

Rhizobacterial genetic diversity associated with *Paspalum atratum* and *Paspalum notatum* by metagenomic analysis

Ailton Ferreira de Paula

Federal University of São Carlos

Paulo Henrique Marques de Andrade

Federal University of São Carlos

Euclides Matheucci Jr

DNA Consult

Marcelo Falsarella Carazzolle

Universidade Estadual de Campinas (UNICAMP)

Lucas Miguel de Carvalho

Universidade Estadual de Campinas (UNICAMP)

Bianca Baccili Zanotto Vigna

Embrapa Pecuária Sudeste

Alberto Carlos de Campos Bernardi

Embrapa Pecuária Sudeste

Alessandra Pereira Fávero

Embrapa Pecuária Sudeste

Anderson Ferreira da Cunha

Federal University of São Carlos

Paulo Teixeira Lacava

pt.lacava@ufscar.br

Federal University of São Carlos

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Abstract

The genus *Paspalum* belongs to the Poaceae family and encompasses several species native to South America. The *Paspalum* Germplasm Bank of Embrapa Pecuária Sudeste has about 450 accessions of 50 species. Among the conserved accessions, *Paspalum notatum* BGP 26 and *Paspalum atratum* BGP 308 were selected for their importance as turfgrass and forage, respectively. The rhizospheric bacterial community of *P. atratum* and *P. notatum* was analyzed via 16S (V3-V4 region) metabarcoding sequencing. The statistical difference found for the Chao ecological index indicates that the bacterial composition and diversity were associated with each species. Thirty-one operational taxonomic units (OTUs) were identified with a predominance higher than 1% from 9 phyla (Proteobacteria n = 11, Acidobacteria n = 8, Actinobacteria n = 3, Planctomycetes n = 2, Nitrospirae n = 2, Verrucomicrobia n = 2, Chloroflexi n = 1, Gemmatimonadetes n = 1, and Bacteroidetes n = 1). Among these, 19 OTUs significantly differed between the species studied. Soil hydrogen-ionic potential (pH) was one of the most important factors influencing the species-specific difference of bacterial populations among accessions. Acidobacteriaceae (Subgroup 1), from the phylum Acidobacteria, was the most abundant family in *P. atratum*. The main group found in *P. notatum* was related to the family Nitrosomonadaceae from the phylum Proteobacteria. Our findings highlight a significant distinction in the rhizospheric bacterial community among the examined *Paspalum* accessions and underscore the existence of a species-specific association. These results can aid soil management practices, particularly in relation to vital economic aspects. Further research could help optimize agricultural strategies and promote sustainable soil health.

INTRODUCTION

The interaction between microorganisms, soil, and plants is vital for increasing crop yield. Plant growth can be promoted by microorganisms directly acting in the plant itself or indirectly when they inhibit the development of another plant that could interfere with plant growth (Eljounaidi et al., 2016; Olanrewaju et al., 2017). The microbiota associated with a particular plant species, also known as the microbiome, plays a crucial role in crop health and productivity. These microorganisms can affect nutrient uptake, disease resistance, and stress tolerance. They can also indicate soil health. Healthy soil is rich in diverse microorganisms that can support plant growth and maintain soil structure and fertility. Imbalances in the microbiome can indicate soil degradation or contamination, which can directly influence plant health and productivity (de Paula et al., 2022; Lacava et al., 2022).

Paspalum is a highly diverse genus from the Poaceae family, native to the Americas (Novo et al., 2016). Over 200 species are found in Brazil (Valls et al., 2023). The *Paspalum* Germplasm Bank (G.B.) is a collection of *Paspalum* accessions maintained by Embrapa Pecuária Sudeste in São Carlos, SP, Brazil. It contains approximately 450 accessions from 50 species (de Paula et al., 2022). The bank is used to evaluate genetic resources in *Paspalum* and can contribute to their use in breeding programs and advanced biotechnology research.

Paspalum notatum and *Paspalum atratum* are economically important species, with worldwide cultivars released (Acuña et al., 2019). Many genotypes of *P. notatum* have agronomic characteristics such as rusticity, low need for pruning, and excellent resistance to leafhoppers. It spreads through rhizomes and is used as a vegetation cover (Amaral & Castilho, 2012; Wallau et al., 2019). The accession BGP 308 of *P. atratum* has high dry matter production and is attractive for forage use. Many genotypes of the species are native, perennial, and highly productive, with excellent forage production and low demands on soil fertility (Karia & de Andrade, 2001; Matta et al., 2023).

Soil microbiota can contribute to nutrient cycling, vegetation diversity, and systems functionality (Leff et al., 2015; Wagg et al., 2014) and are often affected by soil nutrient supply. Vargas et al. (2023) verified that the soil fertility level could define the relative abundance of soil microbial communities beyond the host plant. When studying fungal, archaeal, and bacterial communities in the soil under pastures in different locations around the world, Leff et al. (2015) concluded that the addition of N or P nutrients consistently altered populations due to vegetation responses to nutrient supply.

The microbiota associated with *Paspalum* accessions and the possible interaction between microorganisms and these species are poorly understood (Prabha et al., 2018, 2019). In a recent study, the composition and diversity of bacteria were analyzed in a grazed 'Argentine' bahiagrass (*Paspalum notatum* Flügge) soil supplanted with legume-derived nitrogen. The results showed an increase in *Bradyrhizobium* spp. and other genera involved in biological N₂ fixation or denitrification in pastures with legumes (Guerra et al., 2021). Prabha et al. (2018, 2019) have described the bacterial diversity in the rhizosphere of *P. scrobiculatum* L. (Kodo Millet), a species used for human consumption and nutrition.

Although studies on associated microbiota can help in the development and maintenance of plants and bioprospecting sustainable agriculture and environmental management of species, there are no studies evaluating the *P. atratum* and *P. notatum* associated microbiota. Therefore, we described the rhizospheric microbiota associated with *P. atratum* and *P. notatum* under the same conditions and compared them.

MATERIAL AND METHODS

Biological Material

The plant species were selected based on their economic importance, and the accessions based on their importance for the breeding programs. The genotypes BGP 26 of *P. notatum* and BGP 308 of *P. atratum* are being maintained *ex situ* in the *Paspalum* germplasm bank (GB) located at Embrapa Pecuária Sudeste (21°57'42" S, 47°50'28" W, 860m), São Carlos, Brazil. Soil is a dusky Red Latosol, i.e., Hapludox (Soil Taxonomy) or Rhodic Ferralsol (FAO), according to Calderano Filho et al. (1998). The climate is tropical (Cwa) according to the Köppen-Geiger classification (Kottek et al., 2006), with an average annual rainfall of 1361.6 mm, average minimum temperature, and maximum temperature of 12.1°C (July) and 28.7°C (February), and average annual temperature of 21.5°C. The annual average relative air humidity is 74.3% (EMBRAPA, 2023). Originally, BGP 26 (BGP026/BRA06301/V9684) of *P. notatum* was collected in

Barra do Quaraí/RS (29°43'0" S, 57°1'0" W), Pampa biome, on 12/04/1985. According to Cunha et al. (2001), the municipality's soils are clayey, poorly drained, shallow, and very fertile. It is a narrow-leaf ecotype, very rustic, and can be used as turf in private or public gardens, roadsides, railways, and airport runway sides (EMBRAPA, 2021). Amongst the accessions of the Germplasm Bank, the accession BGP 26 from this species has been registered in Brazil as cultivar Tuim for lawn under the number 36197 in the Ministry of Agriculture, Livestock and Food Supply (RNC, 2023).

The accession BGP 308 (BGP308/BRA30078/VRcMmSv 14525) was collected in Terenos/MS (20°31'0" S, 54°48'60" W), Pantanal biome, on 06/05/2002. It was collected from the same locality as *P. atratum* cv. Pojuca, but there were some morphological differences (Oliveira, 2004).

The plants were identified by Dr. José Francisco Montenegro Valls, a taxonomist specializing in the genus *Paspalum* (Pagliarini et al., 2001; Adamowski et al., 2005; Souza-Chies et al., 2006; Desirée et al., 2011; Reis et al., 2015; Oliveira et al., 2015).

Soil Sampling and Analysis

Soil samples associated with adult plants of the chosen accessions were collected in May 2017 in plots from the GB, which are kept *in vivo* in plots of 3x1 m at a distance of 10 meters from each other. Soil sampling was made at a 0-0.2 m depth in each plot. The chemical properties were determined following Nogueira et al. (1998): soil pH in water and CaCl₂, organic matter (O.M) by wet combustion, available P by the resin method. Exchangeable K⁺, Ca²⁺, Mg²⁺, and H+Al were also measured. Cation exchange capacity (CEC) was measured at the actual soil pH value, and base saturation (%V) was determined. Soil particle size fractions (sand, clay, and silt content) were determined by the hydrometer method. Lime and fertilizer recommendations were calculated. Plant plots were managed in the same way for more than ten years, with two cuts per year (in early spring and late fall). Dolomite lime (Total Neutralizing Power Ratio – TNPR = 70%) was applied at 200 g per m² in the early spring of the 2013-2014 season. Plots were uniformly fertilized with NPK formulation (20-05-20) at 280, 70, and 280 g per m² of N, P₂O₅, and K₂O twice yearly, in late spring and midsummer. Irrigation was provided when necessary for all plots equally.

In the central part of the plots, four samples of rhizospheric soils were collected for each species. The soil collected was at a depth of 0.2 m below the soil surface and adhered at most 3 mm from the roots. After the collection of each plant, the tools used were washed in running water and disinfected with 70% ethyl alcohol to avoid cross-contamination (Batista et al., 2018).

Samples were stored in sterilized plastic bags and transported to the Laboratory of Microbiology and Biomolecules, Department of Morphology and Pathology, Biological and Health Sciences Center, Federal University of São Carlos, São Carlos, SP 13565-905, Brazil.

Metagenomic DNA Extraction and Sequencing

Total DNA from each sample was extracted using the PowerSoil DNA Isolation Kit following the manufacturer's protocol (QIAGEN, Hilden, Germany), using approximately 0.25 g of rhizospheric soil. The integrity of the extracted DNA was evaluated by electrophoresis in a .7% agarose gel stained with Ethidium bromide. Then, DNA quantification and quality were assessed using the NanoVue Plus spectrophotometer (G.E. Healthcare, Marlborough, USA). Samples were diluted to 50 ng/μL and pooled using the same volume for each one (three samples were used to form one pool, resulting in four replicates from each plant accession).

The pooled samples were used to amplify approximately 460 bp of the 16S ribosomal RNA by PCR using specific primers V3 and V4. The full-length primer sequences to follow this region's protocol are 16S Amplicon PCR Forward Primer = 5'

TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG and 16S Amplicon PCR Reverse Primer = 5' GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACHVGGGTATCTAATCC. The PCR products were used to build the metagenomics library for sequencing using the MiSeq Reagent kit v3 (600 cycles) (Illumina Inc.). The sequencing of partial 16S ribosomal RNA was performed using the Illumina MiSeq platform, producing thousands of 300 bp paired-end reads (2 × 300 bp) for each library.

The full data sequence has been registered at NCBI BioProject under the BioProject number PRJNA526512.

Taxonomic Microbial Diversity, Community Composition, and Abundance

The data were analyzed using RStudio (version 3). The alpha diversity data were submitted to the Wilcoxon-Mann-Whitney nonparametric test at a 5% probability value. Following the Shapiro-Wilk normality test, the phylum and operational taxonomic units (OTUs) data were analyzed by T-test.

RESULTS

Soil analysis

The results of the soil chemical analysis determine the nutrient stock and chemical limiting factors, allowing the lime and fertilizer recommendation and the monitoring and evaluation of the nutrient balance in the soil (Havlin et al., 2016). Results obtained from soils cultivated with both species (*P. notatum* and *P. atratum*) indicate that overall soil fertility is better in soil cultivated with *P. notatum*, though varying when considering specific parameters. Although the amounts of lime and fertilizer supplied were the same for all plots, results indicate differences in nutrient uptake and

extraction by species and resulting soil changes. The physical and chemical characteristics of the soils are described in Table 1.

The soil acidity analyzed by both pH in water and CaCl_2 methods was considered very low in *P. notatum* BGP 26 (7.0 and 6.4, respectively) when compared to *P. atratum* BGP 308, which showed 5.6 and 5.0 (respectively for water and CaCl_2) and was considered medium to high. Nutrient availability is influenced by soil pH since macronutrients N, P, K, Ca, Mg, and S, due to indirect or direct effects, have their availability increased at pH values close to neutrality (pH between 6.0 and 6.5). For pH values lower than 5.5, damage to the development of the crop may occur due to the high activity of potentially phytotoxic elements, such as Al and Mn. (Havlin et al., 2016).

The organic matter (O.M.) and K levels were within the values considered medium for the plots of both species, and sulfate was considered high. However, phosphorus was higher in *P. notatum* (19 mg dm^{-3}) compared to *P. atratum* (4.0 mg dm^{-3}) since the highest level was considered “medium” and the other was considered “very low” (Alvarez Venegas et al., 1999; Raij et al., 1996). Phosphorus is a macronutrient that limits plant development in most Brazilian soils. It is scarcely available in low pH conditions due to the retention of the phosphate anion by positively charged particles on the surface of the oxides.

The soil micronutrient levels were all in the same interpretation class (Raij et al., 1996): medium for B and Zn, and high for Cu and Mn. The exceptions were Fe levels, which were considered medium for *P. notatum* and high for *P. atratum* plots. The higher value of Potential Acidity ($\text{H} + \text{Al}$) was observed in *P. atratum* BGP 308 (34 mmolc.dm^{-3}) and the lowest in *P. notatum* (12 mmolc.dm^{-3}). On the other hand, Ca and Mg were higher in *P. notatum* (50 mg.dm^{-3} and 26 mmolc.dm^{-3}) and lower in *P. atratum* (20 mg.dm^{-3} and 9 mmolc.dm^{-3}).

Among the variables that define soil fertility, the cation exchange capacity (CEC) stood out, which is the total amount of cations retained on the surface of soil particles (Havlin et al., 2016). The CEC promotes the retention of cations (K, Ca, and Mg) and decreases losses by leaching these nutrients. Therefore, the CEC values were changed in the observations under the cultivations of the two species, with predominantly high class for the species *P. notatum* and low for *P. atratum*.

The base saturation value (V%) indicates the percentage of negative charges occupied by K, Ca, Mg, Na, and subject to exchange at pH 7.0 when compared to the points occupied by $\text{H} + \text{Al}$ (Raij, 1991). Soils with very low V% require greater amounts of limestone to increase base saturation. V% values were higher in areas under *P. notatum*, being considered high and low for *P. atratum*.

Taxonomic Microbial Diversity, Community Composition, and Abundance

The present study of the rhizosphere microbiota showed an average of 91,272 OTUs in *P. notatum* and 101,554 OTUs in *P. atratum*. The rarefaction curve showed that the sequenced sampling was enough to discover the main OTUs for each studied accession (Figure 1A). The Chao index showed that the diversity in *P. atratum* increased compared to *P. notatum* (Figure 1B). The observed species index showed that *P. atratum* also increased compared to *P. notatum* (Figure 1C).

Thirty-one taxonomic OTUs with an abundance above 0.01 were identified. Of these, 11 OTUs belonged to the phylum Proteobacteria, eight to the phylum Acidobacteria, three to the phylum Actinobacteria, two to each of the phyla Planctomycetes, Nitrospirae, and Verrucomicrobia, and one to each of the phyla Chloroflexi, Gemmatimonadetes, and Bacteroidetes.

The predominance of the groups of microorganisms was completely different between the *P. notatum* and *P. atratum*, at the taxonomic level of phylum, family, and genus (Figure 2). The Figure 2A shows the observed frequency of OTUs for each species. Some OTUs are more frequent in one species than in the other. Proteobacteria and Acidobacteria were the most dominant communities found for both species, with 38% and 25% in *P. notatum* and 37% and 35% in *P. atratum*. Other dominant bacterial phyla found in *P. notatum* were (i) Planctomycetes (9%), (ii) Nitrospirae (7%), (iii) Actinobacteria (6%), (iv) Gemmatimonadetes and Chloroflexi (5%), (v) Verrucomicrobia (4%) and (vi) Bacteroidetes (1%). In *P. atratum*, other dominant bacterial phyla included (i) Actinobacteria (11%), (ii) Verrucomicrobia (6%), (iii) Nitrospirae (4%), (iv) Planctomycetes (3%), (v) Gemmatimonadetes and (vi) Bacteroidetes (2%).

The families and genera distributions are described in Figure 2B. The (i) Acidobacteria Subgroup 6 (10%), (ii) Nitrosomonadaceae (10%), (iii) Desulfurellaceae (6%), and (iv) Planctomycetaceae (6%) were the most dominant communities in *P. notatum*. In *P. atratum*, the most predominant communities belonged to (i) Acidobacteriaceae Subgroup 1 (13%), (ii) Solibacteraceae (Subgroup 3) (13%), and (iii) Xanthobacteraceae (11%).

Among all the genera found in *P. notatum* (Figure 2C), the most dominating communities with 32% of total reads were (i) uncultured derived from Nitrosomonadaceae (10%), (ii) uncultured bacterium derived from Acidobacteria subgroup 2 (10%), (iii) uncultured derived from Planctomycetaceae (6%), and (iv) H16 (6%). In *P. atratum*, the most dominant communities with almost 38% of total reads were (i) uncultured derived from Acidobacteriaceae (Subgroup 1) (14%), (ii) Variibacter (9%), (iii) Candidatus Solibacter (9%), and (iv) Bradyrhizobium (6%).

As for the 31 OTUs with abundance above 1%, the t-test showed that 19 OTUs significantly differed between species. Figure 3 shows that 10 OTUs were more frequent in *P. notatum*; the three most significant OTUs were Bradyrhizobium ($p < 0.0001$), Acidothermus ($p < 0.0001$) and Candidatus Solibacter ($p = 0.0003$), followed by Variibacter ($p = 0.0006$), uncultured derived from Acidobacteriaceae (subgroup 1) ($p = 0.0015$), uncultured derived from Xanthobacteraceae ($p = 0.0035$), Rhizomicrobium ($p = 0.0051$), uncultured bacterium derived from DA101 soil group ($p = 0.0154$), uncultured bacterium derived from Acidobacteria subgroup 2 ($p = 0.0203$), Bryobacter ($p = 0.0408$). Figure 4 shows that out of the 9 OTUs more frequent in *P. atratum*, the three most significant OTUs were RB41 ($p = 0.0001$), uncultured derived

from Nitrosomonadaceae ($p=0.0007$), uncultured bacterium derived from Acidobacteria subgroup 6 ($p=0.0011$), followed by uncultured bacterium derived from Acidobacteria subgroup 6 ($p=0.0014$), uncultured bacterium derived from Xanthomonadales ($p=0.0027$), Nitrospira ($p=0.0046$), uncultured bacterium derived from OM190 ($p=0.0062$), Pedomicrobium ($p=0.0067$), other derived from Acidobacteria Subgroup 6 ($p=0.0234$).

DISCUSSION

Metagenomic analysis of the 16S gene is a widely used technique in molecular biology for studying microbial diversity in different environments, including the rhizosphere of plants (Oliveira et al., 2022). This technique can provide valuable information about the microbial communities interacting with the plant roots, including their composition, abundance, and potential functional roles (Malviya et al., 2022). Using this technology, we developed a metagenomic study involving the rhizosphere of accessions of *P. notatum* BGP 26 and *P. atratum* BGP308 from a germplasm bank. Both accessions of *Paspalum* were cultivated at the same place and were nurtured and managed equally. Thus, the difference observed in the microbiological population could reflect the species-specific OTUs of each accession.

The rhizobacterial community plays a crucial role in the development of plants, making the study of these microorganisms invaluable for the pursuit of economically viable and sustainable agriculture (Backer et al., 2018). Rhizobacteria, residing in the rhizosphere surrounding plant roots, actively contribute to various aspects of plant growth and soil health. Their significance lies in their ability to facilitate nutrient acquisition and assimilation, enhance soil texture, secrete beneficial substances, and regulate ecological processes (Backer et al., 2018). One promising avenue of research is the engineering of rhizobacteria to promote plant growth and increase crop yields. This advancement holds the potential to drive sustainable intensification of agriculture, meeting the growing demand for food while minimizing environmental impact (Haskett et al., 2021).

Furthermore, rhizobacteria play a critical role in regulating geochemical nutrient cycles, ensuring that essential nutrients become readily available to plants. This nutrient availability is vital for maintaining healthy crop growth and maximizing agricultural productivity (Andrade et al., 2023). Recent technological progress in sequencing has enabled significant strides in understanding the rhizosphere microbiomes of various plants. The diversity and composition of the rhizobacterial community are influenced by both the specific plant species and the soil properties in which they thrive (Ling et al., 2022). A higher diversity of rhizobacteria can lead to enhanced soil-plant interactions and increased overall productivity, particularly under resource-limited conditions. This suggests that promoting a diverse and thriving rhizobacterial community can contribute to sustainable agriculture, even in challenging environmental circumstances (Sahib et al., 2020).

Regarding the characteristics of the accessions, the individual short rhizomes of *P. notatum* are tough and stout. The culms (stems) are ascending, usually ranging from 20 to 75 cm tall, and can grow up to 1 m high. The leaves are 4 to 10 mm wide and linear-elongate in shape (Cook et al., 2023b). *Paspalum*

atratum is a rhizomatous perennial tussock grass that can grow up to 2 m when in flower. The leaf blades are elongate, measuring 35-65 cm in length and 7-20 mm in width (Cook et al., 2023a). Considering the interpretation ranges of soil analysis results by Raij et al. (1996) and Alvarez Venegas et al. (1999), *Paspalum* species affect soil parameters. Following the same criteria used by Vargas et al. (2023), soil fertility could be considered poor under *P. atratum* cultivation and medium under *P. notatum*, indicating a change in the edaphic environment due to the growth of both species. The same was observed at the collection site of the species.

Leff et al. (2015) indicated that fertilization with high doses of N and P leads to changes in the species and functionality of soil microbial communities. Vargas et al. (2023) also found that the microorganism populations changed significantly with the soil fertility level rather than with the host plant, and the parameters clay, organic matter, pH, K, and P availability present significant linear correlations with some phyla studied.

Few studies are available in the literature involving rhizosphere metagenomic studies of the *Paspalum* genus plants (Guerra et al., 2021; Prabha et al., 2018, 2019). Prabha et al. (2018, 2019) studied bacterial diversity in the rhizosphere of *P. scrobiculatum* L. (Kodo millet) in Jagdalpur, Chhattisgarh, India. Kodo is fiber-rich and an important food source for subsistence farming communities in many regions in India and Africa (Deshpande et al., 2015). According to Prabha et al. (2019), the microbial communities associated with the *P. scrobiculatum* rhizosphere by metagenomic analysis revealed that Proteobacteria were the second most abundant phylum among the 65 taxonomically diverse phyla identified in the rhizobiome. In our study, the phyla Acidobacteria and Proteobacteria were the most abundant in *P. notatum* (63% of the OTUs) and *P. atratum* (72% of the OTUs). However, *P. notatum* and *P. atratum* presented the phyla Nitrospirae and Gemmatimonadetes that were not present in *P. scrobiculatum* in the study of Prabha et al. (2019). The phyla Firmicutes and Cyanobacteria were present in Prabha et al. (2019) and absent in ours.

Kielak et al. (2016) suggested there is an ecological relationship between Acidobacteria and Proteobacteria groups. These phyla can represent more than 50% of the total groups found in some habitats, being among the most abundant in the soil (Dunbar et al., 2002; Sait et al., 2002). In *P. notatum* (BGP 26), it was observed that 25% (Figure 1A) of the OTUs at the phylum level belonged to Acidobacteria, whereas in *P. atratum* (BGP 308), this value was 37%. Bacteria from the Acidobacteria phylum are predominant in soil with low pH, mainly in subgroup 1 (Sait et al., 2006). However, subgroup 6 can positively or negatively correlate with soil pH (Chan et al., 2006; Mukherjee et al., 2014).

Animals are evolutionarily distant from plants, and studies investigating animal species have shown how nutrition and habitat influence the microbiota of these species. Ting et al. (2020) investigated the difference in microbial diversity of the intestinal tract of *Rhesus macaques* living in the wild and those artificially fed. The results showed no significant difference in microbial diversity between the groups. However, artificial feeding increased the Firmicutes/Bacteroidetes ratio. The observed differences in microbiotas were also related to differences in the species' diet (nutrition) and habitat. In our study,

nutrition and habitat variables were minimized since the two *Paspalum* species were cultivated in the same habitat and received the same nutrition. The soil of both *P. atratum* and *P. notatum* was treated and managed equally. Still, chemical and physical differences were observed between the soil of the two species (Table 1). The soil pH was more acidic in *P. atratum* (Water = 5.6 / CaCl₂ = 5.0) compared to *P. notatum* (Water = 7.0 / CaCl₂ = 6.4). The pH is an important factor to be considered in the soil, as it changes other soil factors, such as the availability of macro and micronutrients (McBride, 1994). Low calcium and magnesium in the soil are correlated with low pH. Calcium and magnesium were lower in *P. atratum* compared to *P. notatum*. Another factor in soil acidity is high H + Al content (Ebeling et al., 2008), which was higher in *P. atratum*.

Of the 19 groups significantly different among species (Figures 3 and 4), 11 belonged to Acidobacteria. In *P. atratum* (BGP 308), families derived from Acidobacteria subgroups 1, 2, and 3 were found, and in *P. notatum* (BGP 26), families derived from Acidobacteria Subgroup 6 were found. Acidobacteriaceae (Subgroup 1) represents one of the most abundant families in *P. atratum* (BGP 308) and belongs to the phylum Acidobacteria. They are heterotrophic and can be aerobic, microaerophilic, or facultatively anaerobic bacteria. They grow on various sugars or polysaccharides; some can use amino acids as carbon sources. They are also acidophilic, growing between pH 3.0 and 7.5; the ideal is usually below pH 6.0. The growing temperatures are between 4 °C and 37 °C (Kielak et al., 2016; Pisa et al., 2011; Wu et al., 2018). The abundance of subgroup 1 is negatively correlated with soil pH (Kielak et al., 2016). In our study, the soil of *P. atratum* was more acidic. The population of Acidobacteriaceae (Subgroup 1) was also significantly higher in *P. atratum* (BGP 308). Data showing the importance of pH for this bacterial group's growth and development is corroborated by Chan et al. (2006) and Mukherjee et al. (2014).

Phosphorus belongs to group 2 of mineral nutrients, which are part of energy storage or structural integrity; it participates in the constitution of phospholipids, nucleic acids, and coenzymes, and has great importance in photosynthesis and cellular respiration (Gyaneshwar et al., 2002; Taiz et al., 2016). Groups belonging to Acidobacteriaceae, such as *Granulicella*, *Acidicapsa*, and *Edaphobacter*, have the potential to produce phosphatase enzymes both in acidic and alkaline conditions; consequently, these enzymes are found in an extracellular form in rhizospheric soils (Rosenberg et al., 2014; Susila et al., 2016). In this present study, Acidobacteriaceae was abundant. Further work is needed to study the growth potential of *P. atratum* in soils rich in inorganic phosphate to obtain phosphate solubilization by non-cultivable rhizobacteria. However, de Paula et al. (2022) aimed to isolate cultivable endophytic and rhizospheric bacteria associated with *P. atratum* and to assess their potential for plant growth improvement. The results demonstrated that the treatments receiving strains of *Pseudomonas* sp. and *Bacillus* sp. were significantly effective in enhancing the solubilization of phosphate in crystalline and sedimentary rocks, respectively. This contribution facilitates the growth of *P. atratum* plants fertilized with insoluble phosphorus sources.

In *P. notatum* (BGP 26), it was observed that 38% of the OTUs at the phylum level belonged to Proteobacteria (Figure 2A). In *P. atratum* (BGP 308), this value was 35%. As for the OTUs that belonged to

Proteobacteria, the family Bradyrhizobiaceae was the most abundant in *P. atratum* (BGP 308), and the family Nitrosomonadaceae was the most abundant in *P. notatum* (BGP 26).

The Bradyrhizobiaceae family was one of the most abundant in *P. atratum* (BGP 308) (Figure 2B). The group Bradyrhizobiaceae belongs to the order Rhizobiales and the Class Alphaproteobacteria. It is usually found in a soil environment or as a host for plants and animals (Ormeño-Orrillo & Martínez-Romero, 2019; Souza et al., 2013). They are isolated with less difficulty from host plant nodules using yeast extract-mannitol agar (YMA medium). Bradyrhizobiaceae participate significantly in biogeochemical cycles, and representatives of the genus *Bradyrhizobium* can use Ammonium salts, nitrate, and amino acids as nitrogen sources. Thus, the Bradyrhizobiaceae have the potential for biological nitrogen fixation, a direct way of promoting plant growth that can be used in agriculture (Ormeño-Orrillo & Martínez-Romero, 2019; Souza et al., 2013). Although there are no specific studies linking the Bradyrhizobiaceae family to *Paspalum* species, the Bradyrhizobiaceae family is known to be associated with plants as hosts and can participate in biological nitrogen fixation, which promotes plant growth (Ormeño-Orrillo & Martínez-Romero, 2019; Souza et al., 2013). Since *Paspalum* is a grass genus, there may be interactions between Bradyrhizobiaceae and *Paspalum* species.

Nitrosomonadaceae are involved in the oxidation and nitrification of ammonia, and this energy source subdivides them into ammonia oxidizers and nitrite oxidizers, playing an essential role in the ammonia cycle (Prosser et al., 2013). Representatives are lithoautotrophic ammonia oxidizers. The two subgroups are isolated from soil and were once considered the major contributors to the oxidation of soil ammonia nitrogen (Prosser et al., 2013). The selective medium can be used to isolate pure cultures of these microorganisms. Still, this isolation is difficult because other microorganisms with a higher growth rate can contaminate the samples with a low growth rate in a medium containing ammonia, which generates organic acids when oxidized. Thus, buffering the medium is necessary, as these microorganisms grow optimally at neutral or moderately alkaline pH, and most do not grow below 7.0 (Prosser et al., 2013).

Paula et al. (2022) evaluated the association between peach trees (*Prunus persica*) and the use of *P. notatum* as ground cover, aiming for vegetation cover to reduce nitrogen loss in the soil by volatilization and leaching. The nitrogen source used was urea and organic compost; results showed that the root system of *P. notatum* is more aggressive than that of peach trees, indicating *P. notatum* absorbed more N. Although the focus of the work was not the nutrition of *P. notatum*, it was observed a good response of this species to fertilization with urea was observed. According to Dias (2021), more than 40% of nitrogen fertilizer is lost to the atmosphere when applied in the field. In addition, nitrogen fertilizer is an expensive input. Further studies can be carried out to explore these microorganisms' ability to carry out the oxidation of ammonia, thus optimizing the use of nitrogen by plants, emphasizing microorganisms of the Nitrosomonadaceae family, which are abundant in *P. notatum* (BGP 308) and play a role in the nitrogen cycle.

Paspalum atratum (BGP 308) and *P. notatum* (BGP 26) are promising species with economic potential that could serve as alternative sources of animal nutrition compared to the current cultivated varieties.

Understanding the current microbiota of these species in the *Paspalum* Germplasm Bank is essential for future comparative studies when these species are in the field, influenced by a new habitat, nutrition, and agrochemicals.

The *Paspalum* Germplasm Bank of Embrapa Pecuária Sudeste has about 450 accessions of 50 species, and this work will serve as a basis for future studies involving new species of *Paspalum*. Future studies on the microbiota of *P. atratum* and *P. notatum* may be directed toward making the most of the species-specific potential of the rhizobacteria present in each species. Studies involving acid soils can be carried out to cultivate *P. atratum*, since this species showed tolerance to acid soil and presented rhizobacteria adapted to this environment.

CONCLUSIONS

Metagenomic analysis of the rhizosphere microbial community of plants provides valuable insights into microbial diversity, including the composition and abundance of different bacterial groups. A total of 19 OTUs showed significant differences between the rhizospheric bacterial population of *P. atratum* and *P. notatum*. The diversity and composition of the rhizobacterial community were influenced by both the specific plant species and the soil properties in which they thrive. Acidobacteria and Proteobacteria are two commonly found bacterial groups in the rhizosphere of *Paspalum* species. Acidobacteria are more abundant in soils with low pH, while Proteobacteria are found in a variety of habitats. The rhizosphere microbiota can be affected by factors such as soil pH, plant nutrition, and agrochemical application, which can impact plant health and crop development. Understanding the microbiota present in *Paspalum* species is essential for future comparative studies when these species are in the field, influenced by a new habitat, nutrition, and agrochemicals.

Declarations

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Author Contributions

A.F.P., A.P.F., A.F.C. and PTL conceived and designed the study. A.F.P., P.H.M.A., E.M.Jr., B.B.Z.V., and A.F.C. conducted the experiments. E.M.Jr., M.F.C., L.M.C. and A.F.C. contributed new reagents and analytical tools. A.F.P., P.H.M.A., E.M.Jr., M.F.C., L.M.C., B.B.Z.V., A.C.C.B., A.P.F., A.F.C. and P.T.L. analyzed the data. A.F.P., A.P.F., A.F.C. and P.T.L. wrote the manuscript. All the authors read, revised, and approved the manuscript.

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Permissions to collect the plants/plant parts

Not applicable.

Consent to participate

Not applicable.

Consent to publish

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Ethical approval

The present study does not require any ethical approval.

Clinical trial number

Not applicable.

Data availability Statement

The datasets generated and/or analyzed during the current study have been deposited in the CEN Herbarium and are publicly accessible under the accession number 112601. The data can be accessed at the following permanent link: (<https://www.embrapa.br/en/web/recursos-geneticos-e-biotecnologia/pesquisa-e-desenvolvimento/bases-de-dados/herbario-cen>). The datasets generated and/or analyzed during the current study have been deposited in the **Alelo Genetic Resources Platform** and are publicly accessible under the accession number BRA number (006301 or BRA006301) or its local code (BGP 26). The data can be accessed at the following permanent link: *Alelo Vegetal Consultas* (https://alelo.cenargen.embrapa.br/index_en.html).

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Table

Table 1. Chemical and physical parameters of soil from the BGP 26 accessions of *Paspalum notatum* and BGP 308 from *Paspalum atratum*.

Descriptor	Sample Description	<i>P. notatum</i> BGP 26	Interpretation*	<i>P. atratum</i> BGP 308	Interpretation*
pH	Water	7	VL	5.6	M
pH	CaCl ₂	6.4	VL	5	H
O.M.	g dm ⁻³	33	M	25	M
P(res)	mg dm ⁻³	19	M	4	VL
K	mmol _c dm ⁻³	2.5	M	1.7	M
Ca	mmol _c dm ⁻³	50	VH	20	M
Mg	mmol _c dm ⁻³	26	VH	9	M
H + Al	mmol _c dm ⁻³	12	L	34	M
Al	mmol _c dm ⁻³	0	VL	3	VL
S.SO ₄	mg dm ⁻³	33		13	
CEC	mmolc dm-3	90	H	65	M
V	%	87	H	47	L
m	%	0	VL	9	VL
B	mg dm-3	0.32	M	0.26	M
Cu	mg dm-3	2.4	H	4.5	H
Fe	mg dm-3	9	M	35	H
Mn	mg dm-3	5.5	H	15	H
Zn	mg dm-3	1.1	M	1.2	M
Total Sand	g kg ⁻¹	379		425	
Clay	g kg ⁻¹	385		429	
Silte	g kg ⁻¹	236		146	
Soil texture**:		Clay loam		Sandy clay	

*VL = very low, L = low, M = medium, H = high, VH = very high, following Raij et al. (1996), and Alvarez Venegas et al. (1999).

** Following USDA–NRCS. (2018).

Figures

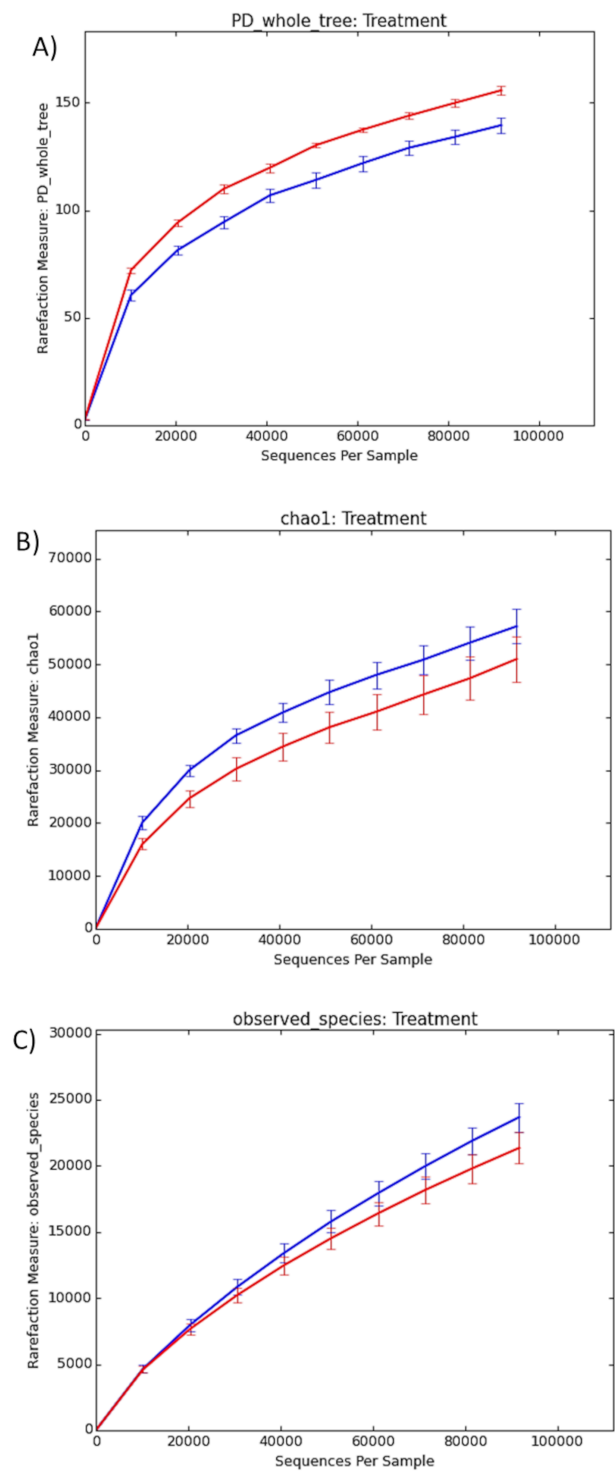


Figure 1

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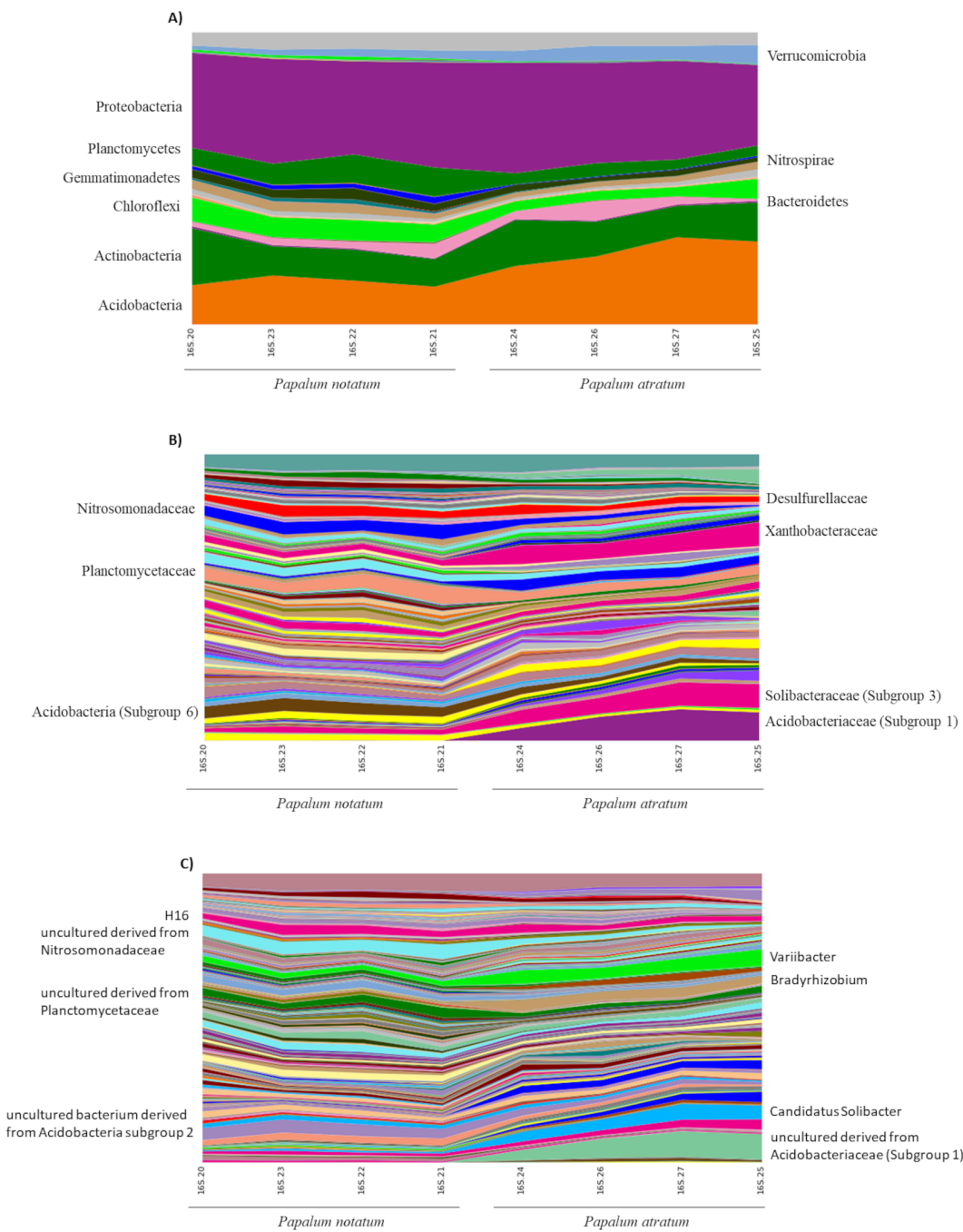


Figure 2

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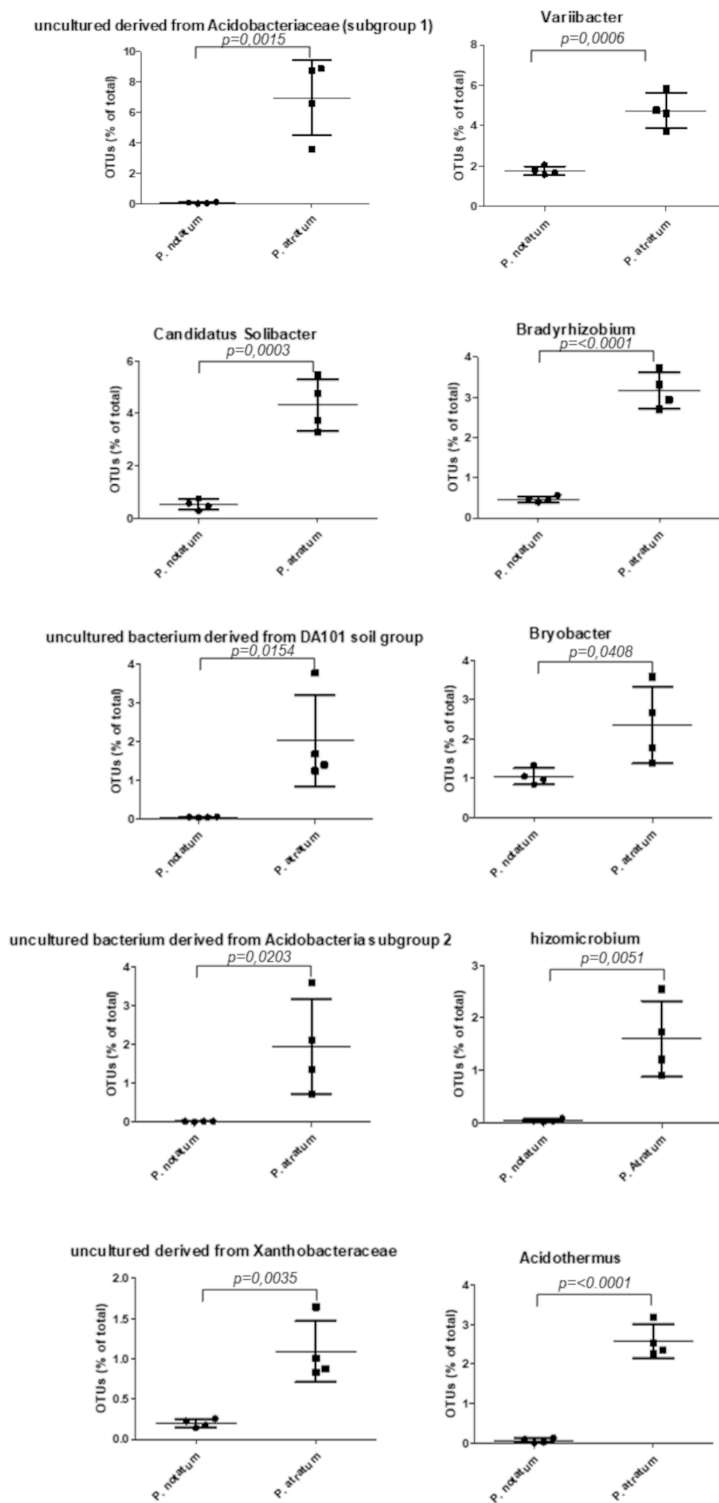


Figure 3

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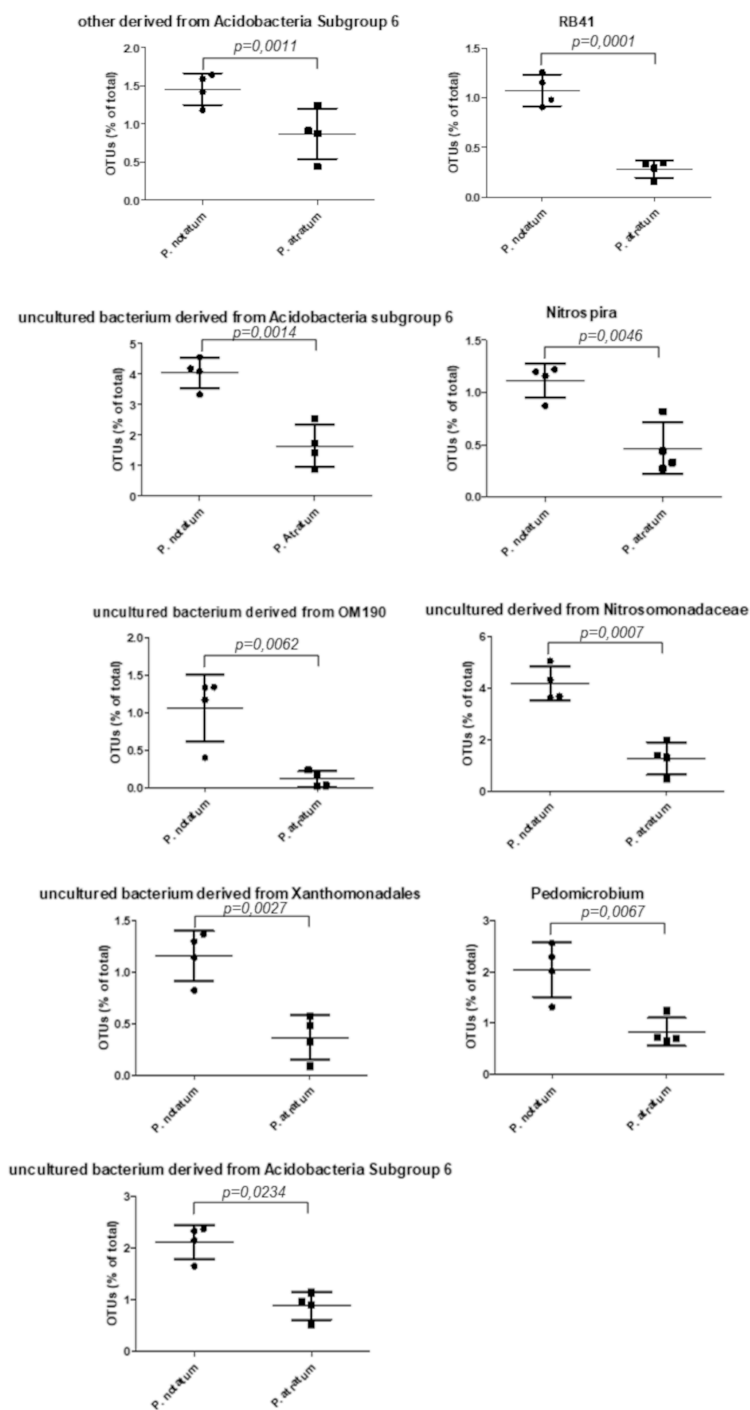


Figure 4

Legend not included with this version.