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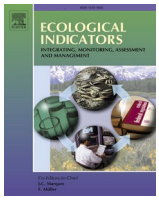

Impacts of deforestation and forest regeneration on soil bacterial communities associated with phosphorus transformation processes in the Brazilian Amazon region

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ABSTRACT

Land-use change has negative impacts on the biodiversity of plants and animals. However, we lack studies and/or information about the impacts of land-use change on the biodiversity of soil microorganisms, especially those involved in the phosphorus (P) transformation processes. This represents a great concern since some microbial groups are extremely important to different P transformation processes and regulate P availability in tropical and subtropical regions. In our study, we used shotgun DNA-metagenomic sequencing and P fractionation analysis to assess the effects of forest-to-pasture conversion on the dynamics of soil bacterial groups involved in the P transformation processes and their potential functions. Additionally, we assessed if the dynamics of these specific soil bacterial groups were recovered after pasture abandonment and with secondary forest establishment. Our results demonstrated that the land-use change altered the total amount of P and its fractions in the soils. The bacterial community structure was affected by changes in soil chemical properties, mainly by changes in the aluminum, total P, and labile P contents. The bacterial groups involved in the P transformation processes and their potential functions were also affected by changes in land use. The pasture soil harbored a distinct bacterial community when compared to the primary and secondary forest sites. In general, forest-to-pasture conversion increased bacterial groups involved in the P mineralization, including Firmicutes, Cyanobacteria, and Gemmatimonadetes. Conversely, we observed an increase of Proteobacteria members (e.g., Bradyrhizobiaceae and Beijerinckiaceae) and genes related to P solubilization and mineralization after pasture abandonment and with the secondary forest re-establishment. Our multi-analytical approach suggests that forest-to-pasture conversion has negative impacts on the biodiversity of bacterial groups involved in the P transformation processes and their potential functions, while the secondary forest re-establishment can stimulate resilience. Taken together, our results indicated that the bacterial groups involved in the P transformation and their potential functions can be gradually recovered and reach intermediate and/or even similar levels to those observed in undisturbed forest sites. This brings new insights and helps to improve our knowledge about the impacts of anthropogenic actions on the soil microbiome in the Amazon region.

1. Introduction

The Brazilian Amazon rainforest has been threatened by the increase in cattle ranching activities (Inpe, 2021; Vale et al., 2019). In 2020, deforestation reached the highest rate in the last 10 years, with more than 11,000 km^2 of pristine forest being converted into pastures (Silva Junior et al., 2020). During this conversion process, farmers extract valuable timber, burn the remaining vegetation (also known as the 'slash-and-burning' method), and seed the area with fast-growing grasses (Navarrete et al., 2015). The slash-and-burning method changes the soil's physical, chemical, and biological properties and favors the initial establishment of pastures in the Amazon region (Melo

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et al., 2017). However, this practice is not sustainable, and after a short period of use, pastures have their productivity drastically reduced, become degraded, and are later abandoned (Souza Braz et al., 2011). Meanwhile, the natural vegetation starts to slowly re-establish itself and form what is known as 'secondary forest' stand (Cenciani et al., 2009). Studies indicated that this practice is becoming common in the Brazilian Amazon region. According to the study of Carvalho et al. (2019), these secondary forest areas are growing, and nowadays, it is estimated that they cover more than 100,000 km^2. Nevertheless, we lack studies and/or knowledge about the re-establishment of these secondary forests, particularly those that deal with soil microorganisms and nutrient cycling (Pedrinho et al., 2020).

Phosphorus (P) is considered an essential nutrient for plant nutrition, and it can limit the primary productivity of many terrestrial environments, including tropical and subtropical forests (Porder et al., 2007). In general, tropical and subtropical forest soils possess high amounts of iron (Fe) and aluminum (Al) oxides due to the strong weathering process typically found in these regions, which have heavy rainfall and high temperatures. These Fe and Al oxides have the geochemical ability to bind P and make it unavailable for the uptake of plants (Gama-Rodrigues et al., 2014; Soltangheisi et al., 2019). Thus, a great number of plants rely on the recycling of P from litterfall and microbial P turnover (Johnson et al., 2003). However, studies demonstrated that land-use change has a negative impact on soil P dynamics in the Amazon region (Chavarro-Bermeo et al., 2022; Soltangheisi et al., 2019). This is because the conversion of a pristine forest to pasture by the slash-and-burning method can alter P-lability and increase P in more recalcitrant pools (Soltangheisi et al., 2019). Also, it can release great amounts of N and P from the forest biomass, which can be lost via leaching and runoff (Garcia-Montiel et al., 2000; Hamer et al., 2013). Conversely, there are few studies and/or information about the effects of secondary forest re-establishment on the P dynamics. So far, studies suggest that secondary forest re-establishment has the potential to promote a slow but continuous recovery of soil P (Fu et al., 2020; Teixeira et al., 2020; Paul, 2014; Teixeira et al., 2020). According to these authors, the increase in plant diversity, the re-establishment of some soil physicochemical properties (e.g., soil pH), and enzymatic activity (e.g., C, N, P, S enzymes) may favor some specific bacterial and fungal groups (e.g., P-mineralizing and P-solubilizing microorganisms), which perform important functions in the P transformation processes and regulate its availability.

In general, these specific bacterial and fungal groups are involved in three main processes in soils: (i) mineralization, (ii) solubilization, and (iii) immobilization (Richardson and Simpson, 2011). Microorganisms involved in the P mineralization can produce enzymes that hydrolyze ester-phosphate bonds and free orthophosphate (PO_4^{3-}) from recalcitrant organic P forms (Arenberg and Arai, 2019). They contain genes coding for enzymes, such as phytase (appA), alkaline phosphatase (phoD), acid phosphatase (olpA), phosphonatase (phnX), and C—P lyases (phn), which possess the ability to mineralize organic-P compounds in soils (Dai et al., 2019; Rodríguez et al., 2006). On the other hand, P-solubilizing microorganisms can produce and release organic acids, such as oxalic acid, malic acid, formic acid, citric acid, and gluconic acid, which possess the ability to solubilize recalcitrant inorganic-P forms in soils (Alori et al., 2017; Rodríguez et al., 2006). They possess the gcd gene (encoding glucose dehydrogenase) and the cofactor pqq gene (encoding pyrroloquinoline quinone) that regulate the solubilization of unavailable mineral P forms (Khan et al., 2007). Lastly, microorganisms involved in the P immobilization can incorporate inorganic P into their biomass, competing with plants for the available P (Richardson and Simpson, 2011). They possess pst and pit transporter genes (e.g., genes coding for P-uptake and transport systems), which can help them to assimilate inorganic P under the P-low and rich conditions. Microbial groups involved in the P transformation are ubiquitous in soils. They have distinctive environmental requirements that can affect their population (size and activity) and, consequently, P transformation processes (Gupta et al., 2017). According to Bi et al. (2019) and Khan et al. (2013), they are also very sensitive to disturbances, especially to changes in soil physicochemical properties. This represents a great concern since previous studies indicated that the conversion of pristine forest to pasture in the Amazon region alters soil physicochemical properties and also the diversity, structure, and composition of the overall soil microbial community (Khan et al., 2019; Mendes et al., 2015a; Pedrinho et al., 2019). Nevertheless, there is a lack of studies and/or knowledge about the impacts of land-use change on microbial groups involved in the P transformation and their potential functions in the Amazon region.

Here, we assessed the effects of land-use change on the dynamics of soil bacterial communities, especially those bacterial groups involved in the P transformation processes and their potential functions. We used shotgun DNA-metagenomic sequencing and P fractionation analysis to assess the diversity, structure, composition, and potential functions of soil bacterial communities involved in different P transformation processes in the Brazilian Amazon region. We hypothesize that forest-to-pasture conversion affects negatively the soil bacterial communities involved in the P transformation due to changes in vegetation and P dynamics (e.g., lability and content). On the other hand, we expect that after the abandonment of degraded pasture, secondary forest re-establishment will lead to a recovery of bacterial groups, especially those involved in the P transformation processes and their potential functions. The following questions were addressed to test our hypotheses: (i) What are the main impacts of land-use change on soil chemical properties and/or P fractions?; (ii) What are the main consequences of land-use change on the structure and diversity of bacterial groups involved in the P transformation processes?; (iii) How does land-use change potentially affect the interactions among bacterial groups involved in the P transformation and their potential functions?; (iv) How does forest regeneration potentially help in the recovery of P dynamics in soils?

2. Material and methods

2.1. Land-use system description and soil sampling

The study was performed in the Tapajós National Forest and its adjacent areas, in Belterra, state of Pará, Brazil (Supplementary Fig. 1). Soil samples were collected from different land-use systems in this region at the beginning of the wet (May) and dry (November) seasons of 2016. Briefly, we selected a i) primary forest (PF − 2°51′23.9″S, 54°57′28.4″W), which comprises a protected rainforest with no history of fire and/or logging activities (e.g., plant species found in this site - Domingues et al., 2007); ii) pasture (PA − 3°07′52.9″S, 54°57′28.1″W), a former rainforest site that was converted to pastureland more than 20 years ago. Pasture is predominantly covered by the grass Urochloa and is characterized by extensive management. The farmer does not use fertilizers and/or animal rotations, and pasture has very low productivity levels (<1 animal unit per ha); and iii) secondary forest (SF − 3°15′47.9″S, 54°53′36.0″W), comprises a site form used for cattle ranching activities and with more than 18–19 years of abandonment. Currently, this area is being naturally re-colonized by rainforest plant species (e.g., small trees and shrubs - more information in Pedrinho et al., 2019).

The soil in these sites is classified as Typic Hapludox with high acidity (average soil pH = 3.95; Table ST1) and very low fertility (Soil Survey Staff, 2014). The climate is classified as Am (Koppen's classification), with an average temperature of 26 °C and precipitation of 2,150 mm. A total of 24 soil samples were collected (three sites × two sampling periods × four replicates per site). At each location, a 200 m transect with four equally spaced sampling points (50 m apart, considered here as replicates) was established. The plant fresh material and/or debris were removed from the soil surface, and then, an auger was used to collect samples at 0 to 0.10 m depth. These included i) 50 g of soil for molecular analysis (e.g., DNA extraction and further shotgun DNA-metagenomic analysis) and ii) 400 g of soil to perform P fractionation

and also to determine chemical properties. Soil samples were transported to the Cell and Molecular Biology Laboratory at the Center for Nuclear Energy in Agriculture (CENA/USP, Piracicaba, Brazil). Samples for molecular analysis were stored at − 80 °C, while samples for P fractionation and chemical analysis were stored at 4 °C until processing.

2.2. Phosphorus fractionation

The soil's chemical properties were determined following the Manual of Soil Analysis Methods (Embrapa, 1997). The soil P fractions were determined by sequential chemical extractions (Condron et al., 1985; Hedley et al., 1982). In this method, inorganic (Pi) and organic (Po) P fractions are progressively extracted (Damian et al., 2019). Briefly, 0.5 g soil was placed in 15 ml centrifuge tubes, and at each step, 10 ml of extractant was added. First of all, we used i) anion exchange resin to extract the most labile P (PAER), followed by ii) sodium bicarbonate (0.5 M NaHCO3) to extract the labile inorganic P (PiBIC) and organic P (PoBIC) sorbed to the soil surfaces, iii) sodium hydroxide (0.1 M NaOH) was added to extract inorganic P associated with amorphous and some crystalline Al and Fe oxides (PiHID-0.1) and organic P associated with humic compounds (PoHID-0.1), iv) hydrochloric acid (1.0 M HCl) was added to extract mainly inorganic P associated with apatite (PiHCl), and v) sodium hydroxide (0.5 M NaOH) was used to extract highly resistant fraction of inorganic P (PiHID-0.5) and organic P (PoHID-0.5). The soil left was dried at 50 °C, grounded, and later digested in sulfuric acid (H2SO4) + hydrogen peroxide (H2O2) to determine the most chemically stable inorganic and organic P forms, here called the residual P (Presidual). Murphy and Riley's (1962) method was used to determine P concentrations. Total P in the alkaline extracts (0.5 M NaHCO3; 0.1 M and 0.5 M NaOH) was measured by digestion in ammonium persulphate ((NH4)2S2O8) + H2SO4 and later autoclaved at 121 °C and 103 KPa for 2 h. Inorganic P fractions (Pi) in alkaline extracts were measured by Dick and Tabatabai's (1977) method. Organic P (Po) was determined by subtracting the total P and Pi in the alkaline fractions. Lastly, soil P fractions were arranged based on their availability such as i) labile (PAER + PiBIC + PoBIC), ii) moderately labile (PiHID-0.1 + PoHID-0.1 + PiHCl), and iii) non-labile (PiHID-0.5 + PoHID-0.5 + Presidual).

2.3. Acid phosphatase analysis

Acid phosphatase enzyme activity was measured by Tabatabai and Bremner's (1969) method. Briefly, 1.0 g of soil was placed in a glass tube and then added of 4 ml of a modified universal buffer solution, 0.25 ml of toluene, and 1 ml of p-nitrophenol phosphate solution. The tubes were swirled for 20 s to assure that the content was well mixed and then incubated at 37 °C for one hour. Afterward, 1 ml of calcium dichloride (CaCl2) and 4 ml of 0.5 M NaOH were added to inactivate the enzyme and extract the p-nitrophenol liberated. Once again, the tubes were swirled, and the soil suspension was passed through Whatman N° 42 filter paper. Lastly, the absorbance of the yellow color of p-nitrophenol was determined in a spectrophotometer at 410 nm.

2.4. Soil DNA extraction and shotgun DNA-metagenomic sequencing

Soil total DNA was extracted using the DNeasy PowerLyzer PowerSoil Kit (Qiagen, Hilden, Germany). To assure good quality and quantity of DNA extracts, we followed all manufacturer's recommendations. Afterward, we ran an agarose gel to confirm DNA quality and used NanoDrop 1000 spectrophotometry (Thermo Scientific, Wilmington, DE, EUA) to assess DNA quantity. The Nextera XT DNA Library Preparation Kit was used to prepare the shotgun DNA-metagenomic libraries. Sequencing was performed on an Illumina HiSeq 2500 platform (2 × 100 bp; Illumina, San Diego, CA, USA), according to the manufacturer's recommendations (for more information check Pedrinho et al., 2019).

2.5. Metagenomic analysis

The FastQC software (version 0.11.5) was used to assess the quality of raw sequences. Afterward, we used Trimmomatic software (version 0.32, Bolger et al., 2014) to trim and remove adapters and low-quality sequences (quality score lower than Q20). The PEAR software (Zhang et al., 2014) was used to initially assemble (R1 and R2) the remaining paired-end sequences. In this step, we chose to remove sequences with<50 nucleotides in length and under Q20. Later, DNA sequences were uploaded in the Metagenomics Rapid Annotation Server (MG-RAST) server v.4 (Meyer et al., 2008). The annotation of taxonomic and functional profiles was performed using Refseq (O'Leary et al., 2016) and KEGG (Kanehisa et al., 2017) databases, respectively (default parameters, minimum alignment length of 15 bp; minimal identity cutoff of 60 %; maximum E-value cutoff of E < 1 × 10–5). Here, we specifically targeted bacterial taxa and genes involved in P transformation as previously described by Bergkemper et al. (2016) and Grafe et al. (2018). Shotgun DNA-metagenomic data can be assessed at MG-RAST under the project ID 'mgp83361'.

2.6. Statistical analysis

The analysis of variance (ANOVA) followed by Tukey's post hoc test for multiple comparisons ($P < 0.05$) was performed to test for significant differences in P fractions and acid phosphatase enzyme activity among the samples from the different land-use systems. The 'agricolae' package in R was used to perform the comparisons. For downstream analyses, we used the normalized matrices from bacterial taxa (Refseq database) and potential functions (KEGG database), respectively. To assess the influences of environmental variables (e.g., soil chemical properties and P fractions) on the bacterial taxonomic and functional structures, we used the distance-based redundancy analysis (dbRDA). Additionally, we used a permutational multivariate analysis of variance (PERMANOVA; Anderson, 2001) to determine if the samples harbored significant differences in structures. The Canoco 4.5 (Biometrics, Wageningen, The Netherlands) software was used to create dbRDA plots. The PAST 4.04 (Hammer et al., 2001) software was used to perform PERMANOVA analyses. Also, we used previous software to calculate the richness and Shannon's alpha diversity indexes.

The differences in the abundance of P bacterial groups and potential functions among the land-use systems were determined using the statistical analysis of metagenomic profiles (STAMP) software (Parks and Beiko, 2010). The frequency tables of bacterial taxons and their potential functions were produced in MG-RAST and used as input. The two-sided Welch's *t*-test (Welch, 1947) was used to calculate P-values and then we used the Benjamini-Hochberg false discovery rate test ($P < 0.05$, Benjamini and Hochberg, 1995) for corrections. To assess the connections among the bacterial taxa involved in the P transformation in the selected land-use systems, we chose to perform the network analysis. For this, we calculated and used the SparCC correlations (Friedman and Alm, 2012) among the bacterial taxa involved in the P transformation process and P fractions in the soils. We highlight that significant ($P < 0.01$) and strong (>0.7 or < − 0.7) correlations were used in each network. Lastly, we used the software Gephi to calculate each network's topological properties and to perform visualization (Bastian et al., 2009).

3. Results

3.1. Phosphatase activity, total P, and its different fractions in the soil

Our results demonstrated that the land-use change altered the total amount of P and its fractions in the soil (Fig. 1A; Supplementary Table 2). The native forest soil had a higher amount of total P in comparison to pasture and secondary forest soils ($P < 0.05$). The majority of P was found in the non-labile fraction followed by the moderate-labile,

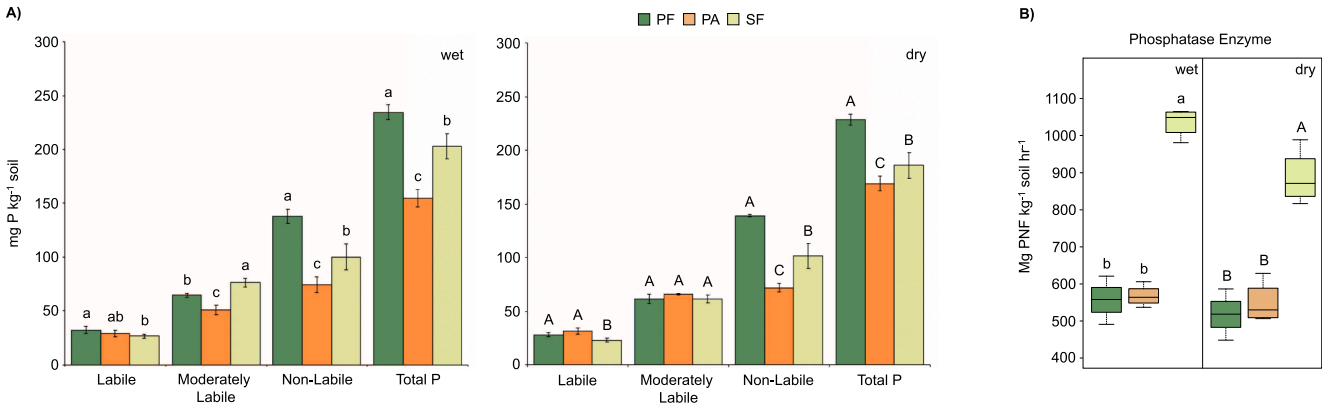

Fig. 1. (A) Phosphorus (P) contents in different fractions of primary forest (PF), pasture (PA), and secondary forest (SF) soils for both wet and dry sampling periods. (B) Acid phosphatase activity of the different land-use systems. Error bars represent the standard deviation of four independent replicates. The different lowercase letters indicate significant differences among the areas during the wet season ($P < 0.05$). The different uppercase letters indicate significant differences among the areas during the dry season ($P < 0.05$). Comparisons were based on the Kruskal-Wallis H test followed by Dunn's multiple comparisons test ($P < 0.05$).

and only a small portion of P was readily available in all land-use systems. We observed a reduction in the total amount of P and its non-labile fraction after forest-to-pasture conversion. Conversely, we observed that after the decline of pasture activities and natural re-establishment of secondary forest, the total amount of P in the soil and its non-labile fraction started to progressively increase, reaching an intermediate level to those observed in the primary forest. Furthermore, we observed lower labile P amounts in the secondary forest soil in comparison to the primary forest. Lastly, we noticed that land-use change negatively affected the activity of the acid phosphatase enzyme (Fig. 1B). Our results demonstrated that secondary forest had higher acid phosphatase activity values in comparison to primary forest and pasture soils ($P < 0.05$).

3.2. The structure of the bacterial community involved in the P transformation

We used the distance-based redundancy analysis (db-RDA) to determine the effects of land-use change on the structure of bacterial groups involved in the P transformation processes. The first two axes of each plot explained more than 75 % of the variation. In general, we observed that samples were grouped according to the land-use system (Fig. 2A, B). The taxonomic structure analysis revealed that bacterial communities involved in the P transformation process were remarkedly different from each other across the different land-use systems (PERMANOVA $F = 13.57$; $P = 0.0001$). Conversely, the potential functional structure analysis demonstrated that microbial functions associated with P transformation in primary and secondary forests were similar between themselves and distinct from pasture (PERMANOVA $F = 10.67$, $P = 0.0003$). Lastly, the general structure of the bacterial community involved in the P transformation was significantly correlated with Al3+ ($F = 9.24$, $P = 0.001$), total P ($F = 2.65$, $P = 0.037$), and the labile P fraction ($F = 2.47$, $P = 0.048$).

3.3. Taxonomic diversity and composition of soil bacterial community

The land-use change altered the richness, diversity, and potential functional diversity of the soil bacterial community involved in the P transformation ($P < 0.05$). The primary and secondary forests presented lower values when compared to pasture (Fig. 3A, B).

In general, the bacterial community related to the P transformation was composed of 24 phyla (Fig. 4). Proteobacteria (55.93 % of total sequences), Actinobacteria (17.41 %), Acidobacteria (9.11 %), Firmicutes (5.34 %), and Cyanobacteria (3.82 %) were the main bacterial phyla in all land-use systems, and together they account for more than 91 % of the soil bacterial community involved in the P transformation.

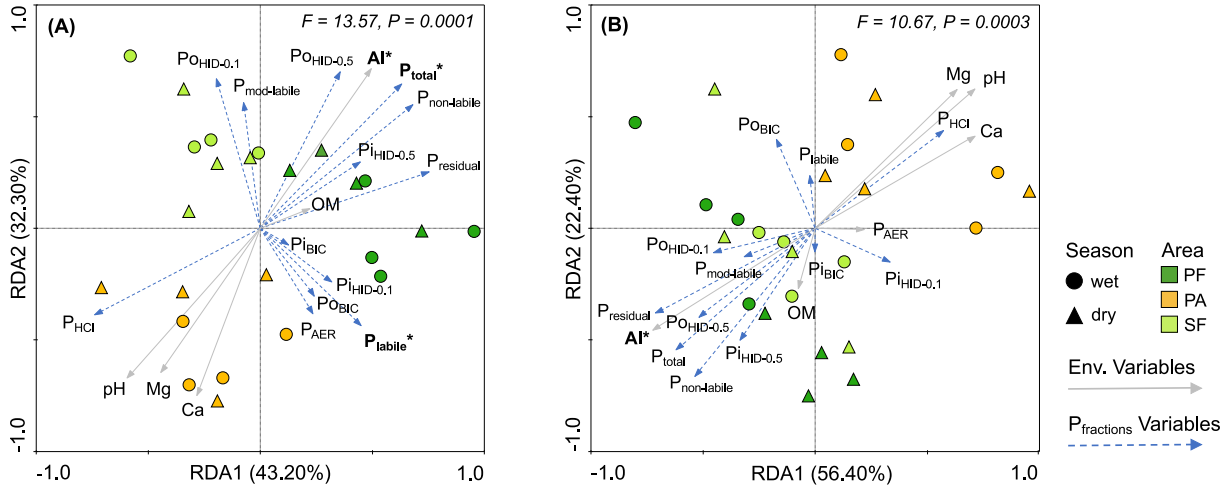

Fig. 2. Redundancy analysis of bacterial community involved in the phosphorus transformation and chemical properties from primary forest (PF), pasture (PA), and secondary forest (SF) soils. (A) Taxonomic analysis using relative abundances based on Refseq database at genera level. (B) Functional analysis using relative abundances based on the KEGG database at the gene level. The significance of these correlations was evaluated via the Monte Carlo permutation test and is indicated by asterisks ($P < 0.05$). Analysis of permutation (PERMANOVA) is indicated in the upper right of each graph. OM organic matter, m aluminum saturation.

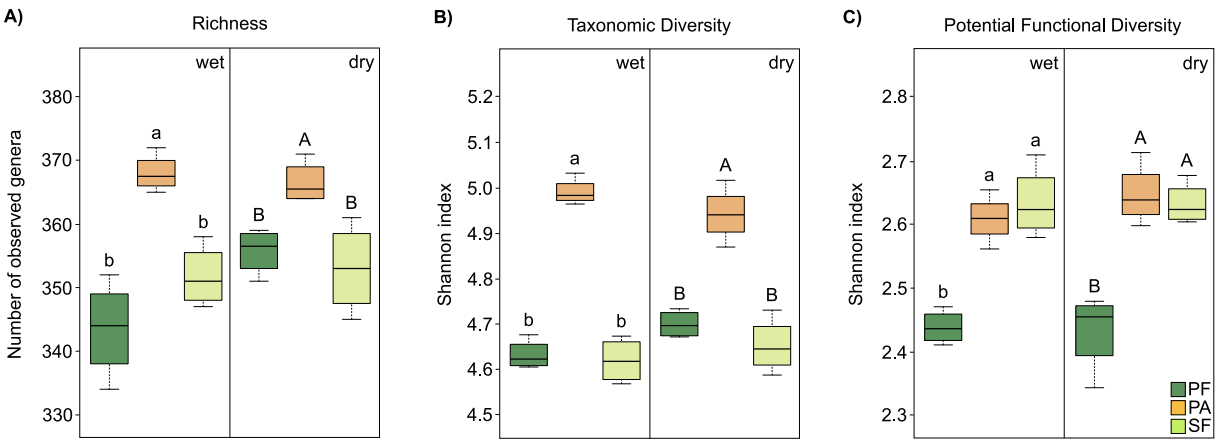

Fig. 3. Diversity measurements of the bacterial community involved in the phosphorus transformation in soils from primary forest (PF), pasture (PA), and secondary forest (SF) for both the wet and dry sampling periods. Taxonomic (A) richness and (B) diversity is based on genera level (Refseq database) and (C) functional diversity is based on gene level (KEGG database). Error bars represent the standard deviation of four independent replicates. The different lowercase letters indicate significant differences among the areas during the wet season. The different uppercase letters indicate significant differences among the areas during the dry season. Comparisons were based on the Kruskal–Wallis H test followed by Dunn's multiple comparisons test ($P < 0.05$).

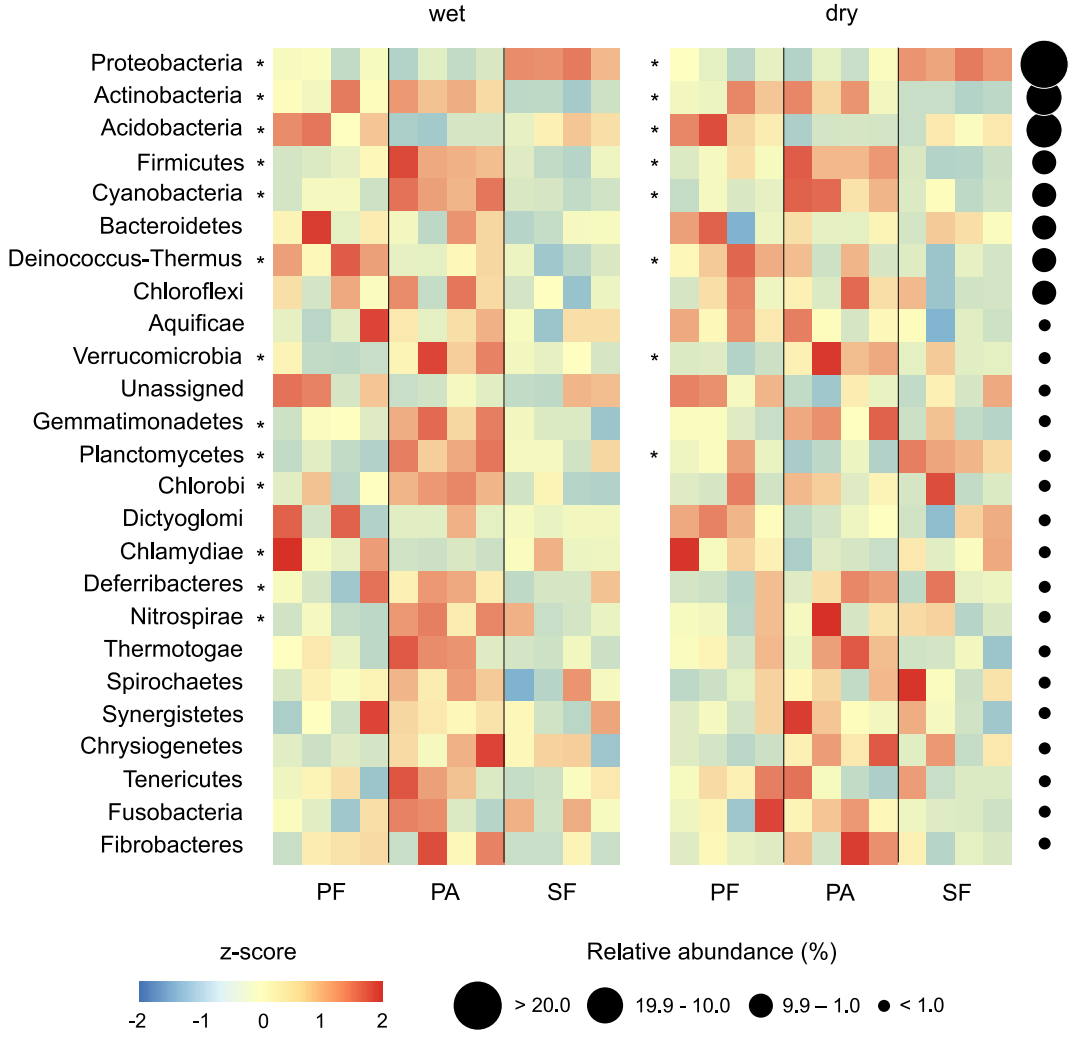

Fig. 4. Heatmaps showing the differential abundance of bacterial phyla involved in the P transformation among primary forest (PF), pasture (PA), and secondary forest (SF) soils for both the wet and dry sampling periods. Asterisks indicate the overrepresented phyla compared with the other land-use systems ($P < 0.05$, after Benjamini–Hochberg correction). The color key relates the heat map colors to the standard score (z-score). The circles are proportional to the relative abundance of each phyla in all samples.

As expected, land-use change also altered the bacterial composition across the studied sites. The forest-to-pasture conversion led to an increase in the abundance of several phyla, such as Firmicutes, Cyanobacteria, Gemmatimonadetes, Verrucomicrobia, Planctomycetes, and Chlorobi. On the other hand, after the decline of pasture activities and the natural re-establishment of secondary forest, the abundance of most of these bacterial phyla returned to levels previously observed in the primary forest. Interestingly, in the secondary forest, we observed that the abundance of Actinobacteria decreased. On the other hand, in this same site, we observed that the abundance of Proteobacteria members increased when compared to primary forest and pasture soils ($P < 0.05$).

The bacterial composition at the family level demonstrated that more than 190 families were involved in the P transformation process (e.g., uptake of extracellular P sources, solubilization, mineralization, and regulation process). Overall, the most abundant families for all land-use systems were Bradyrhizobiaceae (11.61 %), followed by Solibacteraceae (7.42 %), Mycobacteriaceae (6.01 %), Burkholderiaceae (3.59 %), and Comamonadaceae (2.37 %) (Supplementary Fig. 2). The land-use change also affected the bacterial composition at the family level. We observed a high abundance of Mycobacteriaceae in the primary forest. On the other hand, high abundances of Bradyrhizobiaceae, Beijerinckiaceae, and Solibacteraceae families were observed in the secondary forest. Lastly, more than 75 families presented high abundances in the pasture in comparison to native and secondary forests. Here, we call the attention to Bacillaceae, Rhizobiaceae, and Geobacteraceae families.

3.4. Potential functions of the soil bacterial community

The potential functional diversity of the soil bacterial community involved in the P transformation was affected by land-use change. In general, primary forest soil presented a lower potential functional diversity index compared to pasture and secondary forest soils (Fig. 3C). Approximately 0.73 % of the metagenome sequences (~333,400 sequences) were assigned to the P metabolism (Supplementary Fig. 3). The majority of sequences related to the P metabolism were assigned to the uptake of extracellular P sources (69.86 %), solubilization (12.18 %), mineralization (11.56 %), and regulation process (6.40 %) (Fig. 5A-D). In general, land-use change altered significantly the different functional categories. The primary forest soil presented a high abundance of sequences associated with the solubilization and uptake of extracellular P sources when compared to pasture during the wet season ($P < 0.05$). On the other hand, we did not observe significant differences during the dry season (P greater than 0.05). Interestingly, a greater number of sequences affiliated with P mineralization were presented in pasture and secondary forest soils in comparison to the primary forest ($P < 0.05$).

We also compared the relative abundances of bacterial genes encoding proteins that are linked to the uptake of extracellular P sources, solubilization, mineralization, and regulation process across the land-use systems (Fig. 5E; Supplementary Table 3). We highlight that bacterial genes associated with the intracellular P transformation were removed from further analysis. In general, 26 genes were involved in the P transformation process. The genes gcd (glucose dehydrogenase), pho (phosphate regulation), and pst (multimeric ABC-type phosphorus specific transporter) were the most abundant for all studied sites. Also, the majority of the genes involved in the P transformation were affected by land-use change. Among the land-use systems, secondary forest followed by pasture presented a great increase in the number of these genes in comparison to the primary forest ($P < 0.05$; Supplementary Table 3). Here, we especially highlight the increase of phn (phenol hydroxylase component) and pho genes in both land-use systems.

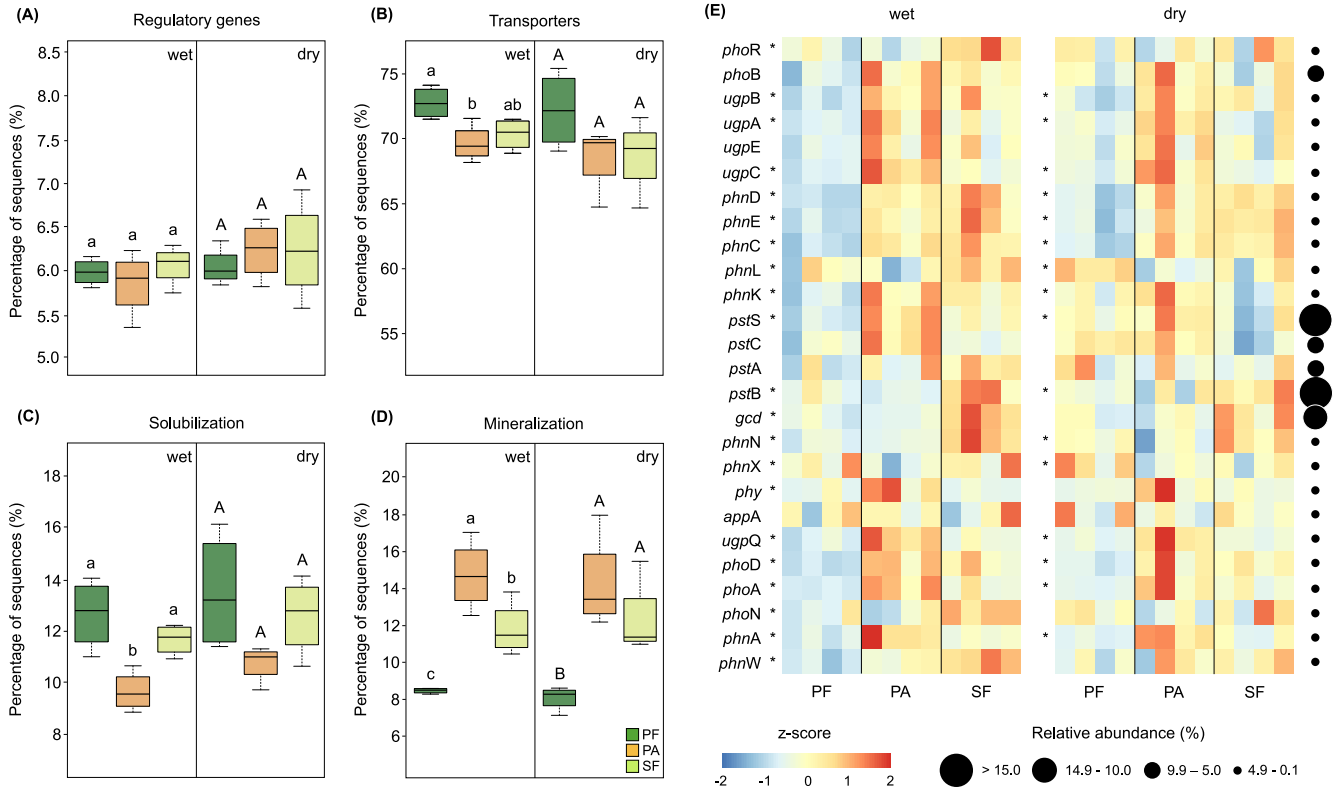

Fig. 5. The proportion of sequences affiliated with the phosphorus transformation processes in the primary forest (PF), pasture (PA), and secondary forest (SF) soils for both wet and dry sampling periods. (A-D) The proportion of sequences affiliated with the level 1 category of phosphorus metabolism. (E) Relative abundance of genes involved in phosphorus transformation processes. The quality-filtered reads were blasted against the KEGG database. Error bars represent the standard deviation of four independent replicates. Different lower- and capital-case letters (A-D) and asterisks (E) refer to significant differences in the abundance of genes across sites in each season based on the Kruskal–Wallis H test followed by Dunn's multiple comparisons test ($P < 0.05$).

3.5. Network analysis

According to Khan et al. (2019), network analysis allows the identification of potential associations among microbial groups, and it can be also used to understand community assembly and ecosystem functioning. Here, we used network analysis to investigate the interaction among the bacterial taxa involved in the P transformation process and P fractions in the soil. The land-use change impacted the network's compositional and topological properties (Fig. 6). The pasture (number of nodes = 99, edges = 162, average degree = 3.27) presented a higher complex network in comparison to the primary forest (number of nodes = 80, edges = 99, average degree = 2.47; Supplementary Table 4). On the other hand, the secondary forest exhibited transitional composition and topology features (number of nodes = 85, edges = 125, average degree = 2.94) between native forest and pasture.

In a further analysis, we tackled bacterial groups with high betweenness centrality values (Supplementary Table 5). According to Khan et al. 2019, these bacterial groups (represented by the nodes) have an essential role in the network as a connector, which represents an important biological and ecological feature. In the primary forest, Methylobacterium, Bradyrhizobium, Candidatus Solibacter, Conexibacter, and Pseudomonas were the top five bacterial genera presenting the highest betweenness centrality values. Also, these bacterial genera presented strong and positive correlations with total P, inorganic, and organic P fractions. Conversely, a significant change in the network properties was noticed after the forest-to-pasture conversion. This time Bradyrhizobium, Acidithiobacillus, Mesorhizobium, Bordetella, and Nocardioides were the bacterial genera that presented the highest and greatest betweenness centrality values. These bacterial genera presented positive correlations with total P and inorganic P fractions. Lastly, the network composition and topology properties changed again after the decline of pasture activities and the natural re-establishment of secondary forest. This time, Granulibacter, Acidobacterium, Gemmatimonas, Rhodomicrobium, and Acidithiobacillus presented the highest values. For this land-use system, the top five bacterial genera presented only a low and positive correlation with total P.

4. Discussion

4.1. Phosphatase activity, total P, and its different fractions in the soil

In general, our results demonstrated that forest-to-pasture conversion alters P-lability and leads to a great reduction in the total amount of P in the soil. According to Solomon et al. (2002), the P transformation process in undisturbed tropical forests is essentially closed with minimal short and middle-term losses and/or gains. On the other hand, the conversion of tropical forest to pasture by the slash-and-burning method alters P-lability and releases a large amount of P (and other nutrients) from the forest biomass, which can be redistributed between living plants and soil microorganisms or lost via runoff and leaching (Hamer et al., 2013; Soltangheisi et al., 2019). Garcia-Montiel et al. (2000) also highlighted that a great reduction of P may occur in pasture soils due to intensive grazing and low use of P and N fertilizers by smallholder farmers in the Amazon region. According to the authors, these practices lead to soil degradation, low productivity levels, and, consequently, the abandonment of these areas. In the meantime, the natural vegetation begins to re-establish itself and form a new forest stand (secondary forest), and some soil properties and/or nutrients (e.g., soil pH, Ca2+, and Mg2+) can be recovered gradually and reach intermediate or even similar levels to those observed in the undisturbed forest sites (Cenciani et al., 2009, Mirsha et al., 2004, Pedrinho et al., 2020). We highlight here that, during the early stages of secondary forest regeneration, there is great competition for nutrients, water, and sunlight among the young forest vegetation – e.g., small trees and shrubs (Rebola et al., 2021). Large amounts of readily available nutrients, especially inorganic C, N, and P pools, are taken up by the dominant species, which grow and establish themselves faster when compared to the others (Kumar et al., 2021). This initial impoverishment of the inorganic P pool may lead to high activity of phosphatase, which is the enzyme that hydrolyzes recalcitrant organic P and make it available to plants and/or microorganisms (Maharjan et al., 2018).

4.2. The structure of the bacterial community involved in the P transformation

Our results demonstrated that the land-use change also impacted the bacterial community structure. Aluminum and total P correlated positively with the bacterial community involved in the P transformation process in the primary forest. According to Sombroek (1984), the

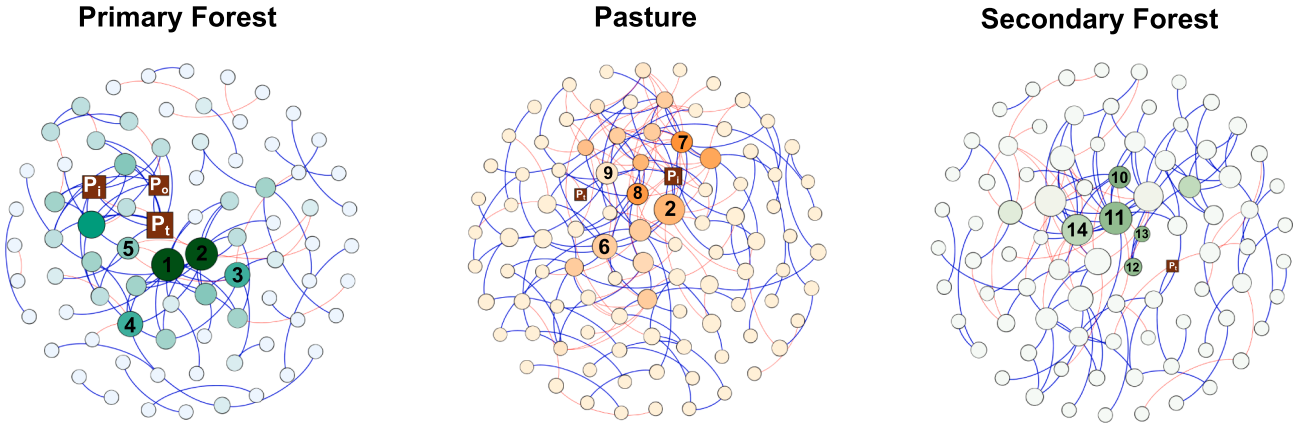

Fig. 6. Network co-occurrence analysis of bacterial communities involved in the phosphorus transformation in soils from primary forest, pasture, and secondary forest. A connection stands for SparCC correlation with magnitude >0.7 (positive correlation–blue edges) or <−0.7 (negative correlation–red edges) and statistically significant ($P \leq 0.01$). Each node represents taxa affiliated at the genus level and the size of the node is proportional to the number of connections (that is, degree). The numbers inside the nodes indicate the genera with more correlations, as follows: (1) Methylobacterium, (2) Bradyrhizobium, (3) Candidatus Solibacter, (4) Conexibacter, (5) Pseudomonas, (6) Acidithiobacillus, (7) Mesorhizobium, (8) Bordetella, (9) Nocardioides, (10) Granulibacter, (11) Acidobacterium, (12) Gemmatimonas, and (13) Rhodomicrobium. Square nodes indicate phosphorus fractions, as follows: Pi = Inorganic Phosphorus; Po = Organic Phosphorus; Pt = Total Phosphorus. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Amazon rainforest soils are naturally rich in Fe and Al oxides due to the strong weathering process typically found in these regions, which have heavy rainfall and high temperatures. These Fe and Al oxides have the geochemical ability to bind P and make it unavailable for the uptake of plants and microorganisms (Gama-Rodrigues et al., 2014). As a consequence, a great number of plants rely on a small group of microorganisms that are capable to mineralize organic P and solubilize inorganic P forms and thus increase P availability in the soil (Shrivastava et al., 2018). In general, these P-solubilizing microorganisms are capable to produce organic acids (e.g., citric acid, malic acid, gluconic acid, etc) and metal chelators that solubilize different P forms in the soil (Mpanga et al., 2019). Furthermore, the great amount of plant litter in the Amazon rainforest soils can support large soil microbial biomass, which is rich in C, N, and P sources (Araújo et al., 2013). According to Khan and Joergensen (2012), the turnover of this soil microbial biomass may contribute to a slow release of inorganic P, that can be accessed and/or used by plants. On the other hand, in the secondary forest, we observed that the bacterial community involved in the P transformation process had a negative correlation with the labile-P. As previously mentioned, the high demand for nutrients (e.g., N and P) by the young forest vegetation during early succession led to a lower amount of labile-P when compared to the primary forest site. Here, we argue that the low availability of labile-P resulted in a more specialized bacterial community, which somehow can use more efficiently organic forms of P and make them available to plants and other microorganisms.

4.3. Taxonomic diversity and composition of soil bacterial community

Our results demonstrated that land-use change can affect the richness and diversity of the soil bacterial community involved in the P transformation process. In general, forest-to-pasture conversion increased both indices. Mendes et al. (2015b) and Pedrinho et al. (2019) observed similar results when studying the impact of land-use change in the Brazilian Amazon region on microbial taxonomic and functional diversities. According to the authors, the stress conditions commonly found in degraded pastures in the Amazon region (e.g., nutrient depletion, great variation of water content, and/or soil temperature) may lead to an increase in richness and taxonomic diversity, which in terms can contribute to the maintenance of soil functioning by the functional redundancy. Furthermore, we observed that land-use change resulted in distinct patterns of relative abundance of bacteria involved in different P transformation processes. The forest-to-pasture conversion led to an increase in the relative abundances of Firmicutes, Cyanobacteria, Gemmatimonadetes, and some other phyla. Previous studies have also observed that the conversion of forest to pasture in the Amazon region increases the abundance of Firmicutes (Mendes et al., 2015b; Rodrigues et al., 2013). According to them, many Firmicutes members are notably resistant to stress conditions, including desiccation, and changes in the soil temperature during the day/night. Firmicute members also participate in different N and P transformation processes. For example, Bacillaceae (one of the Firmicutes' families that present high abundance in the pasture) are saprophyte microorganisms that perform fundamental roles in soil ecology (e.g., organic matter turnover) and plant growth stimulation (e.g., P-solubilization) (Mandic-Mulec et al., 2015). According to Oliveira et al. (2009), Bacillaceae members possess phosphatase and/or phosphate-solubilizing activity. In general, these microorganisms can produce organic acids that are capable to release phosphate from insoluble P sources, and consequently, improve plant nutrition and growth (Oliveira et al., 2009). Cyanobacteria are photosynthetic gram-negative microorganisms, which are well known for having nitrogen-fixation ability (Pedrinho et al., 2020; Singh et al., 2014). Many Cyanobacteria can also perform the solubilization of different P sources (e.g., AlPO4 - aluminum phosphate, FePO4 - ferric orthophosphate, etc) present in soils (Vaishampayan et al., 2001). In general, they can produce extracellular enzymes (e.g., alkaline and acid phosphatases) and organic acids (e.g., lactic, malic, citric, gluconic, and oxalic acid) that enhance mineralization and/or solubilization of P compounds and transformed it into easily available sources to plants and other soil microorganisms (Prasanna et al., 2012). Lastly, Gemmatimonadetes is a cosmopolitan phylum, which inhabits a wide variety of terrestrial environments, including pasture and agricultural soils. Members of this phylum are adapted to dry soil conditions and appear to have the ability to store inorganic P sources that later can be used under low-phosphate availability (DeBruyn et al., 2011; Pascual et al., 2016).

In the secondary forest, the abundance of Actinobacteria members decreased in comparison to primary forest soil. The impacts of land-use change on the abundance of Actinobacteria were previously reported by Zhang et al. (2021). On the other hand, the abundance of Proteobacteria members increased in the secondary forest soil. The shift from pasture to secondary forest vegetation led to a more diverse environment (e.g., plant composition and biomass production) and created suitable conditions (e.g., more diversity of C, N, S, and P sources) for Proteobacteria, which have a well-adapt metabolism and are capable to explore more efficient different niches (Gross et al., 1995; Kazakov et al., 2009). Here, we pay attention to Bradyrhizobiaceae and Beijerinckiaceae (both families belonging to Proteobacteria), which are commonly found in acidic and nutrient-poor forest soils. These microorganisms possess the ability to release organic acids, which help the solubilization of different P sources (Oliverio et al., 2020), and consequently, increase P availability and improve plant nutrition and growth.

4.4. Potential functions of the soil bacterial community

Our results demonstrated that land-use change can affect the potential functions of the soil bacterial community involved in the P transformation process. For all land-use systems, the majority of sequences were assigned to the uptake of extracellular P sources, solubilization, mineralization, and regulation process. A great number of sequences affiliated with the solubilization and uptake of extracellular P sources were observed in the primary forest when compared to the pasture. As previously mentioned, the P transformation process in the primary forest is essentially closed with few losses and/or gains (Solomon et al., 2002), and a large number of plants and microorganisms rely on microbial P turnover and P-solubilizing microorganisms, which are capable to produce organic acids (e.g., malic-, oxalic-, lactic-, and citric acids) that solubilize inorganic P (Mpanga et al., 2019). These microorganisms possess the gcd gene (glucose dehydrogenase) that directly governs the oxidation of glucose to gluconic acid (Liang et al., 2020). Later, this gluconic acid can be used to solubilize inorganically bound fractions of P, and thus, increase P availability in soils (Grafe et al., 2018). In agreement with Grafe et al. (2018), we also noticed that the increase in solubilization was followed by an increase in the P uptake process (e.g., especially pst genes encoding proteins involved in the P uptake) in the primary forest soil. Dai et al. (2019) emphasized that ABC-type phosphorus specific transporter (pst) is a high-affinity transporter that can act in the assimilation process of inorganic P. The soil microorganisms that possess these pst genes are able to use more efficiently P and/or immobilize P in their biomass and, later through microbial P turnover, increase P available to plants (Richardson and Simpson, 2011).

Conversely, pasture and secondary forest soils presented a great number of sequences affiliated with P mineralization in comparison to the primary forest. The P mineralization has a pivotal role in microbial and/or plant nutrition in low-input agrosystems (Oehl et al., 2001). During the mineralization process, some microorganisms can produce enzymes that convert organic P into inorganic forms. According to Dai et al. (2019), these microorganisms usually contain genes encoding for enzymes, such and C—P lyases (phn) and alkaline phosphatase (phoD and phoA), which have a high capacity to release orthophosphate (PO_4^{3-}) from recalcitrant organic P forms in soils. Interestingly, we observed an increase in these genes in both land-use systems. Here, we highlight the importance of this process (and the microorganisms

involved) in degraded land-use systems and suggest that conservation practices and/or restoration measures can increase P levels and improve P availability throughout time.

4.5. Network analysis

Lastly, network analysis revealed that the forest-to-pasture conversion affected the dynamics of interaction in the bacterial community involved in the P transformation process and also altered the compositional and topological properties (Khan et al., 2019; Pedrinho et al., 2020). In general, forest-to-pasture conversion increased network complexity. Mendes et al. (2017) and Pedrinho et al. (2020) highlight that poor management strategy used by smallholder farmers in the Amazon region (e.g., intensive grazing and low use of N and P fertilizers) and stress conditions (e.g., changes in the soil water content and/or temperature during the day/night) usually observed in degraded pasture can increase the taxonomic and functional diversity, which somehow results in a network more complex. In the primary forest, we observed that Methylobacterium presented the highest betweenness centrality value. This suggests that Methylobacterium has great importance in this ecosystem. Previous studies also observed a high abundance of Methylobacterium in forest soils (Zeng et al., 2022). According to Dourado et al. (2015), Methylobacterium is a copiotrophic bacteria capable to promote plant growth (e.g., auxin and cytokinin production) and solubilize organic P, which can be later used by both plants and other microorganisms (Agafonova et al., 2013). Studies also indicated that Methylobacterium can also have a synergistic relationship with some arbuscular mycorrhizal fungi (AMF) (Kim et al., 2010). This interaction between Methylobacterium and AMF could represent an important feature in the primary forest soil. This is because plants can take advantage of the interaction with different soil microorganisms, including some bacteria and AMF, making them more tolerant to abiotic (e.g., salinity, drought, extreme temperatures, etc) and biotic (e.g., plant pathogens) stresses (Lee et al., 2015). Regarding the pasture network, we observed that Bradyrhizobium presented the highest betweenness centrality value. This bacterial group is well-known for its nitrogen fixation ability (Nafis et al., 2019) and studies suggest that Bradyrhizobium has the potential to perform P mineralization (Wei et al., 2019). According to the authors, Bradyrhizobium is a dominant phoD-harboring microorganism in upland soils. They suggest that under P-depleted conditions, Bradyrhizobium can be involved in the mineralization process of soil organic P. This could help us to explain the high number of sequences associated with P mineralization and the high abundance of phoD genes in the pasture. We suggest that after forest-to-pasture conversion, it is very likely that important species were lost. Species such as Methylobacterium could have a key role in soil ecosystem functioning (Khan et al., 2019). Finally, the secondary forest network exhibits transitional composition and topology features between native forest and pasture. In the secondary forest network, Granulibacter was identified as a key bacterial group. This genus is a member of Proteobacteria, which can produce enzymes capable to hydrolyze a variety of phosphomonoesters and release inorganic P (Gaiero et al., 2018).

5. Conclusion

The forest-to-pasture conversion changed the soil's chemical properties, mainly pH, Al3+, total P, and labile P contents. These changes in soil's chemical properties impacted the diversity and structure of the bacterial community involved in the P transformation process. Furthermore, we observed that forest-to-pasture conversion altered the composition of bacterial groups involved in the P transformation and also affected their potential functions. On the other hand, we observed that after the decline of pasture activities and the natural re-establishment of secondary forest, bacterial groups involved in the P transformation and some of their potential functions presented signs of resilience. This suggests that soil biogeochemical processes can be recovered over time. We highlight that we focused our efforts on bacterial groups and their role in different P transformations in soils, even though it is known that fungi, especially AMF, also have a pivotal role in plant nutrition in many tropical and subtropical regions. So, further studies are necessary to understand how land-use change affects this important soil microbial group. Finally, we highlight that this type of study may add relevant information to the development of better restoration practices in the Amazon region, which can provide a fast recovery of the biodiversity of soil microorganisms and their potential functions.

CRediT authorship contribution statement

Alexandre Pedrinho: Conceptualization, Investigation, Data curation, Formal analysis, Writing – original draft, Writing – review & editing. **Lucas William Mendes:** Conceptualization, Methodology, Data curation, Formal analysis, Writing – original draft, Writing – review & editing. **Felipe Martins do Rêgo Barros:** Writing – original draft, Writing – review & editing. **Luis Fernando Merloti:** Writing – original draft, Writing – review & editing. **Mayara Martins e Martins:** Writing – original draft, Writing – review & editing. **Simone Raposo Cotta:** . **Fernando Dini Andreote:** Conceptualization, Writing – review & editing. **Siu Mui Tsai:** Supervision, Conceptualization, Methodology, Writing – review & editing, Funding acquisition.

Declaration of Competing Interest

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

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecolind.2022.109779.

References

Agafonova, N.V., Kaparullina, E.N., Doronina, N.V., Trotsenko, Y.A., 2013. Phosphate-solubilizing activity of aerobic Methylobacteria. Microbiology 82, 864–867. https://doi.org/10.1134/S0026261714010020.

Alori, E.T., Glick, B.R., Babalola, O.O., 2017. Microbial phosphorus solubilization and its potential for use in sustainable agriculture. Front. Microbiol. 8, 971. https://doi.org/10.3389/fmicb.2017.00971.

Anderson, M.J., 2001. A new method for non-parametric multivariate analysis of variance. Austral Ecol. 26, 32–46. https://doi.org/10.1111/j.1442-9993.2001.01070. pp. x.

Araújo, A.S.F., Cesarz, S., Leite, L.F.C., Borges, C.D., Tsai, S.M., Eisenhauer, N., 2013. Soil microbial properties and temporal stability in degraded and restored lands of Northeast Brazil. Soil Biol. Biochem. 66, 175–181. https://doi.org/10.1016/j.soilbio.2013.07.013.

Arenberg, M.R., Arai, Y., 2019. Uncertainties in soil physicochemical factors controlling phosphorus mineralization and immobilization processes, first ed. Elsevier, London. 10.1016/bs.agron.2018.11.005.

Bastian, M., Heymann, S., Jacomy, M., 2009. Gephi: An open source software for exploring and manipulating networks. Proceedings of the Third International ICWSM Conference, AAAI Publications.

Benjamini, Y., Hochberg, Y., 1995. Controlling the false discovery rate: a practical and powerful approach to multiple testing. J. R. Stat. Soc. 57, 289–300. https://www.jstor.org/stable/2346101.

Bergkemper, F., Schöler, A., Engel, M., Lang, F., Krüger, J., Schloter, M., Schulz, S., 2016. Phosphorus depletion in forest soils shapes bacterial communities towards phosphorus recycling systems. Environ. Microbiol. 18, 1988–2000. https://doi.org/10.1111/1462-2920.13442.

Bi, Q.F., Li, K.J., Zheng, B.X., Liu, X.P., Li, H.Z., Jin, B.J., Ding, K., Yang, X.R., Lin, X.Y., Zhu, Y.G., 2019. Partial replacement inorganic phosphorus (P) by organic manure reshapes phosphate mobilizing bacterial community and promotes P bioavailability in a paddy soil. Sci. Total Environ. 03, 134977 https://doi.org/10.1016/j.scitotenv.2019.134977.

Bolger, A.M., Lohse, M., Usadel, B., 2014. Trimmomatic: A flexible trimmer for Illumina sequence data. Bioinformatics 30, 2114–2120. https://doi.org/10.1093/bioinformatics/btu170.

Carvalho, R., Adami, M., Amaral, S., Bezerra, F.G., de Aguiar, A.P.D., 2019. Changes in secondary vegetation dynamics in a context of decreasing deforestation rates in Pará, Brazilian Amazon. Appl. Geogr. 106, 40–49. https://doi.org/10.1016/j.apgeog.2019.03.001.

Cenciani, K., Lambais, M.R., Cerri, C.C., de Azevedo, L.C.B., Feigl, B.J., 2009. Bacteria diversity and microbial biomass in forest, pasture and fallow soils in the southwestern amazon basin. Rev. Bras. Cienc. Solo 33, 907–916. https://doi.org/10.1590/S0100-06832009000400015.

Chavarro-Bermeo, J.P., Arruda, B., Mora-Motta, D.A., Bejarano-Herrera, W., Ortiz-Morea, F.A., Somenahally, A., Silva-Olaya, A.M., 2022. Responses of soil phosphorus fractions to land-use change in Colombian Amazon. Sustainability 14, 2285. https://doi.org/10.3390/su14042285.

Condron, L.M., Goh, K.M., Newman, R.H., 1985. Nature and distribution of soil phosphorus as revealed by a sequential extraction method followed by 31P nuclear magnetic resonance analysis. J. Soil Sci. 36, 199–207. https://doi.org/10.1111/j.1365-2389.1985.tb00324.x.

Dai, Z., Liu, G., Chen, H., Chen, C., Wang, J., Ai, S., Wei, D., Li, D., Ma, B., Tang, C., Brookes, P.C., Xu, J., 2019. Long-term nutrient inputs shift soil microbial functional profiles of phosphorus cycling in diverse agroecosystems. ISME J. 14, 757–770. https://doi.org/10.1038/s41396-019-0567-9.

Damian, J.M., Firmano, R.F., Cherubin, M.R., Pavinato, P.S., Marchi-Soares, T., Paustian, K., Cerri, C.E.P., 2019. Changes in soil phosphorus pool induced by pastureland intensification and diversification in Brazil. Sci. Total Environ. 703, 135463 https://doi.org/10.1016/j.scitotenv.2019.135463.

DeBruyn, J.M., Nixon, L.T., Fawaz, M.N., Johnson, A.M., Radosevich, M., 2011. Global biogeography and quantitative seasonal dynamics of gemmatimonadetes in soil. Appl. Environ. Microbiol. 77, 6295–6300. https://doi.org/10.1128/AEM.05005-11.

Dick, W.A., Tabatabai, M.A., 1977. Determination of orthophosphate in aqueous solutions containing labile organic and inorganic phosphorus compounds. J. Environ. Qual. 6, 82–85. https://doi.org/10.2134/jeq1977.00472425000600010018x.

Domingues, T.F., Martinelli, L.A., Ehleringer, J.R., 2007. Ecophysiological traits of plant functional groups in forest and pasture ecosystems from eastern Amazônia, Brazil. Plant Ecol 193, 101–111. https://doi.org/10.1007/s11258-006-9251-z.

Dourado, M.N., Neves, A.P.C., Santos, D.S., Araújo, W.L., 2015. Biotechnological and agronomic potential of endophytic pink-pigmented methylotrophic Methylobacterium spp. BioMed Res. Int. 2015, 909016 https://doi.org/10.1155/2015/909016.

Embrapa Brazilian Agricultural Research Corporation, 1997. Methods of Soil Analysis. Center National for Soil Research, Rio de Janeiro. Friedman, J., Alm, E.J., 2012. Inferring correlation etworks from genomic survey data. PLoS Comput. Biol. 8, e1002687. https://doi.org/10.1371/journal.pcbi.1002687.

Fu, D., Wu, X., Duan, C., Chadwick, D.R., Jones, D.L., 2020. Response of soil phosphorus fractions and fluxes to different vegetation restoration types in a subtropical mountain ecosystem. Catena 193, 104663. https://doi.org/10.1016/j.catena.2020.104663.

Gaiero, J.R., Bent, E., Fraser, T.D., Condron, L.M., Dunfield, K.E., 2018. Validating novel oligonucleotide primers targeting three classes of bacterial non-specific acid phosphatase genes in grassland soils. Plant Soil 427, 39–51. https://doi.org/10.1007/s11104-017-3338-2.

Gama-Rodrigues, A.C., Sales, M.V.S., Silva, P.S.D., Comerford, N.B., Cropper, W.P., Gama-Rodrigues, E.F., 2014. An exploratory analysis of phosphorus transformations in tropical soils using structural equation modeling. Biogeochemistry 118, 453–469. https://doi.org/10.1007/s10533-013-9946-x.

Garcia-Montiel, D.C., Neill, C., Melillo, J., Thomas, S., Steudler, P.A., Cerri, C.C., 2000. Soil phosphorus transformations following forest clearing for pasture in the Brazilian Amazon. Soil Sci. Soc. Am. J. 64, 1792–1804. https://doi.org/10.2136/sssaj2000.6451792x.

Grafe, M., Goers, M., von Tucher, S., Baum, C., Zimmer, D., Leinweber, P., Vestergaard, G., Kublik, S., Schloter, M., Schulz, S., 2018. Bacterial potentials for uptake, solubilization and mineralization of extracellular phosphorus in agricultural soils are highly stable under different fertilization regimes. Environ. Microbiol. Rep. 10, 320–327. https://doi.org/10.1111/1758-2229.12651.

Gross, K.L., Pregitzer, K.S., Burton, A.J., 1995. Spatial variation in nitrogen availability in three successional plant communities. J. Ecol. 83, 357–367. https://doi.org/10.2307/2261590.

Gupta, A., Gupta, R., Singh, R.L., 2017. Principles and applications of enviromental biotechnology for sustainable future, first ed. Springer, Singapore. 10.1007/978-981-10-1866-4.

Hamer, U., Potthast, K., Burneo, J.I., Makeschin, F., 2013. Nutrient stocks and phosphorus fractions in mountain soils of Southern Ecuador after conversion of forest to pasture. Biogeochemistry 112, 495–510. https://doi.org/10.1007/s10533-012-9742-z.

Hammer, Ø., Harper, D., Ryan, P.D., 2001. PAST: Paleontological statistics software: package for education and data analysis. Palaeontol. Electron. 4, 1–9.

Hedley, M.J., Stewart, J.W.B., Chauhan, B.S., 1982. Changes in inorganic and organic soil phosphorus fractions induced by cultivation practices and by laboratory incubations. Soil Sci. Soc. Am. J. 46, 970–976. https://doi.org/10.2136/sssaj1982.03615995004600050017x.

INPE, 2021. National Institute for Space Research. http://www.inpe.br/.

Johnson, A.H., Frizano, J., Vann, D.R., 2003. Biogeochemical implications of labile phosphorus in forest soils determined by the Hedley fractionation procedure. Oecologia 135, 487–499. https://doi.org/10.1007/s00442-002-1164-5.

Kanehisa, M., Furumichi, M., Tanabe, M., Sato, Y., Morishima, K., 2017. KEGG: new perspectives on genomes, pathways, diseases and drugs. Nucleic Acids Res. 45, D353–D361. https://doi.org/10.1093/nar/gkw1092.

Kazakov, A.E., Rodionov, D.A., Alm, E., Arkin, A.P., Dubchak, I., Gelfand, M.S., 2009. Comparative genomics of regulation of fatty acid and branched-chain amino acid utilization in Proteobacteria. J. Bacteriol. 191, 52–64. https://doi.org/10.1128/JB.01175-08.

Khan, M.S., Ahmad, E., Zaidi, A., Oves, M., 2013. Functional aspect of phosphate solubilizing bacteria: importance in crop production. In: Maheshwari, D.K. (Ed.), Bacteria in Agrobiology: Crop Productivity. Springer, Berlin. https://doi.org/10.1007/978-3-642-37241-4_10.

Khan, M.A.W., Bohannan, B.J.M., Nüsslein, K., Tiedje, J.M., Tringe, S.G., Parlade, E., Barberán, A., Rodrigues, J.L.M., 2019. Deforestation impacts network co-occurrence patterns of microbial communities in Amazon soils. FEMS Microbiol. Ecol. 95, fiy230. https://doi.org/10.1093/femsec/fiy230.

Khan, K.S., Joergensen, R.G., 2012. Relationships between P fractions and the microbial biomass in soils under different land use management. Geoderma 173–174, 274–281. https://doi.org/10.1016/j.geoderma.2011.12.022.

Khan, M.S., Zaidi, A., Wani, P.A., 2007. Role of phosphate-solubilizing microorganisms in sustainable agriculture - a review. Agron. Sustain. Dev. 27, 29–43. https://doi.org/10.1051/agro:2006011.

Kim, K., Yim, W., Trivedi, P., Madhaiyan, M., Boruah, H.P.D., Islam, R., Lee, G., Sa, T., 2010. Synergistic effects of inoculating arbuscular mycorrhizal fungi and Methylobacterium oryzae strains on growth and nutrient uptake of red pepper (Capsicum annuum L.). Plant Soil 327, 429–440. https://doi.org/10.1007/s11104-009-0072-4.

Kumar, A., Kumar, M., Pandey, R., ZhiGuo, Y., Cabral-Pinto, M., 2021. Forest soil nutrient stocks along altitudinal range of Uttarakhand Himalayas: An aid to Nature Based Climate Solutions. Catena 207, 105667. https://doi.org/10.1016/j.catena.2021.105667.

Lee, Y., Krishnamoorthy, R., Selvakumar, G., Kim, K., Sa, T., 2015. Alleviation of salt stress in maize plant by co-inoculation of arbuscular mycorrhizal fungi and Methylobacterium oryzae CBMB20. J. Korean Soc. Appl. Biol. Chem. 58, 533–540. https://doi.org/10.1007/s13765-015-0072-4.

Liang, J.L., Liu, J., Jia, P., Yang, T., Zeng, Q., Zhang, S., Liao, B., Shu, W., Li, J., 2020. Novel phosphate-solubilizing bacteria enhance soil phosphorus cycling following ecological restoration of land degraded by mining. ISME J. 14, 1600–1613. https://doi.org/10.1038/s41396-020-0632-4.

Maharjan, M., Maranguit, D., Kuzyakov, Y., 2018. Phosphorus fractions in subtropical soils depending on land use. Eur. J. Soil Biol. 87, 17–24. https://doi.org/10.1016/j.ejsobi.2018.04.002.

Mandic-Mulec, I., Stefanic, P., van Elsas, J.D., 2015. Ecology of bacillaceae. Microbiol. Spectrum 3, 2. https://doi.org/10.1128/microbiolspec.TBS-0017-2013.

Melo, V.F., Orrutéa, A.G., Motta, A.C.V., Testoni, S.A., 2017. Land use and changes in soil morphology and physical-chemical properties in Southern Amazon. Rev. Bras. Cienc. Solo 41, 1–14. https://doi.org/10.1590/18069657rbcs20170034.

Mendes, L.W., Brossi, M.J.L., Kuramae, E.E., Tsai, S.M., 2015a. Land-use system shapes soil bacterial communities in Southeastern Amazon region. Appl. Soil Ecol. 95, 151–160. https://doi.org/10.1016/j.apsoil.2015.06.005.

Mendes, L.W., Tsai, S.M., Navarrete, A.A., de Hollander, M., van Veen, J.A., Kuramae, E.E., 2015b. Soil-borne microbiome: linking diversity to function. Microbi. Ecol. 70, 255–265. https://doi.org/10.1007/s00248-014-0559-2.

Mendes, L.W., Braga, L., Navarrete, A.A., de Souza, D.G., Silva, G.G.Z., Tsai, S.M., 2017. Using metagenomics to connect microbial community biodiversity and functions. Curr. Issues Mol. Biol. 24, 103–118. https://doi.org/10.21775/cimb.024.103.

Meyer, F., Paarmann, D., D'Souza, M., Olson, R., Glass, E.M., Kubal, M., Paczian, T., Rodriguez, A., Stevens, R., Wilke, A., Wilkening, J., Edwards, R.A., 2008. The metagenomics RAST server–a public resource for the automatic phylogenetic and functional analysis of metagenomes. BMC Bioinf. 9, 1–8. https://doi.org/10.1186/1471-2105-9-386.

Mirsha, A., Sharma, S.D., Pandey, R., 2004. Amelioration of degraded sodic soil by afforestation. Arid. Land Res. Manag 18, 13–23. https://doi.org/10.1080/15324980490244960.

Mpanga, I.K., Nkebiwe, P.M., Kuhlmann, M., Cozzolino, V., Piccolo, A., Geistlinger, J., Berger, N., Ludewig, U., Neumann, G., 2019. The form of N supply determines plant

growth promotion by P-solubilizing microorganisms in maize. Microorganisms 7, 38. https://doi.org/10.3390/microorganisms7020038.
Murphy, J., Riley, J.P., 1962. A modified single solution method for the determination of phosphate in natural waters. Anal. Chim. Acta 27, 31–36. https://doi.org/10.1016/S0003-2670(00)88444-5.
Nafis, A., Raklami, A., Bechtaoui, N., El Khalloufi, F., El Alaoui, A., Glick, B.R., Hafidi, M., Kouisni, L., Ouhdouch, Y., Hassani, L., 2019. Actinobacteria from extreme niches in Morocco and their plant growth- promoting potentials. Diversity 11, 139. https://doi.org/10.3390/d11080139.
Navarrete, A.A., Tsai, S.M., Mendes, L.W., Faust, K., de Hollander, M., Cassman, N.A., Raes, J., van Veen, J.A., Kuramae, E.E., 2015. Soil microbiome responses to the short-term effects of Amazonian deforestation. Mol. Ecol. 24, 2433–2448. https://doi.org/10.1111/mec.13172.
Oehl, F., Oberson, A., Sinaj, S., Frossard, E., 2001. Organic phosphorus mineralization studies using isotopic dilution techniques. Soil Sci. Soc. Am. J. 65, 780–787. https://doi.org/10.2136/sssaj2001.653780x.
O'Leary, N.A., Wright, M.W., Brister, J.R., Ciufo, S., Haddad, D., McVeigh, R., Rajput, B., Robbertse, B., Smith-White, B., Ako-Adjei, D., Astashyn, A., Badretdin, A., Bao, Y., Blinkova, O., Brover, V., Chetvernin, V., Choi, J., Cox, E., Ermolaeva, O., Farrell, C. M., Goldfarb, T., Gupta, T., Haft, D., Hatcher, E., Hlavina, W., Joardar, V.S., Kodali, V.K., Li, W., Maglott, D., Masterson, P., McGarvey, K.M., Murphy, M.R., O'Neill, K., Pujar, S., Rangwala, S.H., Rausch, D., Riddick, L.D., Schoch, C., Shkeda, A., Storz, S.S., Sun, H., Thibaud-Nissen, F., Tolstoy, I., Tully, R.E., Vatsan, A. R., Wallin, C., Webb, D., Wu, W., Landrum, M.J., Kimchi, A., Tatusova, T., DiCuccio, M., Kitts, P., Murphy, T.D., Pruitt, K.D., 2016. Reference sequence (RefSeq) database at NCBI: current status, taxonomic expansion, and functional annotation. Nucleic Acids Res. 44, 733–745. https://doi.org/10.1093/nar/gkv1189.
Oliveira, C.A., Alves, V.M.C., Marriel, I.E., Gomes, E.A., Scotti, M.R., Carneiro, N.P., Guimarães, C.T., Schaffert, R.E., Sá, N.M.H., 2009. Phosphate solubilizing microorganisms isolated from rhizosphere of maize cultivated in an oxisol of the Brazilian Cerrado Biome. Soil Biol. Biochem. 41, 1782–1787. https://doi.org/10.1016/j.soilbio.2008.01.012.
Oliverio, A.M., Bissett, A., McGuire, K., Saltonstall, K., Turner, B.L., Fierer, N., 2020. The role of phosphorus limitation in shaping soil bacterial communities and their metabolic capabilities. MBio 11, e01718–e2020. https://doi.org/10.1128/mBio.01718-20.
Parks, D.H., Beiko, R.G., 2010. Identifying biologically relevant differences between metagenomic communities. Bioinformatics 26, 715–721. https://doi.org/10.1093/bioinformatics/btq041.
Pascual, J., García-López, M., Bills, G.F., Genilloud, O., 2016. Longimicrobium terrae gen. nov., sp. nov., an oligotrophic bacterium of the under-represented phylum Gemmatimonadetes isolated through a system of miniaturized diffusion chambers. Int. J. Syst. Evol. Microbiol. 66, 1976–1985. https://doi.org/10.1099/ijsem.0.000974.
Paul, E.A., 2014. Soil Microbiology, Ecology and Biochemistry, fourth ed. Academic Press, London.
Pedrinho, A., Mendes, L.W., Merloti, L.F., Fonseca, M.C., Cannavan, F.S., Tsai, S.M., 2019. Forest-to-pasture conversion and recovery based on assessment of microbial communities in Eastern Amazon rainforest. FEMS Microbiol. Ecol. 95, fiy236. https://doi.org/10.1093/femsec/fiy236.
Pedrinho, A., Mendes, L.W., Merloti, L.F., Andreote, F.D., Tsai, S.M., 2020. The natural recovery of soil microbial community and nitrogen functions after pasture abandonment in the Amazon region. FEMS Microbiol. Ecol. 96, fiaa149. https://doi.org/10.1093/femsec/fiaa149.
Porder, S., Vitousek, P.M., Chadwick, O.A., Chamberlain, C.P., Hilley, G.E., 2007. Uplift, erosion, and phosphorus limitation in terrestrial ecosystems. Ecosystems 10, 159–171. https://doi.org/10.1007/s10021-006-9011-x.
Prasanna, R., Joshi, M., Rana, A., Shivay, Y.S., Nain, L., 2012. Influence of co-inoculation of bacteria-cyanobacteria on crop yield and C-N sequestration in soil under rice crop. World J. Microbiol. Biotechnol. 28, 1223–1235. https://doi.org/10.1007/s11274-011-0926-9.
Rebola, L.C., Paz, C.P., Gamarra, L.V., Burslem, D.F.R.P., 2021. Land use intensity determines soil properties and biomass recovery after abandonment of agricultural land in an Amazonian biodiversity hotspot. Sci. Total Environ. 801, 149487 https://doi.org/10.1016/j.scitotenv.2021.149487.
Richardson, A.E., Simpson, R.J., 2011. Soil microorganisms mediating phosphorus availability update on microbial phosphorus. Plant Physiol. 156, 989–996. https://doi.org/10.1104/pp.111.175448.
Rodríguez, H., Fraga, R., Gonzalez, T., Bashan, Y., 2006. Genetics of phosphate solubilization and its potential applications for improving plant growth-promoting bacteria. Plant Soil 287, 15–21. https://doi.org/10.1007/978-1-4020-5765-6_2.
Shrivastava, M., Srivastava, P.C., D'Souza, S.F., 2018. Phosphate-Solubilizing Microbes: Diversity and Phosphates Solubilization Mechanism. in: Meena V. (Eds.) Role of Rhizospheric Microbes in Soil. Springer, Singapore, pp. 137-165. 10.1007/978-981-13-0044-8_5.
Silva Junior, C.H.L., Pessôa, A.C.M., Carvalho, N.S., Reis, J.B.C., Anderson, L.O., Aragão, L.E.O.C., 2020. The Brazilian Amazon deforestation rate in 2020 is the greatest of the decade. Nat. Ecol. Evol. 5, 144–145. https://doi.org/10.1038/s41559-020-01368-x.
Singh, H., Khattar, J.I.S., Ahluwalia, A.S., 2014. Cyanobacteria and agricultural crops. Int. J. Plant Res. 27, 37. https://doi.org/10.5958/j.2229-4473.27.1.008.
Soil Survey Staff, 2014. Keys to Soil Taxonomy. Natural Resources Conservation Service, Washington. USDA —.
Solomon, D., Lehman, J., Mamo, T., Fritzsche, F., Zech, W., 2002. Phosphorus forms and dynamics as influenced by land use changes in the sub-humid Ethiopian highlands. Geoderma 105, 21–48. https://doi.org/10.1016/S0016-7061(01)00090-8.
Soltangheisi, A., Moraes, M.T., Cherubin, M.R., Alvarez, D.O., Souza, L.F., Bieluczyk, W., Navroski, D., Teles, A.P.B., Pavinato, P.S., Martinelli, L.A., Tsai, S.M., Camargo, P.B., 2019. Forest conversion to pasture affects soil phosphorus dynamics and T nutritional status in Brazilian Amazon. Soil Till. Res. 194, 104330 https://doi.org/10.1016/j.still.2019.104330.
Sombroek, W.G., 1984. Soils of the Amazon region. In: Sioli, H. (Ed.), The amazon, Limnology and Landscape Ecology of a Mighty Tropical River and Its Basin. Dr. W. Junk Publishers, Boston, pp. 521–535.
Souza Braz, A.M., Fernandes, A.R., Alleoni, L.R.F., 2011. Soil attributes after the conversion from forest to pasture in Amazon. Land Degrad. Dev. 24, 33–38. https://doi.org/10.1002/ldr.1100.
Tabatabai, M.A., Bremner, J.M., 1969. Use of p-nitrophenyl phosphate for assay of soil phosphatase activity. Soil Biol. Biochem. 1, 301–307. https://doi.org/10.1016/0038-0717(69)90012-1.
Teixeira, H.M., Cardoso, I.M., Bianchi, F.J.J.A., Silva, A.C., Jamme, D., Peña-Claros, M., 2020. Linking vegetation and soil functions during secondary forest succession in the Atlantic forest. Forest Ecol. Manag. 457, 117696 https://doi.org/10.1016/j.foreco.2019.117696.
Vaishampayan, A., Sinha, R.P., Hader, D.P., Dey, T., Gupta, A.K., Bhan, U., Rao, A.L., 2001. Cyanobacterial biofertilizers in rice agriculture. Bot. Rev. The 67, 453–516. https://doi.org/10.1007/BF02857893.
Vale, P., Gibbs, H., Vale, R., Christie, M., Florence, E., Munger, J., Sabaini, D., 2019. The expansion of intensive beef farming to the Brazilian Amazon. Glob. Environ. Change 57, 101922. https://doi.org/10.1016/j.gloenvcha.2019.05.006.
Wei, X., Hu, Y., Razavi, B.S., Zhou, J., Shen, J., Nannipieri, P., Wu, J., Ge, T., 2019. Rare taxa of alkaline phosphomonoesterase-harboring microorganisms mediate soil phosphorus mineralization. Soil Biol. Biochem. 131, 62–70. https://doi.org/10.1016/j.soilbio.2018.12.025.
Welch, B.L., 1947. The generalization of student's' problem when several different population variances are involved. Biometrika 34, 28–35. https://doi.org/10.2307/2332510.
Zeng, Q., Mei, T., Delgado-Baquerizo, M., Wang, M., Tan, W., 2022. Suppressed phosphorus-mineralizing bacteria after three decades of fertilization. Agric. Ecosyst. Environ. 323, 107679 https://doi.org/10.1016/j.agee.2021.107679.
Zhang, Y., Finn, D., Bhattacharyya, R., Dennis, P.G., Doolette, A.L., Smernik, R.J., Dalal, R.C., Meyer, G., Lombi, E., Klysubun, W., Jones, A.R., Wang, P., Menzies, N. W., Kopittke, P.M., 2021. Long-term changes in land use influence phosphorus concentrations, speciation, and cycling within subtropical soils. Geoderma 393, 115010. https://doi.org/10.1016/j.geoderma.2021.115010.
Zhang, J., Kobert, K., Flouri, T., Stamatakis, A., 2014. PEAR: a fast and accurate Illumina Paired-End reAd mergeR. Bioinformatics 30, 614–620. https://doi.org/10.1093/bioinformatics/btt593.

Further reading

R Development Core Team, 2016. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna http://www.R-project.org.