from Archaea and 31 from Bacteria (Fig. 2A). The five most abundant phyla were Actinobacteriota (28% of all sequences), followed by Proteobacteriota (27%), Acidobacteriota (12%), Chloroflexi (7%) and Crenarchaeota (5%). Together they represented 79% of all sequences. Furthermore, we found that the ITS sequences were classified into 13 fungal phyla (Fig. 2B). The most 5 abundant ones were Ascomycota (72%), followed by Basidiomycota (13%), Chytridiomycota (3%), Mortierellomycota (3%), and Glomeromycota (2%). Together, these groups represented 93% of all sequences.

A Simper analysis was performed to check the percentage of the soil microbial dissimilarity carried out by the varieties of *Brachiaria* compared to the Control (Fig. 2C). For the prokaryotic community, the variety BR showed the most dissimilar community with 32%, followed by BI (23%), BP (22%), and BM (21%). For the fungi community, it was found a similar pattern. The BR showed the most dissimilar community with 68%, followed by BM (64%), BI (62%), and BP (58%). The 5 main taxa that contributed to Simper's dissimilarity analysis for each variety are represented in Supplementary Fig. 4. Regarding the fungal community, the specie *Hydnophlebia meloi* and the genera *Conocybe* and *Cladosporium* contributed to the dissimilarity in all varieties. The genus *Trichoderma* and the specie *Phaeosphaeria caricis* contributed to the dissimilarity of the BP variety. The family Chytridiaceae contributed to the dissimilarity of the BM variety, while the species *Talaromyces columbiensis* and *Fusarium acutatum* to the BI variety. Considering the prokaryotic taxa, the family Nitrososphaeraceae contributed to the dissimilarity of all varieties. The order of Gaiellales and the genus *Conexibacter* contributed to the dissimilarity of the BP, BM, and BI varieties. The genus *Sphingomonas* contributed to the dissimilarity of the BR and BM varieties. The genera *Pseudarthrobacter* and *Pseudomonas* contributed to the dissimilarity of the BR variety. The class Holophagae contributed to the dissimilarity of the BP variety and the families Chthoniobacteraceae and Xanthobacteraceae to the BI variety.

Soil microbial functional profile

The prokaryotic functional profile was obtained using the FAPROTAX database, which assigned putative functions according to the microbial compositional profile (Fig. 3A). The Control showed lower values than the varieties for microbial metabolism functions such as chemohetorotrophy and aerobic chemoheterotrophy. However, the phototrophy, photoheterotrophy, photoautotrophy, and anoxygenic photoautotroph functions were lower in the BR variety compared to the other treatments. Also, the BR variety showed a lower % for functions related to N-metabolism. The nitrification, aerobic ammonia oxidation, nitrate respiration, nitrogen respiration, nitrate denitrification, nitrite denitrification, nitrous oxide

denitrification, denitrification, and ureolysis functions were lower in the BR variety compared to the other treatments. However, the cellulolysis function was higher in the BI variety compared to the BP variety. The fungi functions were signed as guilds by the FUNGuild database. Considering the “Trophic-level” classification (Fig. 3B), we found that the Control showed a higher % of Saprotroph but lower values for Pathotroph-Saprotroph. The Saprotroph-Symbiotroph and Symbiotroph were higher in the BI variety. Finally, according to the “Guild” classification, the BI variety has a higher % for Endophyte, Ectomycorrhizal, and Arbuscular Mycorrhizal (Supplementary Fig. 5).

Microbial marker-genes abundance and their correlations with soil and plant characteristics

The quantification of marker genes indicated that the abundance of microbial communities changed according to the treatments. The bacterial abundance (Fig. 4B) was lower in the BI and BP varieties compared to the other treatments. However, the BI and BM varieties presented the highest archaeal abundance (Fig. 4C). The size of the fungi communities also changed. The BP showed the highest abundance compared to the other treatments (Fig. 4A). Regarding the ammonia-oxidizers, we found that the BI variety showed the lowest value for oxidizing bacteria (Fig. 4E). However, the BI and BP varieties showed the highest values for oxidizing archaea (Fig. 4A). Finally, the microbial N-fixers were lowest in the Control and highest in the BR variety (Fig. 4D).

A correlogram was carried out to assess the correlation between the gene’s abundance to soil and plant characteristics (Supplementary Fig. 6). The bacterial abundance correlated positively with AOB and *nif*H genes. However, it correlated negatively with the AOA gene, SOC, N-total, and N-Organic from the soil, and N-Leaf and N-SPAD from plants. The archaeal abundance correlated positively with the soil characteristics P and S and the plant Root-Biomass, Leaf-Biomass, N-Leaf, and N-SPAD. Nonetheless, the gene correlated negatively with the soil characteristics Mg^{2+}, SB, NO_3^-, N-Inorganic, Density, and Micro-Porosity. The fungal abundance correlated positively with the gene *nif*H and the soil P, K, and NH_4^+. However, the gene correlated negatively with NO_3^- and Micro-Porosity. The bacterial ammonia-oxidizers correlated positively with the *nif*H gene and soil NH_4^+. However, the gene correlated negatively with the gene AOA and the soil characteristics SOC, Ca^{2+}, Mg^{2+}, CEC, N-Total, and N-Inorganic. The archaeal ammonia-oxidizers correlated positively with SOC, Mg^{2+}, N-Total, N-Organic, Leaf-Biomass, N-Leaf, and N-SPAD but negatively with the gene *nif*H and the NH_4^+ from the soil. Lastly, the *nif*H gene correlated positively with the H-Al, NH_4^+, Macro-porosity, and Root-Biomass. However, the gene correlated negatively with the pH, Ca^{2+}, Mg^{2+}, SB, CEC, $V_{\%}$, N-Total, N-Organic, and Micro-Porosity.

Discussion

Brachiaria *varieties growth, N acquisition, and influence on soil physicochemical properties*

In our study, the soil physicochemical characteristics changed according to the varieties of *Brachiaria*. We found that the presence of the plants increased the soil P and SOC, and both positively correlated with the plant biomass. *Brachiaria* varieties are characterized by a high biomass production that, after decomposing, can increase SOC and soil fertility (Brandan et al. 2017). Also, the *Brachiaria* roots can exudate organic acids, OH^- and H^+, to increase the soil P availability (Merlin et al. 2016), which explains the increase of nutrients in the treatments with the plant in our study. In general, the utilization of *Brachiaria* sp. can provide a wide range of beneficial services to tropical agroecosystems (Baptistella et al. 2020). Its versatility allows it to be introduced as an isolated crop (pasture), used as a cover crop (predecessor crop), combined with annual or perennial crops (e.g., coffee and orange), or incorporated into intercropping systems (e.g., maize). The soil quality legacy left by *Brachiaria* cultivation can be decisive in increasing crop productivity in tropical agricultural systems (Baptistella et al. 2020).

Interestingly, the BR variety increased the soil acidity and decreased many nutrients dependent on pH, such as K, Ca^{2+}, and Mg^{2+}. The soil pH is considered one of the main factors responsible for regulating the availability of nutrients in the soil and, consequently, making them available for plant and microbes' absorption (Fernández and Hoeft 2009). Also, the BR variety can exudate high rates of organic acids, C-based compounds, and H^+ to make P available in conditions of scarcity of this element (Chigira and Oyama 2000; Louw-Gaume et al. 2017; Almeida et al. 2020). In our study, this root exudation ability linked with cation-based nutrient uptake may explain why the acidity increased in the BR variety compared to the others. The soil nitrogen dynamics also changed according to the varieties of *Brachiaria* and revealed different strategies of N acquisition, microbial abundance, and plant growth. Plants and microbes can absorb N in their soil-available forms (NH_4^+ and NO_3^-), which commonly enter into agricultural lands through N-based fertilizers, biological N fixation (BNF), and organic matter mineralization (Robertson and Vitousek 2009). Therefore, the last two were the only N source in this study, considering there was no N-fertilizer application in the field during the experiment. The highest NO_3^- amount in the Control treatment indicated the influence of the plants on the availability of this nutrient in the soil. The element is the final product of soil nitrification and has high mobility in the system soil-plant, been quickly uptake by plants, immobilized by microbes, or susceptible to denitrification and leaching (Powlson 1993; Momesso et al. 2022). Considering the difference between varieties, the BR and BI showed the most contrasting results for soil N characteristics. The BR variety showed an accumulation of soil NH_4^+. Interestingly, this variety also showed a higher abundance of microbial N-fixers and accumulated more SOC than the Control, suggesting the plant uses multiple strategies to acquire the nutrient. (Rocha et al. 2020) and (Bossolani et al. 2020) also found an increase in the soil N-Fixers in the BR variety. They highlighted the plant's potential to improve biological N fixation in the rotational agriculture system. Also, the highest amount of N-inorganic found in soil and the lower amount of N accumulation in the leaves of the BR variety indicated a less pronounced need for N to its growth.

The BR variety did not show the highest values of shoot-biomass in this study. However, this variety decreased soil density and increased porosity, indicating high root growth, leading to better soil physical structuration. These soil changes provided by BR root development are a management tool to make the

soil suitable for crop rotation systems (Favilla et al. 2021). This legacy from the previous cultivation of forage grasses is essential for reducing soil compaction and increasing soil porosity (Silva et al. 2021). On the other hand, the BI variety presented a contrasting result regarding the soil N-cycle. This variety presented the lowest values for the soil N-Inorganic, NH_4^+, and NO_3^-. However, these nutrients accumulated in the plant contributed to its growth, confirmed by the highest values of N-Leaf concentration and plant biomass. In addition, we also found an increase in soil porosity and a decrease in soil bulk density. Forage grasses, in general, have a high potential to accumulate leaf nutrients and produce high plant biomass (below- and aboveground), but some varieties require soils with high fertility and N amendments to maintain their growth (Valle et al., 2017; (Camargo et al. 2022). In addition, the BI variety had more microbial N-fixers than the Control but less than the BR variety, as well as, had a smaller influence on the accumulation of SOC compared to other treatments. Together, these results suggested that this variety can extract more of the required N from soil organic matter than through the association with the microbes (BNF).

The selection of the microbial community by Brachiaria *varieties.*

Prokaryotic community

The prokaryotic community changed according to the varieties of *Brachiaria*, which explained almost 60% of the data variation (based on the db-RDA analysis). Also, the soil's chemical and physical characteristics strongly influenced this selection. It was found that the Control samples were segregated from *Brachiaria* samples. The SOC, P, NH_4^+, and Macro- and Total-Porosity indicated a strong correlation with the Control samples. Interestingly, all these parameters increased with the presence of the plants in this study (except the NH_4^+). One of the reasons that explain these changes is that *Brachiaria* plants can have profound root growth in the soil, increasing their contact area with soil particles and enabling a high uptake of water and nutrients (Rao et al. 1996; Santos et al. 2013). The aggressive *Brachiaria* growth allows the plants to produce a high amount of biomass and root-soil colonization (Oliveira et al. 2019; Silva et al. 2021). Consequently, we can find an increase in SOC metabolization and soil porosity, favoring C decomposers and aerobic microbes (Uribe et al. 2022; Abán et al. 2022). Besides improving soil properties, *Brachiaria* varieties can improve their nutrition by modulating specific soil microbial groups. For example, the *Brachiaria* plants can extract P retained in the soil colloids by exudation of organic acids and associating with P-solubilizing bacteria groups (Merlin et al. 2016; Oliveira et al. 2021) and mycorrhizal fungi (Clark and Zeto 2000). Also, the plants can inhibit microbial ammonia-oxidizers (a process known as biological nitrification inhibition or BNI), increasing their advantage in the competition for soil N uptake against microbes (Moreta et al. 2014; Nakamura et al. 2020). We found that *Brachiaria* samples were strongly associated with soil nutrients (Ca^{2+}, Mg^{2+}, and N) and indicators of soil acidity (pH and H_+Al). In our study, most of these soil characteristics increased in the presence of the plants (except for the BR variety). Soil pH is considered one of the main drivers of soil microbiome due to its indirect effects on the availability of nutrients and toxic elements that can change microbial growth (Lammel et al. 2018). Simper's analysis highlighted the main taxa for differentiating each *Brachiaria* variety from the Control.

The archaeal family *Nitrososphaeraceae* appeared among the top 5 taxa in all varieties. The group harbors aerobic microbes and ammonia-oxidizers (Stieglmeier et al. 2014). The taxa *Gaiellales* and *Conexibacter* contributed to BP, BM, and BI varieties as one of the top 5 taxa. Both are described as aerobics, but the order *Gaiellales* includes chemoorganotrophic microbes (Albuquerque et al. 2011), while the *Conexibacter* is a taxon capable of reducing nitrate to nitrite (Monciardini et al. 2003; Schumann 2015). The *Sphingomonas* genus was one of the most important for the BR and BM varieties, being able to produce plant growth hormones such as gibberellins and indole acetic acid (Asaf et al. 2020). The BR variety presented the most dissimilar community compared to the Control, while the BI variety showed the most similar community. The main taxa responsible for the BR dissimilarity were the genera *Pseudarthrobacter* and *Pseudomonas*, which include BNF, siderophore producers, and plant growth promoters (Mutai et al. 2017; Tshishonga and Serepa-Dlamini 2020).

Fungal community

The *Brachiaria* varieties also changed the soil fungi communities and explained 42% of their variation (based on the db-RDA analysis). Soil acidity characteristics (pH and V%), many pH-dependent nutrients (N-Total, Ca^{2+}, Mg^{2+}), and the soil components SB and CEC contributed to the observed segregation from the Control samples. While the pH can indirectly and directly modulate the bacterial community (Lammel et al. 2018), some studies suggested that soil fungi communities are less responsible for variations in soil pH and more correlated to soil nutrients (Lauber et al. 2008; Rousk et al. 2010). The soil porosity (Micro- and Macro-Porosity), nutrients (N-Inorganic, P), and SOC contributed to the segregation of *Brachiaria* varieties samples. The increase in soil porosity can contribute to aerobic activity from bacteria and, especially, fungi communities and consequently increase SOC metabolization (Yang et al. 2019). The positive correlation between *Brachiaria* species and the P availability in the soil is not only due to the association with bacterial communities' groups (Merlin et al. 2016; Oliveira et al. 2021) but also with AMF. These fungi groups supply their C needs by associating with plant roots and, in exchange, provide P and other nutrients to their hosts (Clark and Zeto 2000). The AMF also benefits from the BNI process performed by a wide variety of *Brachiaria* (Nakamura et al. 2020). The increase in the NH_4^+ availability can supply the plant needs and the AMF, mainly when N and P supplies are scarce in the soil (Teutscherova et al. 2019). The BR variety showed the most dissimilar fungi community compared to the Control (as found for the prokaryotic community). Also, between the top 5 fungi taxa in each *Brachiaria* variety, we found the *Hydnophlebia meloi* species and *Conocybe* and *Cladosporium* genera, which include many endophytic and spore-forming groups (Amandeep 2015; Teasdale et al. 2018).

Brachiaria varieties influence microbial soil potential functions

Prokaryotic community

The presence of the plants increased the prokaryotic activity metabolism, indicated by the higher percentage of chemoheterotroph and aerobic-chemoheterotroph in the *Brachiaria* soil samples. The findings converged with prokaryotic structure and composition results that showed a strong correlation of

microbial aerobic groups with the improvements in soil structure (porosity and density) performed by plants' presence. Besides, the improvements in soil nutrient availability by *Brachiaria* plants can contribute to soil microbial activity (Brandan et al. 2017; Cardozo Junior et al. 2018). Interestingly, we found a decrease in phototroph metabolism, nitrification, and denitrification in the BR variety. The increased soil acidity and toxic elements in the BR soil can explain those changes. These elements can directly affect the growth of the microbial community responsible for those functions (Sauze et al. 2017; Bossolani et al. 2020; Naz et al. 2022).

Fungal community

The presence of plants also decreased the percentage of Saprotroph fungi but increased the Pathotroph-Saprotroph ones. The results indicate that the presence of plants can make other functional groups more competitive in the soil than saprotrophs (Schmidt et al. 2019). The BI variety showed a higher percentage for the Saprotroph-Symbiotroph and Symbiotroph trophic levels. Also, the guild classification showed a higher percentage for Endophyte, Ectomycorrhizal, and Arbuscular Mycorrhizal. Interestingly, all of them are related to bringing nutritional benefits to their hosts (Clark and Zeto 2000; Nakamura et al. 2020). However, most BI variety studies focused on plant productivity and their potential for animal nutrition (Valle et al., 2017; (Camargo et al. 2022). Our results indicated that the BI variety also presented a higher association with the fungi community, highlighting its potential to recruit the soil microbiome to help its nutrition.

The abundance of microbial groups and N-cycle genes

The size of the bacterial community decreased in the BP and BI varieties. The positive correlation of the bacterial abundance with ammonia-oxidizer bacteria and N-fixers abundance indicated these functional groups' contribution to the soil bacterial community size. The archaeal abundance increased in the BM and BI varieties. The gene correlated positively with P and S, highlighting the importance of these elements for archaeal growth. For example, the S is important for archaeal groups due to their need for the element as a cofactor for soil methanogenesis (Liu et al. 2012). And more recently, the archaea were characterized as drivers of soil stoichiometry in phosphorus-deficient habitats (Wang et al. 2022). The size of the fungal community increased in the BP variety. It correlated positively with N-fixers, P, K, and NH_4^+, reinforcing their role in N and P soil cycle on the fungi community (Teutscherova et al. 2019). Also, the negative correlation with micro-porosity indicates that soil structure can play a role in the fungi community (Yang et al. 2019). Our results showed that the *Brachiaria* varieties could recruit N-fixing microorganisms in different quantities. Different genotypes of *Brachiaria* can recruit different groups of N fixers (Reis et al. 2001). However, the negative correlation of the gene with soil acidity characteristics and the positive with soil porosity reinforce the preferences of these groups for non-acidic and aerobic soils (Bossolani et al. 2020). Interestingly, the N-fixers and NH_4^+ availability influenced our study's soil ammonia oxidizers abundance. The bacterial (AOB) and archaeal ammonia-oxidizers (AOA) display an important role in nitrification by converting NH_4^+ to NO_3^- and increasing the availability of nutrients in the soil (Prosser et al. 2020). Both microbial groups compete for the same N subtract. Still, the archaeal group

has limited growth in soils rich in NH_4^+ due to their low tolerance to the element (French et al. 2012) and has a growth advantage in acid and oligotrophic soils (Sims et al. 2012). Also, the bacterial group can have an advantage in agricultural lands due to the liming and nutrient availability found in these soils (Banning et al. 2015). Interestingly, the higher abundance of N-fixers in the BR and BP, and consequently the increase in NH_4^+ availability, favored the AOB abundance and inhibited the AOA in our results. Also, we found the contrary pattern on BP and BI varieties. Both had less N-fixer abundance, which may have given a competitive advantage to archaeal ammonia oxidizers over the bacterial group. The explanation was reinforced by decreased AOB gene abundance and increased AOA in the soils from BP and BI varieties.

Conclusions

In this study, we investigated the impact of four *Brachiaria* varieties on soil properties and microbial communities. All treatments improved soil P, SOC, and soil porosity. However, the BR and BI varieties presented the most contrasting results. The BR variety led to a decrease in soil pH and nutrients and lower plant biomass, while the BI variety exhibited higher biomass but depleted nitrogen from the soil. The main drivers of microbial community structure were changes in soil acidity, nutrient availability, and soil porosity. The taxa *Nitrososphaeraceae*, *Gaiellales*, *Conexibacter*, Sphingoonas, *Hydnophlebia meloi*, *Conocybe*, and *Cladosporium* were associated with the dissimilarities between the *Brachiaria* varieties and the Control treatment. The presence of the plants increased microbial functions such as chemoheterotroph, aerobic-chemoheterotroph, and Pathotroph-Saprotroph. The BR variety decreased prokaryotic phototroph metabolism, nitrification, and denitrification, while the BI variety showed a higher proportion of symbiotrophic fungi. The abundance of bacteria, archaea, and fungi varied based on the varieties and positively correlated with functional bacterial genes, P, N, and soil porosity. Also, the different abilities of the varieties to recruit N-fixing microorganisms dictated the abundance and competition between bacterial and archaeal ammonia-oxidizing. Selecting an efficient *Brachiaria* variety with superior nutrient acquisition capabilities and the ability to leave a positive legacy in the soil can be a highly effective strategy for improving agricultural systems and increasing food production. These varieties can contribute to sustainable and productive agriculture in tropical regions by improving soil properties and promoting beneficial microbial communities. This approach can also reduce the need for external inputs such as fertilizers and pesticides, leading to a more cost-effective and environmentally friendly agricultural system. Overall, prioritizing the selection of such varieties can significantly impact agricultural productivity and environmental sustainability.

Declarations

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References

1. Abán CL, Larama G, Ducci A et al (2022) Soil Properties and Bacterial Communities Associated with the Rhizosphere of the Common Bean after Using Brachiaria brizantha as a Service Crop: A 10-Year Field Experiment. Sustainability 15:488. https://doi.org/10.3390/su15010488
2. Albuquerque L, França L, Rainey FA et al (2011) Gaiella occulta gen. nov., sp. nov., a novel representative of a deep branching phylogenetic lineage within the class Actinobacteria and proposal of Gaiellaceae fam. nov. and Gaiellales ord. nov. Syst Appl Microbiol 34:595–599. https://doi.org/10.1016/j.syapm.2011.07.001
3. Almeida DS, Delai LB, Sawaya ACHF, Rosolem CA (2020) Exudation of organic acid anions by tropical grasses in response to low phosphorus availability. Sci Rep 10:16955. https://doi.org/10.1038/s41598-020-73398-1
4. Almeida DS, Rosolem CA (2016) Ruzigrass Grown in Rotation with Soybean Increases Soil Labile Phosphorus. Agron J 108:2444–2452. https://doi.org/10.2134/agronj2015.0478
5. Amandeep K (2015) Diversity of species of the genus Conocybe (Bolbitiaceae, Agaricales) collected on dung from Punjab, India. Mycosphere 6:19–42. https://doi.org/10.5943/mycosphere/6/1/4
6. Asaf S, Numan M, Khan AL, Al-Harrasi A (2020) Sphingomonas: from diversity and genomics to functional role in environmental remediation and plant growth. Crit Rev Biotechnol 40:138–152. https://doi.org/10.1080/07388551.2019.1709793
7. Banning NC, Maccarone LD, Fisk LM, Murphy DV (2015) Ammonia-oxidising bacteria not archaea dominate nitrification activity in semi-arid agricultural soil. Sci Rep 5:11146. https://doi.org/10.1038/srep11146
8. Baptistella JLC, de Andrade SAL, Favarin JL, Mazzafera P (2020) Urochloa in Tropical Agroecosystems. Front Sustain Food Syst 4:119. https://doi.org/10.3389/fsufs.2020.00119
9. Bieluczyk W, Piccolo M, de Pereira C MG, et al (2020) Integrated farming systems influence soil organic matter dynamics in southeastern Brazil. Geoderma 371:114368. https://doi.org/10.1016/j.geoderma.2020.114368
10. Bossolani JW, Crusciol CAC, Merloti LF et al (2020) Long-term lime and gypsum amendment increase nitrogen fixation and decrease nitrification and denitrification gene abundances in the rhizosphere and soil in a tropical no-till intercropping system. Geoderma 375:114476. https://doi.org/10.1016/j.geoderma.2020.114476
11. Brandan CP, Chavarría D, Huidobro J et al (2017) Influence of a tropical grass (Brachiaria brizantha cv. Mulato) as cover crop on soil biochemical properties in a degraded agricultural soil. Eur J Soil Biol 83:84–90. https://doi.org/10.1016/j.ejsobi.2017.10.009

12. Brody JR, Kern SE (2004) Sodium boric acid: a Tris-free, cooler conductive medium for DNA electrophoresis. Biotechniques 36:214–216. https://doi.org/10.2144/04362BM02
13. Callahan B (2017) Asv Tables Inferred By Dada2 From The Tara Oceans V9 Metabarcoding Dataset
14. Camargo FC, Difante G dos, Montagner S DB, et al (2022) Morphogenetic and structural traits of Ipyporã grass subjected to nitrogen fertilization rates under intermittent grazing. Ciênc Rural 52:e20201074. https://doi.org/10.1590/0103-8478cr20201074
15. Cantarella H, van Raij B, Quaggio JA (1998) Soil and plant analyses for lime and fertilizer recommendations in Brazil. Commun Soil Sci Plant Anal 29:1691–1706. https://doi.org/10.1080/00103629809370060
16. Cardozo Junior FM, Carneiro RFV, Rocha SMB et al (2018) The Impact of Pasture Systems on Soil Microbial Biomass and Community-level Physiological Profiles: Pasture Systems on Microbial Biomass and Community-level Profiles. Land Degrad Dev 29:284–291. https://doi.org/10.1002/ldr.2565
17. Chigira M, Oyama T (2000) Mechanism and effect of chemical weathering of sedimentary rocks. Developments in Geotechnical Engineering. Elsevier, pp 267–278
18. Clark RB, Zeto SK (2000) Mineral acquisition by arbuscular mycorrhizal plants. J Plant Nutr 23:867–902. https://doi.org/10.1080/01904160009382068
19. Crusciol CAC, Momesso L, Portugal JR et al (2021) Upland rice intercropped with forage grasses in an integrated crop-livestock system: Optimizing nitrogen management and food production. Field Crops Res 261:108008. https://doi.org/10.1016/j.fcr.2020.108008
20. De Mendiburu F, Simon R (2015) Agricolae - Ten years of an open source statistical tool for experiments in breeding, agriculture and biology. PeerJ PrePrints
21. Dias-Filho MB (2016) Uso de Pastagens para a Produção de Bovinos de Corte no Brasil: Passado, Presente e Futuro. Embrapa Amazônia Oriental. Documentos 418. Embrapa Amazônia Oriental, Belém-PA. https://www.infoteca.cnptia.embrapa.br/infoteca/bitstream/doc/1042092/1/DOCUMENTOS418.pdf. Accessed 22 Mar 2023
22. EMBRAPA - Empresa Brasileira de Pesquisa Agropecuária (1984) Brachiaria brizantha cv. Marandu. Documentos 21. Centro Nacional de Pesquisa de Gado de Corte, Campo Grande-MS. CNPGC 31 p. https://www.infoteca.cnptia.embrapa.br/bitstream/doc/317899/1/Brachiariabrizantha.pdf. Accessed 22 Mar 2023
23. Eri M, da Silva Junior CA, Lima M et al (2020) Capitalizing on opportunities provided by pasture sudden death to enhance livestock sustainable management in Brazilian Amazonia. Environ Dev 33:100499. https://doi.org/10.1016/j.envdev.2020.100499
24. Euclides VPB, Montagner DB, Barbosa RA et al (2016) Animal performance and sward characteristics of two cultivars of Brachiaria brizantha (BRS Paiaguás and BRS Piatã). Rev Bras Zootec 45:85–92. https://doi.org/10.1590/S1806-92902016000300001

25. Favilla HS, Tormena CA, Cherubin MR (2021) Detecting near-surface Urochloa ruziziensis (Braquiaria grass) effects on soil physical quality through capacity and intensity indicators. Soil Res 59:214. https://doi.org/10.1071/SR20148
26. Fernández FG, Hoeft RG (2009) Managing soil pH and crop nutrients. Ill Agron Handb 24:91–112
27. French E, Kozlowski JA, Mukherjee M et al (2012) Ecophysiological Characterization of Ammonia-Oxidizing Archaea and Bacteria from Freshwater. Appl Environ Microbiol 78:5773–5780. https://doi.org/10.1128/AEM.00432-12
28. Klute A, Dirksen C (1986) Water retention: laboratory methods. Methods of soil analysis: part 1—physical and mineralogical methods,(methodsofsoilan1). Soil Sci Soc Am Am Soc Agron
29. Kluthcouski J, Cobucci T, Aidar H, Yokoyama LP, de Oliveira IP, da Costa JL, da S, Silva JG, da, Vilela L, Barcellos A, de de O, Magnabosco C (2000) U Sistema Santa Fé-Tecnologia Embrapa: integração lavoura- pecuária pelo consórcio de culturas anuais com forrageiras, em áreas de lavoura, nos sistemas direto e convencional. Circular Técnica 38. Emprapa Arroz e Feijão, Santo Antônio do Goiás-GO. ISSN 1516–8476; 38. https://ainfo.cnptia.embrapa.br/digital/bitstream/CNPAF/17323/1/circ_38.pdf. Accessed 22 Mar 2023
30. Kõljalg U, Nilsson RH, Abarenkov K et al (2013) Towards a unified paradigm for sequence-based identification of fungi. Mol Ecol 22:5271–5277. https://doi.org/10.1111/mec.12481
31. Lammel DR, Barth G, Ovaskainen O et al (2018) Direct and indirect effects of a pH gradient bring insights into the mechanisms driving prokaryotic community structures. Microbiome 6:106. https://doi.org/10.1186/s40168-018-0482-8
32. Lauber CL, Strickland MS, Bradford MA, Fierer N (2008) The influence of soil properties on the structure of bacterial and fungal communities across land-use types. Soil Biol Biochem 40:2407–2415. https://doi.org/10.1016/j.soilbio.2008.05.021
33. Liu Y, Beer LL, Whitman WB (2012) Sulfur metabolism in archaea reveals novel processes: Sulfur metabolism in archaea. Environ Microbiol 14:2632–2644. https://doi.org/10.1111/j.1462-2920.2012.02783.x
34. Louca S, Parfrey LW, Doebeli M (2016) Decoupling function and taxonomy in the global ocean microbiome. Science 353:1272–1277. https://doi.org/10.1126/science.aaf4507
35. Louw-Gaume AE, Schweizer N, Rao IM et al (2017) Temporal differences in plant growth and root exudation of two Brachiaria grasses in response to low phosphorus supply. Trop Grassl-Forrajes Trop 5:103. https://doi.org/10.17138/TGFT(5)103-116
36. Malavolta E, Vitti GC, Oliveira AS (1997) Avaliação do estado nutricional das plantas: princípios e aplicações, 2nd edn. POTAFOS, Piracicaba-SP, p 309
37. Merlin A, Rosolem CA, He Z (2016) Non-labile phosphorus acquisition by Brachiaria. J Plant Nutr 39:1319–1327. https://doi.org/10.1080/01904167.2015.1109117
38. Momesso L, Crusciol CAC, Leite MFA et al (2022) Forage Grasses Steer Soil Nitrogen Processes, Microbial Populations, and Microbiome Composition in A Long-term Tropical Agriculture System.

Agric Ecosyst Environ 323:107688. https://doi.org/10.1016/j.agee.2021.107688

39. Monciardini P, Cavaletti L, Schumann P et al (2003) Conexibacter woesei gen. nov., sp. nov., a novel representative of a deep evolutionary line of descent within the class Actinobacteria. Int J Syst Evol Microbiol 53:569–576. https://doi.org/10.1099/ijs.0.02400-0
40. Moreta DE, Arango J, Sotelo M et al (2014) Biological nitrification inhibition (BNI) in Brachiaria pastures: A novel strategy to improve eco-efficiency of crop-livestock systems and to mitigate climate change. Trop Grassl-Forrajes Trop 2:88–91
41. Mutai C, Njuguna J, Ghimire S (2017) Brachiaria Grasses (Brachiaria spp.) harbor a diverse bacterial community with multiple attributes beneficial to plant growth and development. MicrobiologyOpen 6:e00497
42. Nakamura S, Saliou PS, Takahashi M et al (2020) The contribution of root turnover on biological nitrification inhibition and its impact on the ammonia-oxidizing archaea under Brachiaria cultivations. Agronomy 10:1003
43. Naz M, Dai Z, Hussain S et al (2022) The soil pH and heavy metals revealed their impact on soil microbial community. J Environ Manage 321:115770
44. Nguyen NH, Song Z, Bates ST et al (2016) FUNGuild: an open annotation tool for parsing fungal community datasets by ecological guild. Fungal Ecol 20:241–248
45. Oksanen J, Blanchet FG, Kindt R et al (2013) Package 'vegan'. Community Ecol Package Version 2:1–295
46. Oliveira JT, Pereira A, Souza AJ et al (2021) Plant growth-promoting mechanisms and genetic diversity of bacteria strains isolated from Brachiaria humidicola and Brachiaria decumbens.An Acad Bras Ciênc93
47. de Oliveira SM, de Almeida REM, Pierozan C et al (2019) Contribution of corn intercropped with Brachiaria species to nutrient cycling.Pesqui Agropecuária Trop49
48. Pizarro EA, do Valle C, Keller-Grein G et al (1996) Regional experience with Brachiaria: tropical America-savannas.Brachiaria Biol Agron Improv Calli CIAT225–246
49. Powlson D (1993) Understanding the soil nitrogen cycle. Soil Use Manag 9:86–93
50. Project MapBiomas – Mapeamento Anual de Cobertura e Uso da Terra do Brasil – Coleção 7. https://mapbiomas-br-site.s3.amazonaws.com/MapBiomas_Pastagem_2022_30_11.pdf. Accessed 28 Feb 2023
51. Prosser JI, Hink L, Gubry-Rangin C, Nicol GW (2020) Nitrous oxide production by ammonia oxidizers: physiological diversity, niche differentiation and potential mitigation strategies. Glob Change Biol 26:103–118
52. Quast C, Pruesse E, Yilmaz P et al (2012) The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Res 41:D590–D596
53. R Core Team (2021) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/

54. Rao IM, Kerridge P, Macedo M (1996) Nutritional requirements of Brachiaria and adaptation to acid soils.Brachiaria Biol Agron Improv53
55. Reis VM, dos Reis FB Jr, Quesada DM et al (2001) Biological nitrogen fixation associated with tropical pasture grasses. Funct Plant Biol 28:837–844
56. Robertson GP, Vitousek PM (2009) Nitrogen in agriculture: balancing the cost of an essential resource. Annu Rev Environ Resour 34:97–125
57. Rocha KF, Kuramae EE, Borges BMF et al (2020) Microbial N-cycling gene abundance is affected by cover crop specie and development stage in an integrated cropping system. Arch Microbiol 202:2005–2012
58. Rousk J, Bååth E, Brookes PC et al (2010) Soil bacterial and fungal communities across a pH gradient in an arable soil. ISME J 4:1340–1351
59. Santos PM, Cruz PG da, de Araujo LC et al (2013) Response mechanisms of Brachiaria brizantha cultivars to water deficit stress. Rev Bras Zootec 42:767–773
60. Sauze J, Ogée J, Maron P-A et al (2017) The interaction of soil phototrophs and fungi with pH and their impact on soil CO2, CO18O and OCS exchange. Soil Biol Biochem 115:371–382
61. Schmidt R, Mitchell J, Scow K (2019) Cover cropping and no-till increase diversity and symbiotroph:saprotroph ratios of soil fungal communities. Soil Biol Biochem 129:99–109. https://doi.org/10.1016/j.soilbio.2018.11.010
62. Schumann P (2015) Conexibacteraceae. In: Whitman WB, Rainey F, Kämpfer P et al (eds) Bergey's Manual of Systematics of Archaea and Bacteria, 1st edn. Wiley, pp 1–3
63. da Silva RF, Severiano E, da Oliveira C et al (2021) Changes in soil profile hydraulic properties and porosity as affected by deep tillage soil preparation and Brachiaria grass intercropping in a recent coffee plantation on a naturally dense Inceptisol. Soil Tillage Res 213:105127. https://doi.org/10.1016/j.still.2021.105127
64. Sims A, Horton J, Gajaraj S et al (2012) Temporal and spatial distributions of ammonia-oxidizing archaea and bacteria and their ratio as an indicator of oligotrophic conditions in natural wetlands. Water Res 46:4121–4129. https://doi.org/10.1016/j.watres.2012.05.007
65. Smith KA, Mullins CE (eds) (2001) Soil and environmental analysis: physical methods, 2nd edn. rev.expanded. M. Dekker, New York
66. Stieglmeier M, Klingl A, Alves RJE et al (2014) Nitrososphaera viennensis gen. nov., sp. nov., an aerobic and mesophilic, ammonia-oxidizing archaeon from soil and a member of the archaeal phylum Thaumarchaeota. Int J Syst Evol Microbiol 64:2738–2752. https://doi.org/10.1099/ijs.0.063172-0
67. Subbarao GV, Nakahara K, Hurtado MP et al (2009) Evidence for biological nitrification inhibition in Brachiaria pastures. Proc Natl Acad Sci 106:17302–17307. https://doi.org/10.1073/pnas.0903694106
68. Teasdale SE, Caradus JR, Johnson LJ (2018) Fungal endophyte diversity from tropical forage grass Brachiaria. Plant Ecol Divers 11:611–624. https://doi.org/10.1080/17550874.2019.1610913

69. Teutscherova N, Vazquez E, Arevalo A et al (2019) Differences in arbuscular mycorrhizal colonization and P acquisition between genotypes of the tropical Brachiaria grasses: is there a relation with BNI activity? Biol Fertil Soils 55:325–337. https://doi.org/10.1007/s00374-019-01353-y
70. Tshishonga K, Serepa-Dlamini MH (2020) Draft Genome Sequence of Pseudarthrobacter phenanthrenivorans Strain MHSD1, a Bacterial Endophyte Isolated From the Medicinal Plant Pellaea calomelanos. Evol Bioinforma 16:117693432091325. https://doi.org/10.1177/1176934320913257
71. Uribe M, Peñuela GA, Pino NJ (2022) Megathyrsus maximus and Brachiaria decumbens improve soil characteristics and select promising rhizobacteria during rhizoremediation of petroleum hydrocarbons. Rhizosphere 22:100517. https://doi.org/10.1016/j.rhisph.2022.100517
72. USDA - Natural Resources Conservation Service - Soil Survey Staff (2022) Keys to Soil Taxonomy, 13th edn. https://www.nrcs.usda.gov/sites/default/files/2022-09/Keys-to-Soil-Taxonomy.pdf. Accessed 22 Mar 2023
73. Valle CB, Do EVPB, Montagner DB, Valerio JR, Mendes-Bonatto AB, Verzignassi JR, Torres FZV, Macedo MCM, Fernandes CD, Barrios SCL, Dias Filho MB, Machado LAZ, Zimmer AH (2017) BRS Ipyporã (" belo começo" em guarani): híbrido de Brachiaria da Embrapa. Comunicado Técnico 137. Brasília-DF. https://ainfo.cnptia.embrapa.br/digital/bitstream/item/159958/1/BRS-Ipypora-belo-comeco-em-guarani.pdf. Accessed 22 Mar 2023
74. Wang J-T, Zhang Y-B, Xiao Q, Zhang L-M (2022) Archaea is more important than bacteria in driving soil stoichiometry in phosphorus deficient habitats. Sci Total Environ 827:154417. https://doi.org/10.1016/j.scitotenv.2022.154417
75. Wei T, Simko V (2021) R package 'corrplot': Visualization of a Correlation Matrix (Version 0.92). https://github.com/taiyun/corrplot. Accessed 22 Mar 2023
76. Wickham H ggplot2: Elegant Graphics for Data Analysis, 2nd ed. 2016. Springer International Publishing: Imprint: Springer, ChamYang C, Liu N, Zhang Y (2016) (2019) Soil aggregates regulate the impact of soil bacterial and fungal communities on soil respiration. Geoderma 337:444–452. https://doi.org/10.1016/j.geoderma.2018.10.002

Tables

Table 1. Soil and Plant characteristics from soil samples of varieties of *Brachiaria*. The average and standard deviation values are based on 4 replicates.

	Treatments					
Soil chemical characteristics	**Control**	**BR**	**BP**	**BM**	**BI**	**P-Value**
pH ($CaCl_2$)	5.98± 0.10 a	5.50± 0.00 c	5.93± 0.13 a	5.88±0.10 ab	5.70± 0.08 bc	<0.01
S.O.C. (g kg^{-1})	8.90± 0.50 b	9.3±2.1 ab	11.4±1.9 ab	9.6±2.1 ab	12.7±0.5 a	0.02
P (mg kg^{-1})	26.75± 1.71 b	34.25± 4.35 a	41.25± 1.89 a	38.50± 3.70 a	38.75± 2.99 a	<0.01
K (mmol kg^{-1})	4.0± 0.23 c	3.80± 0.00 c	5.50± 0.24 a	4.58± 0.15 b	3.15± 0.10 d	<0.01
Ca^{2+} (mmol kg^{-1})	27.0± 1.41 a	18.25± 0.50 b	26.50± 2.08 a	25.50± 1.0 a	25.75± 0.96 a	<0.01
Mg^{2+} (mmol kg^{-1})	19.0± 0.82 a	13.0± 1.15 c	18.25± 1.26 a	15.75± 0.5 b	16.0± 0.0 b	<0.01
H+Al (mmol kg^{-1})	18.0± 0.0 d	28.0± 0.0 a	16.0± 0.0 e	19.50± 1.0 c	21.50± 1.0 b	<0.01
Al (mmol kg^{-1})	0.0± 0.0	0.75± 0.50	0.0± 0.0	0.50± 0.58	0.50± 0.58	0.09
S.B. (mmol kg^{-1})	50.0± 0.0 a	35.25± 1.50 c	50.25± 3.40 a	46.0± 1.41 ab	44.75± 0.96 b	<0.01
C.E.C. (mmol kg^{-1})	68.0± 2.16	63.25± 1.50	66.25± 3.40	66.50± 1.73	66.25± 0.50	0.07
V (%)	73.50± 1.0 a	55.75± 0.96 c	76.0± 0.82 a	70.25± 1.26 b	67.75± 1.50 b	<0.01
m (%)	0.0± 0.0	2.25± 1.50	0.0± 0.0	1.0± 1.15	1.0± 1.15	0.06
S (mg/ Kg)	11.50± 1.91 bc	8.25± 2.22 c	10.75±1.71 bc	18.0±2.0 a	13.0±1.63 b	<0.01
N-Total (mg kg^{-1})	1015± 40 abc	785± 101 c	1137± 105 a	840± 99 bc	1050± 128 ab	<0.01
N-Organic (mg kg^{-1})	922± 31 abc	752± 100 c	1124± 103 a	831.9± 103 bc	1047± 128 ab	<0.01
N-Inorganic (mg kg^{-1})	93.14± 11.24 a	32.68± 5.53 b	13.32± 3.38 c	8.17± 8.27 c	2.60± 1.57 c	<0.01
NH_4^+ (mg kg^{-1})	0.0± 0.0 c	23.04± 4.64 a	12.16± 2.76 b	3.34± 2.98 c	0.0± 0.0 c	<0.01
NO_3^- (mg kg^{-1})	93.35± 11.24 a	9.65± 4.43 b	1.16± 0.72 b	4.82± 7.54 b	2.60± 1.57 b	0.01

Soil physics characteristics						
Density (g cm^{-3})	1,50±0.01 a	1.30±0.06 b	1.46±0.03 a	1.47±0.03 a	1.25±0.07 b	<0.01
Total Porosity (cm^3 cm^{-3})	0.40±0.01 c	0.51±0.02 ab	0.45±0.01 abc	0.44±0.01 bc	0.53±0.02 a	<0.01
Micro-porosity (cm^3 cm^{-3}	0.34±0.02	0.28±0.01	0.30±0.01	0.30±0.01	0.30±0.02	0.05
Macro-porosity (cm^3 cm^{-3})	0.06±0.01 b	0.23±0.03 a	0.15±0.02 ab	0.15±0.02 ab	0.23±0.04 a	<0.01
Plant characteristics						
N-Leaf (unit)	-	14.88±0.75 b	21.34±1.44 a	18.04±2.58 ab	21.54±1.45 a	<0.01
N-SPAD	-	32.05±2.23 c	43.25±2.88 ab	35.92±4.75 bc	44.77±2.22 a	<0.01
Shoot-Biomass (T ha^{-1})	-	7.6± 0.7 b	9.17± 1.8 b	8.6± 1.6 b	12± 0.9 a	<0.01
Root-Biomass (T ha^{-1})	-	0.96± 0.1 ab	0.49± 0.3 b	0.98± 0.2 ab	1.28± 0.2 a	<0.01

Lower-case letters indicate statistical differences based on the One-Way ANOVA test, followed by the LSD test to compare groups with p-value adjusted by Bonferroni correction. **Ctrl** – Control; **BR** - *Brachiaria ruziziensis*; **BP** - *Brachiaria brizantha* cv. BRS Paiaguás; **BM** - *Brachiaria brizantha* cv. Marandu; **BI** - *Brachiaria* spp. cv. BRS Ipyporã.

Figures

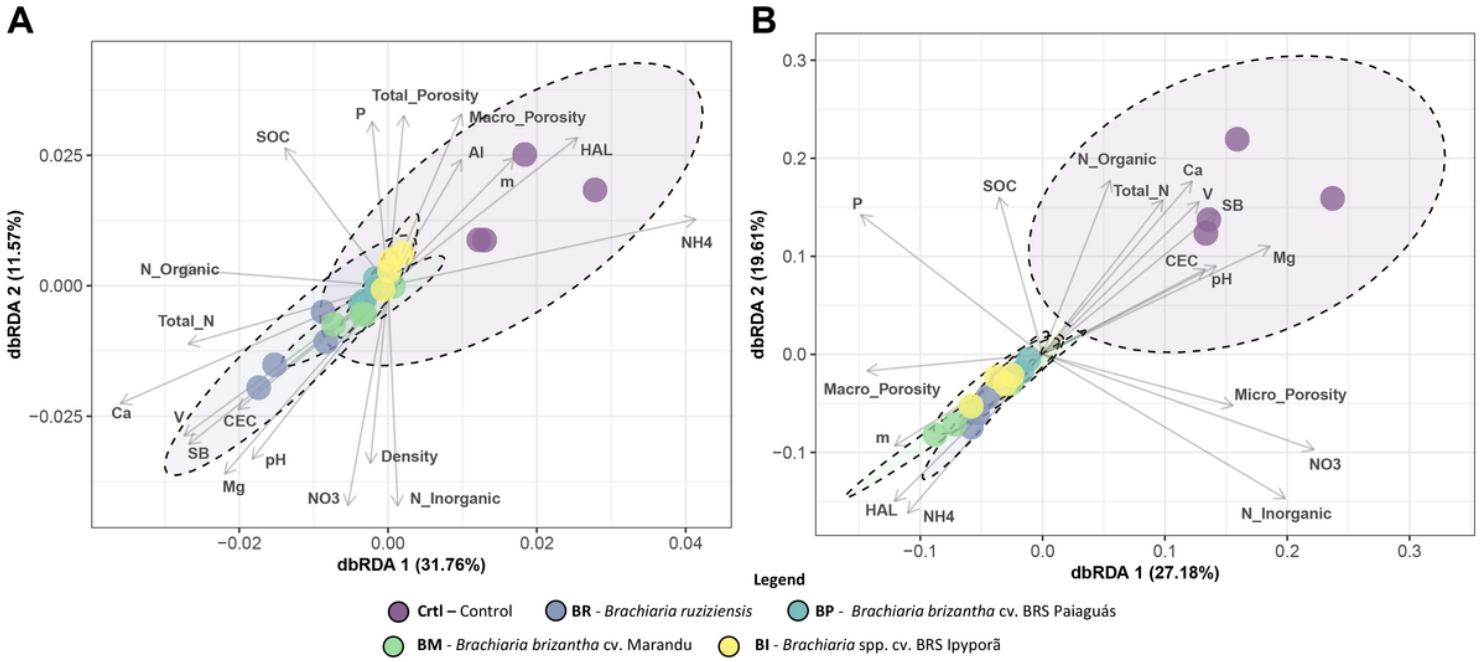

Figure 1

Distance-based redundancy analysis (db-RDA) of prokaryotic (**A**) and fungi (**B**) communities and their correlation with soil characteristics. The analysis was based on the Bray-Curtis distance. The arrows indicate soil characteristics significantly correlating with microbial community composition (based on 999 permutations and P<0.05). The dashed lines indicated significant sample clusters based on the multivariate Adonis test (999 permutations).

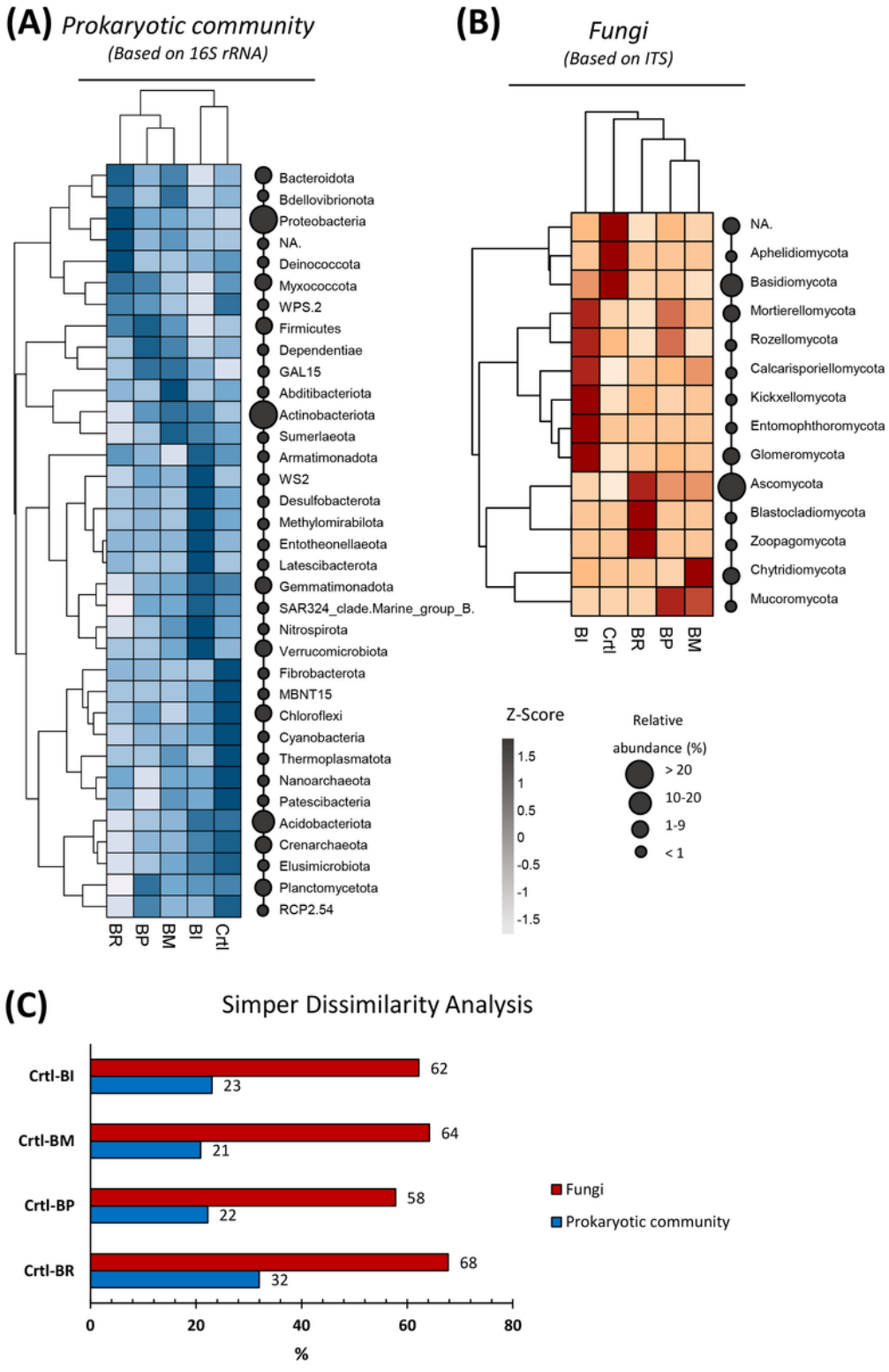

Figure 2

Heat maps showing the relative differential abundance of the prokaryotic community **(A)** and Fungi **(B)** phyla. **(C)** Simper dissimilarity analysis for Fungal and prokaryotic communities between the Control and each variety of *Brachiaria*. **Ctrl** – Control; **BR** - *Brachiaria ruziziensis*; **BP** - *Brachiaria brizantha* cv. BRS Paiaguás; **BM** - *Brachiaria brizantha* cv. Marandu; **BI** - *Brachiaria* spp. cv. BRS Ipyporã.

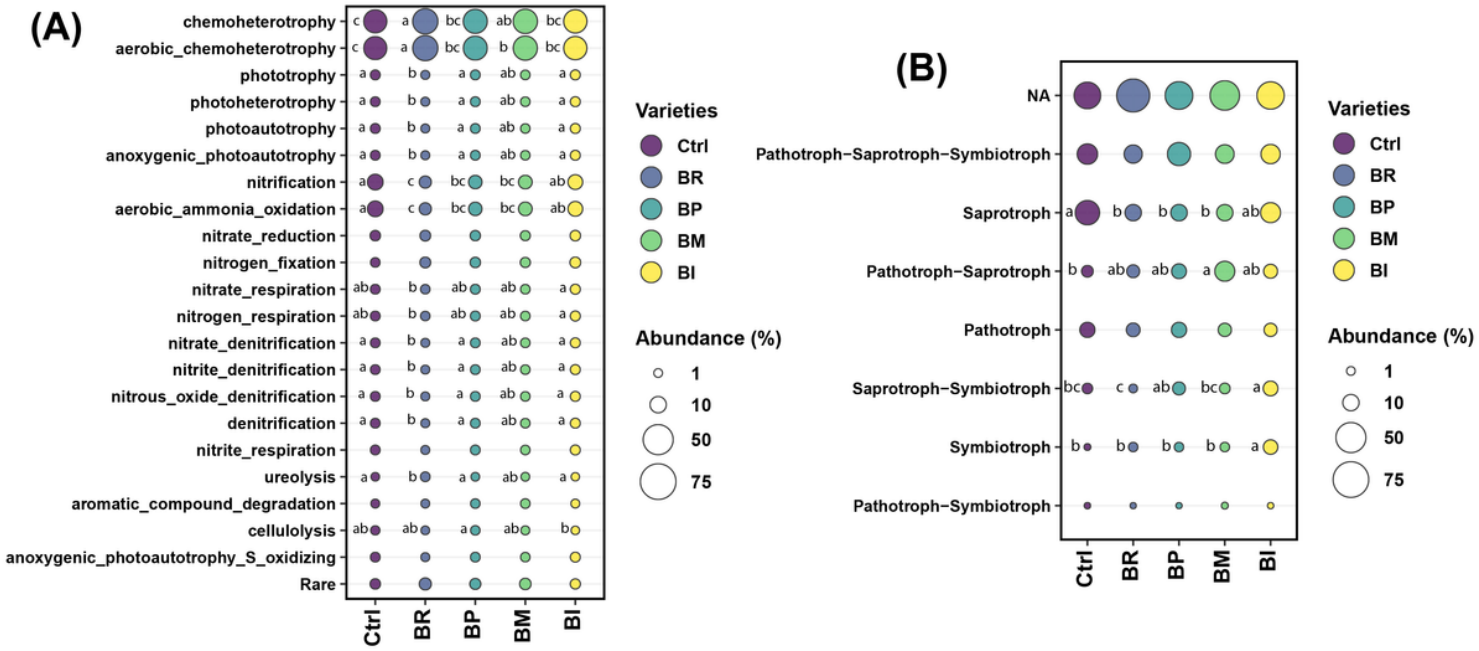

Figure 3

(A) Bubble-plot representing the differences in the abundance (%) of potential prokaryotic functions according to the FAPROTAX database (based on 16S rRNA sequencing). The “Rare” function is based on the sum of functions that represents less than 1% (Supplementary Table 6). **(B)** Bubble-plot representing the differences in the abundance (%) of fungal functional groups (trophic mode) according to the FUNGuild database (based on *ITS* sequencing). The lower-case letters represent functions with a statistical difference based on the One-Way ANOVA, followed by the post-hoc Tukey-Kramer test ($P < 0.05$) and after the Benjamini-Hochberg FDR correction. **Ctrl** – Control; **BR** - *Brachiaria ruziziensis*; **BP** - *Brachiaria brizantha* cv. BRS Paiaguás; **BM** - *Brachiaria brizantha* cv. Marandu; **BI** - *Brachiaria* spp. cv. BRS Ipyporã.

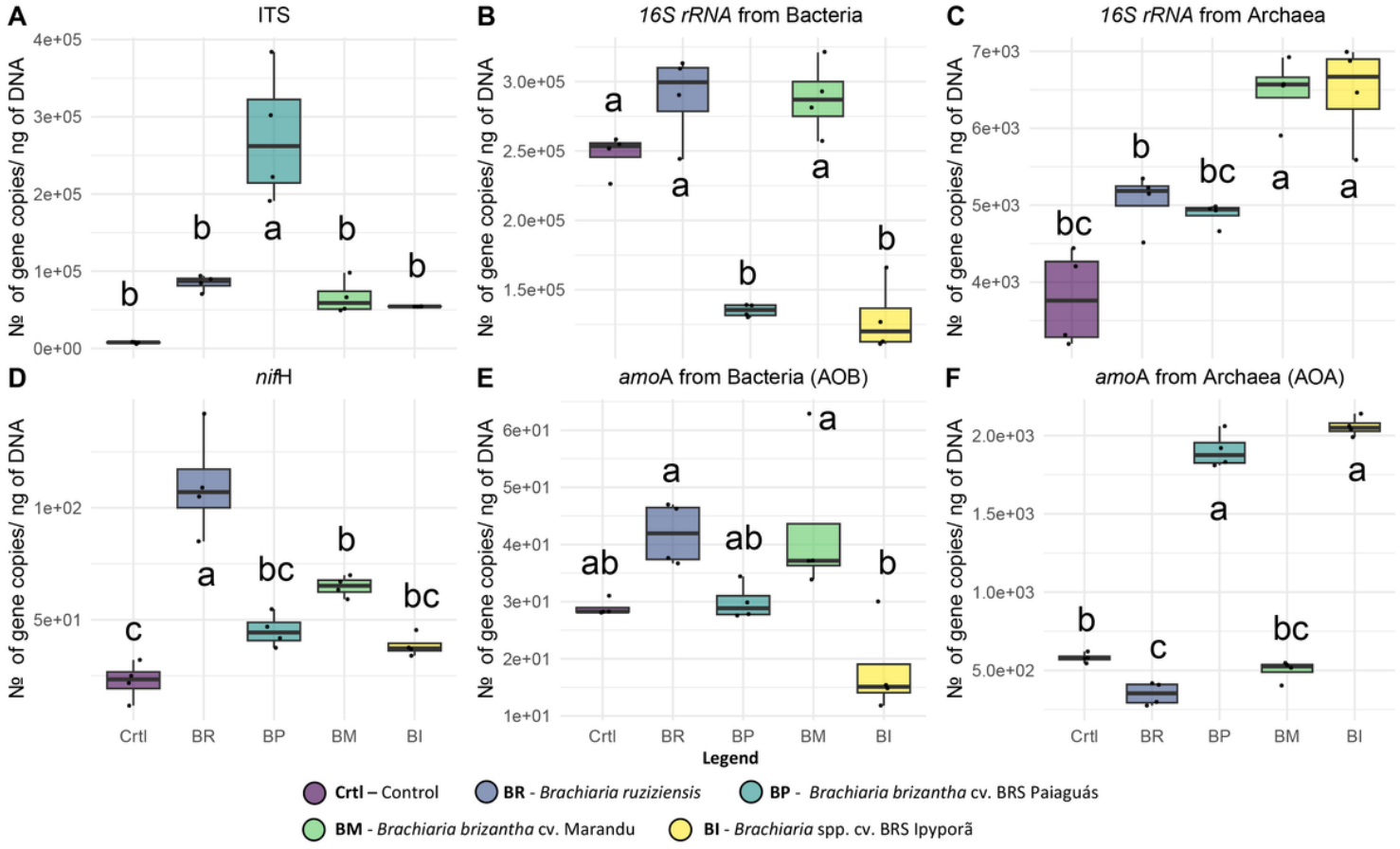

Figure 4

Box-plot representing the abundance of the marker genes *16S rRNA* of *Bacteria* **(A)** and *Archaea* **(B)**, ITS **(C),** *amo*A *of Bacteria* **(D)** and *Archaea* **(E),** and *nif*H. Lower case letters indicate statistical differences based on the One-Way ANOVA test, followed by the LSD test to compare groups with p-value adjusted by Bonferroni correction. All P-Values were smaller than 0.01.

Supplementary Files

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- Supplementarymaterial.docx