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From deforestation to regeneration: How do land-use changes shape soil microbes and methane-cycling genes in the Eastern Amazon?

Jéssica A. Mandro [a], Júlia B. Gontijo [a,b], Fernanda M. Nakamura [a], Clovis D. Borges [a], Raimundo Cosme de Oliveira Jr [c], Erika Berenguer [d], Brendan J.M. Bohannan [e], Klaus Nüsslein [f], Jorge L. Mazza Rodrigues [b,g], Siu M. Tsai [a], Andressa M. Venturini [a,h,i,j,*]

[a] Cell and Molecular Biology Laboratory, Center for Nuclear Energy in Agriculture, University of São Paulo, Piracicaba, SP, Brazil
[b] Department of Land, Air and Water Resources, University of California, Davis, CA, USA
[c] EMBRAPA Amazônia Oriental, Belém, PA, Brazil
[d] Environmental Change Institute, University of Oxford, Oxford, UK
[e] Department of Biology, Institute of Ecology and Evolution, University of Oregon, Eugene, OR, USA
[f] Department of Microbiology, University of Massachusetts, Amherst, MA, USA
[g] Environmental Genomics and Systems Biology Division, Lawrence Berkeley National Laboratory, Berkeley, CA, USA
[h] Princeton Institute for International and Regional Studies, Princeton University, Princeton, NJ, USA
[i] Department of Biology, Stanford University, Stanford, CA, USA
[j] Department of Environmental Science, American University, Washington, DC, USA

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ABSTRACT

Land-use change in the Amazon Rainforest impacts soil properties and belowground microbial communities, with far-reaching implications for soil ecosystem services, including methane cycling processes. However, it is unclear whether the known methane sink-to-source shift observed after forest-to-pasture conversion occurs consistently throughout the year, or whether forest regeneration can help restore this crucial ecosystem process. Here, we assessed the impacts of forest-to-pasture conversion and forest regeneration in the Amazon Rainforest on its soil properties and microbial communities, focusing on methane-related microbiota, using 16S rRNA sequencing and quantitative real-time PCR. Conversion resulted in significant changes in soil chemistry and microbial communities, while seasonality and its interaction with land use intensified these differences. Land-use change also increased the abundance of methanotrophs and methanogens, but the ratio between both groups was altered, consistent with pastures as potential sources of methane and forests as sinks. Seasonality further increased the impact of the conversion on methane-cycling microorganisms. Our findings also highlight the potential of passive forest regeneration to restore certain soil chemical and microbiological patterns similar to those of primary forests, including methane-related genes. Although these results provide strategies to support methane mitigation, they indicate that the functional and taxonomic potential of microbial communities may not be equally recovered. This highlights the importance of maintaining primary forests to preserve critical ecosystem services.

1. Introduction

The Amazon rainforest is the largest reservoir of biodiversity in the world (Dirzo and Raven, 2003), and more than half of its extension is located in Brazil (Strand et al., 2018). However, until 2018, approximately 14% of the Amazon had been deforested, corresponding to 870,000 km^2 (Berenguer et al., 2021), a process mainly driven by the expansion of pastures and croplands (Marengo et al., 2018; Nobre et al., 2016). In the Brazilian Amazon, c. 15 Mha of deforested land are now covered by secondary forests (Silva Junior, 2020). However, most of these forests do not survive more than four years, as they suffer the same fate as primary forests: deforestation (Wang et al., 2020). Between 1987

Forest regeneration may support the recovery of microbial functions, as indicated by a higher abundance of the soluble methane monooxygenase gene (*mmoX*) in secondary forests.

* Corresponding author at: Department of Environmental Science, American University, Washington, DC, USA.
E-mail address: aventurini@american.edu (A.M. Venturini).

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and 2017, for example, approximately 20 Mha of secondary forests of the region were cleared, representing 45.5% of the deforestation of primary forest areas detected in the same period (42.9 Mha) (Nunes et al., 2020). In fact, between 2012 and 2014, the loss of secondary forests exceeded that of primary forests in almost all states of the biome, which places the loss of secondary vegetation as a crucial factor to be considered in Amazonian studies.

Deforestation negatively impacts a series of ecosystem services that forests provide, such as climate and hydrological regulation (Strand et al., 2018), C storage, and habitat provision (Borma et al., 2022; Foley et al., 2007). Many of these vital functions derive from activities performed by soil microorganisms (Coban et al., 2022), and they respond quickly to environmental variations, constituting an important tool for understanding ecological feedback after deforestation. Microbes associated with methane (CH_4), a potent greenhouse gas (GHG), with a warming potential 27 times higher than that of carbon dioxide (CO_2) over a 100-year time horizon (IPCC, 2022), are also impacted by land-use change. Microbiological studies have found a common pattern for CH_4 fluxes in the region: while consumption is favored in primary forests, production is commonly observed in pastures (Paula et al., 2014; Meyer et al., 2017; Meyer et al., 2020; Kroeger et al., 2018; Kroeger et al., 2020; Fonseca de Souza et al., 2022; Venturini et al., 2022; Obregón Alvarez et al., 2023; Venturini et al., 2023).

The biological production of CH_4 is mainly carried out by certain archaeal groups during the final stage of the anaerobic degradation of organic matter through a process known as methanogenesis (Nazaries et al., 2013). Methanogenic archaea, hereafter referred to as methanogens, are anaerobic microorganisms that can use different substrates (and pathways), including CO_2, carbon monoxide (CO), methanol (CH_3OH), methylamines (CH_3NH_2 or CH_5N), and acetate ($C_2H3O_2^-$) (Buan, 2018). Regardless of the metabolic pathway, CH_4 production is mediated by the methyl-coenzyme M reductase (MCR) complex, which catalyzes the last reduction step to produce CH_4 (Berghuis et al., 2019). Since the MCR complex is highly conserved, the *mcrA* gene, which encodes the α subunit of this enzyme, can be used as a functional marker of the process (Kietäväine and Purkamo, 2015). The production of CH_4 through methanogenesis represents the largest biogenic source of this gas on Earth (Vanwonterghem et al., 2016; Thauer et al., 2008), and about 40% of the CH_4 produced by microorganisms is released into the atmosphere, while the remainder is consumed by methanotrophs, microorganisms that perform the aerobic and anaerobic oxidation of CH_4 (Conrad, 2009; Lyu et al., 2018).

Methanotrophs are microorganisms capable of oxidizing CH_4 as their primary source of carbon and energy (Hanson and Hanson, 1996; Bowman, 2006) and are, therefore, key biological regulators of atmospheric CH_4, contributing to the mitigation of greenhouse gas emissions (Kalyuzhnaya et al., 2019). Aerobic methanotrophs are predominantly bacteria, whereas anaerobic methane-oxidizing archaea (ANME) are also known to perform CH_4 oxidation in anoxic environments (Guerrero-Cruz et al., 2021; Ouboter et al., 2024). In aerobic methanotrophs, the ability of methanotrophs to metabolize CH_4 is due to the presence of the methane monooxygenase enzyme (MMO), the first in the methanotrophic pathway, and responsible for the oxidation of CH_4 to methanol (Tinberg and Lippard, 2011). This enzyme can be found in two structurally and biochemically distinct forms: a cytoplasmic or soluble form (sMMO) and a membrane-bound or particulate form (pMMO) (Hanson and Hanson, 1996; Auman and Lidstrom, 2002; Liebner and Svenning, 2013; Khmelenina et al., 2018). Almost all methanotrophs have pMMO, while some also possess sMMO (Liebner and Svenning, 2013) or both (Knief, 2015). The conserved genes *pmoA* and *mmoX* encode the β and α subunits of the enzymes pMMO and sMMO, respectively, and are used as functional markers of aerobic CH_4 oxidation (McDonald et al., 2008; Ali et al., 2006).

While it is clear that soil CH_4 microbial communities and fluxes are altered by the forest-to-pasture conversion in the Amazon, we are still trying to understand if forest regeneration can help recover this biogeochemical cycle in already deforested areas. Forest recovery can play an important role in mitigating climatic changes, improving ecosystem services (Nunes et al., 2020) and recuperating soil microbial processes (Pedrinho et al., 2019, Pedrinho et al., 2020). For the Amazon, the CH_4 consumption in secondary forests suggests the recovery of methanotrophy with forest re-establishment (Meyer et al., 2020). However, considering the variability of secondary forests in the region and how strongly CH_4 microbes are influenced by the water regime (Venturini et al., 2022), it is still unclear if these responses are consistent across seasons and if this recovery can represent a typical pattern across the basin.

Here, we evaluated the impacts of land-use change and seasonality on soil microbial communities of the Eastern Amazon, focusing on the organisms related to CH_4 production and consumption. To this end, we characterized the abundance, structure, composition, and functional potential of these communities – through large-scale amplicon sequencing and quantitative PCR of key marker genes – in forest, pasture, and secondary forest sites. We hypothesize that forest-to-pasture conversion in the Amazon alters methane-cycling microbial communities, increasing their potential for CH_4 emission due to shifts in the abundance of methanogens and methanotrophs. We also hypothesize that 15 years of natural forest regeneration partially restores these communities and their functions and that seasonality modulates these patterns.

2. Material and methods

2.1. Soil description and sampling

Soil samples were collected in Amazonian forests and pastures located in the municipality of Belterra, State of Pará, Eastern Amazon, Brazil. The climate of the region is classified as tropical humid (Am) according to the Köppen–Geiger classification, with a mean annual temperature of approximately 27 °C and little seasonal variation. The rainfall regime is monsoonal, with an annual total of approximately 2,200 to 2,400 mm and a short dry season occurring between August and November (Alvares et al., 2013). The soil in the studied areas was classified as Oxisol (Soil Survey Staff, 2022). Soil samples were collected from different land-use types at the beginning of the wet (July) and dry (November) seasons of 2015. The sampling was carried out in two locations for each land use: well-preserved primary forests, without signs of human disturbance (PF1, 2°51′18.4″ S 54°57′27.2″ W; PF2, 3°17′47.2″ S 54°57′48.4″ W); pastures planted with *Urochloa* spp. (PA1, 3°18′49.9″ S 54°54′35.1″ W; PA2, 3°07′52.9″ S 54°57′28.1″ W, ages between 10 and 20 years, respectively); and secondary forests (SF1, 3°15′28.0″ S 54°53′19.2″ W; SF2, 3°15′47.9″ S 54°53′36.0″ W), with more than 15 years of abandonment. At each site, after the removal of the litter layer, the topsoil (0–10 cm layer) was collected in a transect of five points separated by 50 m each, totaling 10 sampling points per land use per season.

2.2. Soil chemical and physical analysis

The chemical and physical properties of the soil samples were determined at the Department of Soil Science, Luiz de Queiroz College of Agriculture (ESALQ/USP), University of São Paulo, Piracicaba, Brazil. The following soil attributes were determined according to standard protocols (van Raij et al., 2001): pH in calcium chloride ($CaCl_2$); phosphorus (P), potassium (K), magnesium (Mg), and calcium (Ca), extracted with ion-exchange resin; sulfur (S), extracted with 0.01 M calcium phosphate [$Ca_3(PO_4)_2$]; exchangeable aluminum (Al), extracted with 1 M potassium chloride (KCl); potential acidity (H+Al), determined with the Shoemaker-McLean-Pratt (SMP) buffer; boron (B), extracted with hot water; iron (Fe), manganese (Mn), zinc (Zn), and copper (Cu), extracted with diethylenetriaminepentaacetic acid (DTPA) and determined by atomic absorption spectrophotometry. Soil organic matter

(OM) was determined by the dichromate/titrimetric method. Based on these results, the sum of exchangeable bases (SEB), cation-exchange capacity (CEC), base saturation (V%), and aluminum saturation (m%) were also calculated. For the wet season samples, total nitrogen (N) content and soil physical properties were determined using the Kjeldahl method and according to the recommendations of Embrapa – Empresa Brasileira de Pesquisa Agropecuária (1997), respectively, using undisturbed soil cores. Soil density was measured using Kopecky's ring method, total porosity by the saturation method, microporosity by the tension table method, and macroporosity by subtracting microporosity from total porosity.

2.3. Soil DNA extraction

Total DNA was extracted in duplicate from 250 mg of each soil sample using the PowerLyzer PowerSoil DNA Isolation Kit (Qiagen, Hilden, Germany), following an optimized protocol for Amazonian soils (Venturini et al., 2020). DNA quality and quantity were analyzed using a NanoDrop 2000c spectrophotometer (Thermo Fisher Scientific, Inc., Waltham, MA, USA) and visualized by agarose gel electrophoresis, and samples were stored at -20 °C.

2.4. Quantitative PCR

The abundance of methanogens, methanotrophs, Archaea, and Bacteria was quantified using quantitative PCR (qPCR) assays targeting the marker genes: *mcrA* (methanogens), *pmoA* and *mmoX* (methanotrophs), and archaeal and bacterial 16S rRNA. For each gene, a standard curve was constructed from the PCR-amplified product and its serial dilution ranging from 10^0 to 10^{10} copies of the amplicon of interest. The cycling conditions, primers, and references are described in Supplementary Table S1. All reactions were performed in triplicate on a StepOnePlus Real-Time PCR System (Thermo Fisher Scientific, Inc., Waltham, MA, USA), according to the following specifications: 5 μL of SYBR Green ROX qPCR Master Mix (Thermo Fisher Scientific, Inc., Waltham, MA, USA), 1 μL of each primer (5 pmols), 0.2 μL of Bovine Serum Albumin (BSA, 20 mg mL^{-1}) (Thermo Fisher Scientific, Inc., Waltham, MA, USA), 1 μL of DNA (10 ng/μL), 1.8 μL of ultrapure H_2O. The results were analyzed using the StepOnePlus™ Real-Time software v2.3 (Thermo Fisher Scientific, Inc., Waltham, MA, USA).

2.5. 16S rRNA sequencing and bioinformatics

Amplicon sequencing targeting the V4 region of the 16S rRNA gene from Archaea and Bacteria was performed at the Center for Functional Genomic Research (ESALQ/USP), Piracicaba, Brazil. Soil samples from points 1, 3, and 5 of all areas in both seasons were sequenced, totaling 36 libraries. The V4 region was amplified with the primers 515F-Y (Parada et al., 2016) and 806R (Apprill et al., 2015) (Supplementary Table S1) and sequenced on an Illumina MiSeq platform (Illumina Inc., San Diego, CA, USA) with 250 bp paired-end reads. The computational processing of the raw sequences was performed in RStudio 4.0.2 (RStudio Team, 2016) using the DADA2 1.16.0 package (Callahan et al., 2016). Only the forward reads were used due to the low quality of the reverse reads. Forward sequences with mean Phred quality score lower than 20 and length shorter than 220 bp were removed, as well as the sequences of the primers. The remaining sequences were error-corrected, de-replicated, and chimera-removed. Taxonomic assignment was performed using the SILVA database (release 138, non-redundant) (Quast et al., 2013). Absolute abundances of the amplicon sequence variants (ASVs) were converted to relative abundance by total sum scaling. Sequences of methanogenic and methanotrophic microbial groups were manually filtered and grouped at the genus level using the PhyMet2 (Michał et al., 2018; http://phymet2.biotech.uni.wroc.pl/) and Methanotroph Commons (http://www.methanotroph.org/wiki/taxonomy/) databases. For this analysis, only pastures were considered due to the absence of sequences of these groups in the other study areas.

2.6. Statistical analyses

All statistical analyses were performed in RStudio 4.0.2 (RStudio Team, 2016). Soil chemical properties were aligned rank-transformed and analyzed using linear mixed-effects models with the ARTool v0.10.8 package (Kay and Wobbrock, 2020), while for soil physical properties and nitrogen, only the effects of land use were considered since the physical properties of the samples were determined in only one season. Non-metric multidimensional scaling (NMDS) and permutational multivariate analysis of variance (PERMANOVA) were used to assess the structure of soil chemical properties (Gower distance) and microbial community composition (Bray-Curtis dissimilarity) across treatments, using the vegan package 2.5-7 (Oksanen et al., 2015) ($p < 0.05$). The algorithm coefficients of the envfit function were obtained through the vegan package 2.5-7 (Oksanen et al., 2015) to identify the soil chemical properties that were significantly associated with the modulations of the microbial communities ($p < 0.05$). Pairwise comparisons were performed with the pairwiseAdonis package 0.0.1 (Martinez Arbizu, 2017) ($p < 0.05$). Parameters of the microbial communities, including species richness, Shannon diversity, and Pielou evenness, were calculated using the vegan package 2.5-7 (Oksanen et al., 2015). We also performed the random forest analysis on the microeco package 0.14.1 (Liu et al., 2021) to predict the most important phyla associated with each land use. The multinomial species classification method (CLAM) ($p < 0.05$, coverage limit = 10) was used to analyze niche occupation (specialists vs. generalists) of microbial communities at the ASV level, according to land uses and seasonality through the vegan package 2.5-7 (Oksanen et al., 2015).

The prediction of functions related to the CH_4 cycle was performed using PICRUSt2 (Douglas et al., 2020). The qPCR results, the abundance of each phylum of archaea and bacteria (considering a relative abundance higher than 1% for bacteria), the parameters of the microbial communities, and predicted functional abundances were aligned rank-transformed and analyzed by linear mixed-effects models using ARTool 0.10.8 (Kay and Wobbrock, 2020) ($p < 0.05$). Plots were generated using the ggplot2 package 3.3.3 (Wickham, 2016). Spearman's rank correlation coefficient was used to determine the correlations between the qPCR abundance of CH_4 genes and soil chemical properties related to acidity and fertility using the ggpubr package 0.0.6 (Kassambara, 2017). Correlation analyses included all measured soil chemical parameters and gene abundances, and the results were visualized using the corrplot package 0.92 (Wei and Simko, 2021).

3. Results

3.1. Soil chemical and physical characteristics

Pasture soils presented, in general, the greatest differences in attributes when compared to the two other forest classes, while most of the chemical properties had similar values between primary and secondary forests. In pastures, pH was higher (Supplementary Tables S2 and S3), also showing higher levels of Ca and decreased amounts of Al ($p < 0.05$). Although land use was the main factor influencing soil properties ($R^2 = 0.618$, $p = 0.001$), seasonality also explained a small fraction of the variability ($R^2 = 0.036$, $p = 0.048$) (Fig. 1; Table 1). Mg and OM contents increased during the dry season, while B was higher during the wet season ($p < 0.05$). The contents of P, S, K, H+Al, CEC, V, m, Cu, and Zn were influenced by the interaction between seasonality and land use ($p < 0.05$). Although no difference in the physical properties was observed among land uses ($p < 0.05$) (Supplementary Table S5), in pasture, we can observe a trend towards a decrease in soil total, micro-, and macroporosity (Supplementary Table S4). In the secondary forests, we noticed a tendency for recovery of the total porosity values to levels close to those of the primary forests.

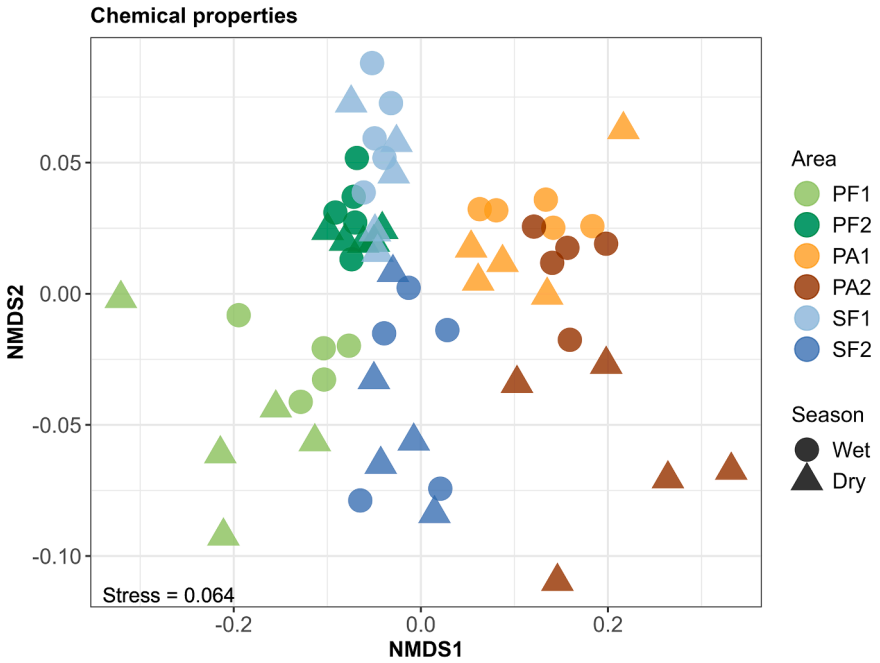

Fig. 1. Non-metric multidimensional scaling (NMDS, Gower distance) of the chemical properties of primary forest (PF1 and PF2), pasture (PA1 and PA2), and secondary forest (SF1 and SF2) soils in the wet and dry seasons. PF1/PF2, PA1/PA2, and SF1/SF2 represent replicate sampling areas within each land-use type. Axes represent ordination space. The ordination stress value is displayed in the figure.

Table 1
Permutational multivariate analysis of variance (PERMANOVA) of the chemical properties and the 16S rRNA sequencing data (Archaea and Bacteria) of primary forest, pasture, and secondary forest soils in the wet and dry seasons.

Data	Land use			Season			Land use x Season		
	R^2	F	p-value	R^2	F	p-value	R^2	F	p-value
Chemical properties	0.618	27.476	**0.001**	0.036	3.220	**0.048**	0.008	0.364	0.868
Archaea	0.311	7.082	**0.001**	0.008	0.371	0.851	0.020	0.475	0.875
Bacteria	0.618	27.080	**0.001**	0.016	1.439	0.211	0.023	1.008	0.389

Bold values indicate statistical significance at $p < 0.05$ (p-value). Distance indices used were Gower distance (chemical properties) and Bray-Curtis dissimilarity (amplicon sequencing). R^2: percentage of the variance explained. F: F-values.

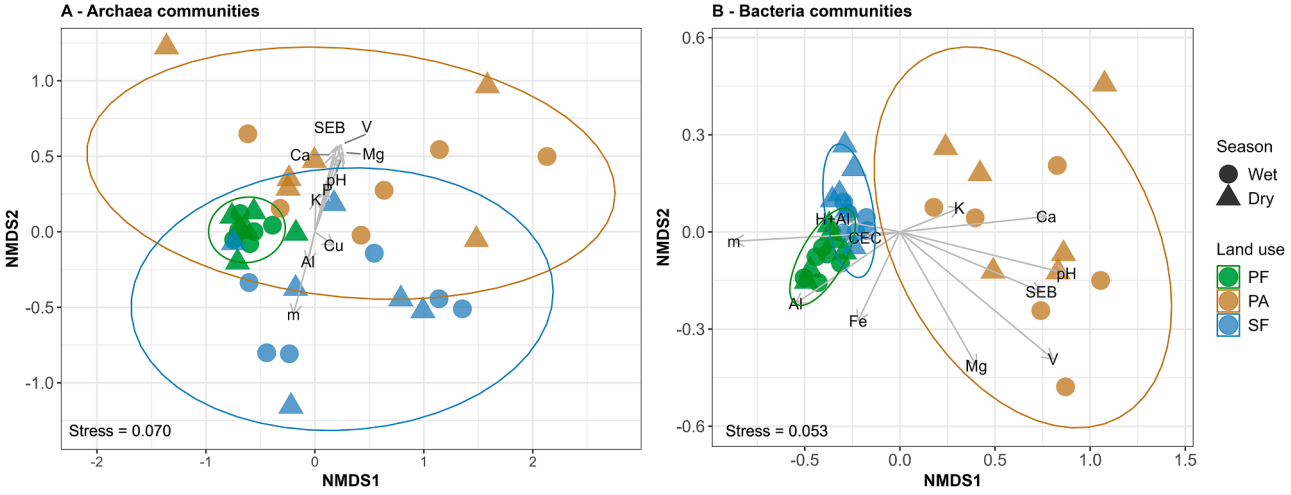

Fig. 2. Non-metric multidimensional scaling (NMDS, Bray–Curtis dissimilarity) of Archaea (A) and Bacteria (B) communities in primary forest (PF), pasture (PA), and secondary forest (SF) soils during the wet and dry seasons. Environmental vectors were added using envfit, and only factors significantly correlated with community structure ($p < 0.05$) are displayed. Environmental variables include: Al, exchangeable aluminum; Ca, calcium; CEC, cation exchange capacity; Cu, copper; Fe, iron; K, potassium; m, aluminum saturation; Mg, magnesium; Mn, manganese; pH, soil pH; H+Al, potential acidity; SEB, sum of exchangeable bases; P, phosphorus; and V, base saturation. Analyses were performed at the ASV level. The ordination stress value is shown in the figure.

3.2. Microbial community structure

PERMANOVA (Table 1), NMDS, and envfit (Fig. 2, Supplementary Table S6) analyses ($p < 0.05$) indicated differences in archaeal and bacterial communities according to different land uses but not between seasons. Although archaeal communities showed dissimilarity in the NMDS (Fig. 2a), the multivariate analysis indicated that communities did not differ between forests (primary and secondary forests) whereas pasture communities differed from the other land uses (Supplementary Table S7). In pastures, the taxonomic composition (ASV-level) of archaeal communities was mainly correlated with high levels of pH, Ca, Mg, SEB, and V, while in secondary forests, they were associated with m and Al levels. On the other hand, the structure of the bacterial communities differed between land uses, which accounted for 61% of the observed variation (Table 1). The NMDS showed that pasture communities were more distinct from those in forests (Fig. 2b, Supplementary Table S7). Similar to archaeal communities, after forest-to-pasture conversion, bacterial communities were correlated with high levels of pH, V, SEB, and macronutrients, such as Ca and Mg (Supplementary Table S6). In primary forests, they were correlated with Al and m, while in secondary forests, they were correlated with H+Al and CEC.

3.3. Diversity of the archaeal and bacterial communities

A total of 3.4 million 16S rRNA sequences were obtained (considering only the forward sequences used in the analysis), with an average of 95,000 sequences per sample. After quality control, 2,475,537 sequences were maintained, with an average lenght of 201 bp. Following the DADA2 pipeline, 13,839 ASVs were obtained, of which 98.55% were attributed to Bacteria, 1.28% to Archaea, and 0.17% to unclassified sequences and Eukaryotes. We observed an increase in both Shannon diversity and Pielou evenness ($p < 0.05$) of archaeal ASVs after conversion, particularly during the wet season, while the opposite trend was observed during the dry season (Fig. 3, Table 2). Similarly, we noticed a tendency for richness to increase in pasture soils during the wet season.

Moreover, irrespective of the season, we observed a higher Shannon diversity and richness of bacterial ASVs in pasture soils. However, the NMDS ordination revealed greater dispersion among pasture samples and stronger separation from forest samples, indicating higher variability in community composition within pasture soils.

3.4. Composition of the archaeal and bacterial communities

The microbial communities were composed of 42 phyla, with five attributed to Archaea and 37 to Bacteria (Fig. 4). Proteobacteria (average considering all land uses and seasons = 27%), Actinobacteriota (16%), Acidobacteriota (13%), Planctomycetota (11%), Chloroflexi (8%), Firmicutes (7%), Verrucomicrobiota (6%), Crenarchaeota (4%), Candidatus WPS-2 (or Candidatus Eremiobacterota) (3%), and Myxococcota (2%) were the most abundant phyla for all soil samples and together represent about 97% of the microbial community. Crenarchaeota was the most abundant archaeal phylum across all land uses and seasons. However, its abundance was considered similar among

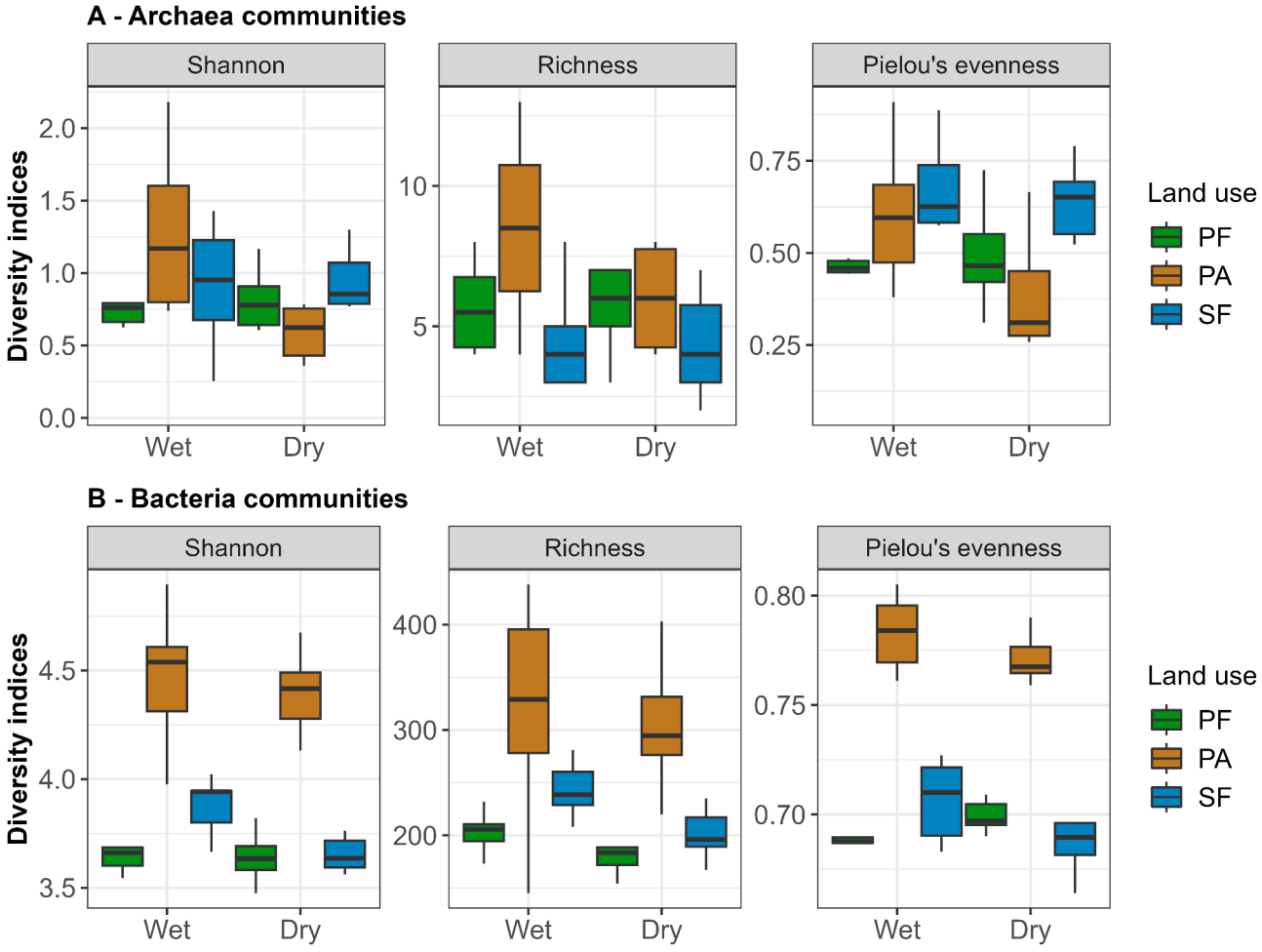

Fig. 3. Diversity indices of archaeal (A) and bacterial (B) communities at the ASV level in primary forest (PF), pasture (PA), and secondary forest (SF) soils during the wet and dry seasons. The panels show Shannon diversity, richness, and Pielou evenness for each land-use type.

Table 2
Results of linear mixed-effects models for diversity indices, qPCR gene quantification, and PICRUSt2 predicted functions (ASV-level) in primary forest, pasture, and secondary forest soils during the wet and dry seasons.

Data	Land use			Season			Land use x Season		
	df	F	p-value	df	F	p-value	df	F	p-value
Archaea ASVs									
Shannon	2	0.151	0.865	1	7.172	0.017	2	6.367	0.009
Richness	2	1.226	0.407	1	4.013	0.063	2	1.982	0.172
Pielou evenness	2	0.568	0.617	1	3.919	0.066	2	8.278	0.003
Bacteria ASVs									
Shannon	2	32.567	0.009	1	6.721	0.020	2	2.370	0.127
Richness	2	1998.166	< 0.001	1	5.341	0.035	2	0.557	0.584
Pielou evenness	2	9.751	0.048	1	2.272	0.152	2	3.322	0.063
Gene quantification									
mcrA	2	10.891	0.042	1	14.984	< 0.001	2	6.001	0.006
pmoA	2	5.087	0.108	1	48.286	< 0.001	2	21.758	< 0.001
mmoX	2	5.467	0.099	1	12.938	0.001	2	0.112	0.894
Ratio: *mcrA:pmoA+mmoX*	2	2.719	0.211	1	4.164	0.051	2	3.366	0.049
Functional predictions									
Methanogenesis, acetate -> methane	2	3.345	0.172	1	3.301	0.089	2	0.222	0.803
Methanogenesis, CO_2 -> methane	2	1.066	0.446	1	0.049	0.827	2	0.459	0.640
Methanogenesis, dimethylamine -> methane	2	10.745	0.444	1	23.129	< 0.001	2	10.345	0.001
Methanogenesis, methanol -> methane	2	0.646	0.584	1	1.750	0.205	2	0.266	0.769
Methanogenesis, methylamine -> methane	2	10.745	0.444	1	23.422	< 0.001	2	10.052	0.001
Methanogenesis, trimethylamine -> methane	2	0.518	0.640	1	0.003	0.953	2	0.101	0.904
Methane oxidation, methane -> methanol	2	31.664	0.009	1	0.229	0.639	2	4.107	0.037
Methane oxidation, methane -> formaldehyde	2	0.847	0.510	1	3.103	0.098	2	0.583	0.570

Values in bold indicate statistical significance at $p < 0.05$ (p-value). df: degrees of freedom. F: F-value.

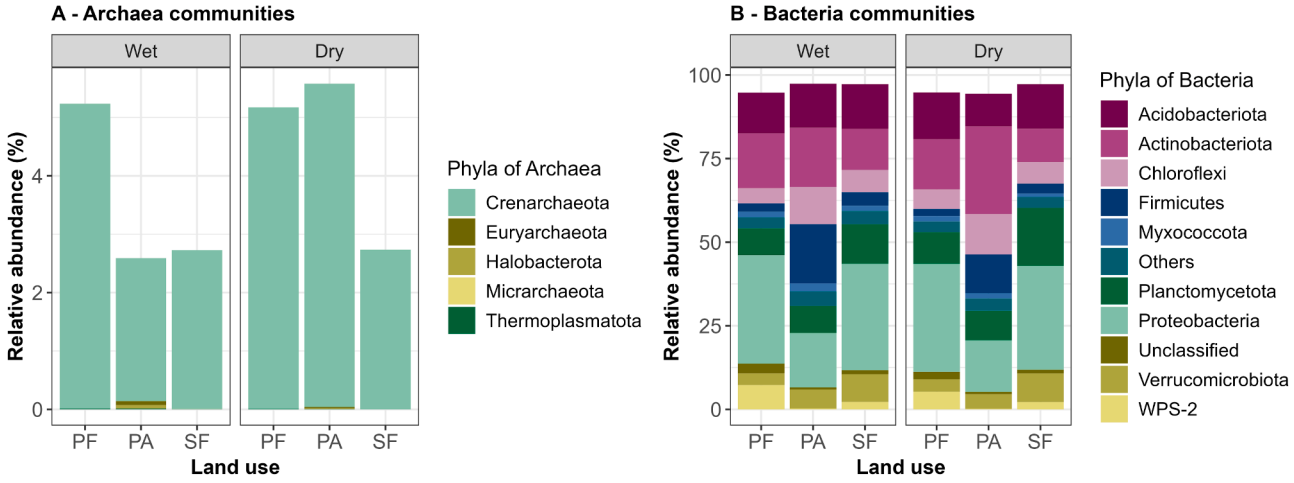

Fig. 4. Relative abundance of archaeal (A) and bacterial (B) phyla in primary forest (PF), pasture (PA), and secondary forest (SF) soils during the wet and dry seasons. Bacterial phyla with relative abundances below 1% were grouped into "Others". "Unclassified" represents sequences that could not be assigned to any phylum during taxonomic classification.

land uses ($p < 0.05$) (Supplementary Table S8 and Fig. 4).

On the other hand, after forest-to-pasture conversion, there was an increase in Actinobacteriota, Chloroflexi, Firmicutes, and Verrucomicrobiota and a decrease in Proteobacteria and WPS-2, while Planctomycetota and Verrucomicrobiota showed even higher in secondary forests. When we evaluated the main biomarker phyla of each land use through the random forest analysis (Fig. 5), we verified that, in fact, WPS-2 and Proteobacteria were the main biomarkers for primary forest, Actinobacteriota and Firmicutes for pasture, and Verrucomicrobiota and Planctomycetota for secondary forest. When we analyzed methanogenic and methanotrophic groups, we noticed their presence only in the pasture (Supplementary Figure S2). We found six genera of methanogenic archaea (*Methanobacterium, Methanobrevibacter, Methanocella, Rice Cluster I, Methanosarcina*, and *Methanomassiliicoccus*) and four genera of methanotrophic bacteria (*Methylocystis, Methyloparacoccus, Methylomicrobium*, and Sh765B-TzT-35).

3.5. Niche occupancy

Niche occupation was also affected by forest-to-pasture conversion and forest recovery. When comparing different land uses, pasture showed the highest proportion of specialist organisms (Fig. 6). Compared to the primary forest, pasture had 29.4% and 31.2% of specialists in the wet and dry seasons, respectively, while we observed a small reduction in pasture specialists compared to the secondary forest: 24.3% and 27.3% in the wet and dry seasons. On the other hand, a lower presence of specialists was detected when comparing both forests: 1.2% and 7.7% for primary and secondary forests in the wet season and 3.2% and 6.8% in the dry season. The presence of generalists was also greater in this comparison, with 43.5% (wet) and 41.3% (dry) of groups shared between both forest types. The comparison between primary forest and pasture had the lowest share of generalist groups: 14.7% and 14.3% in the wet and dry seasons, respectively. Secondary forest and pasture shared 20.1% and 17.2% of generalists in the wet and dry seasons.

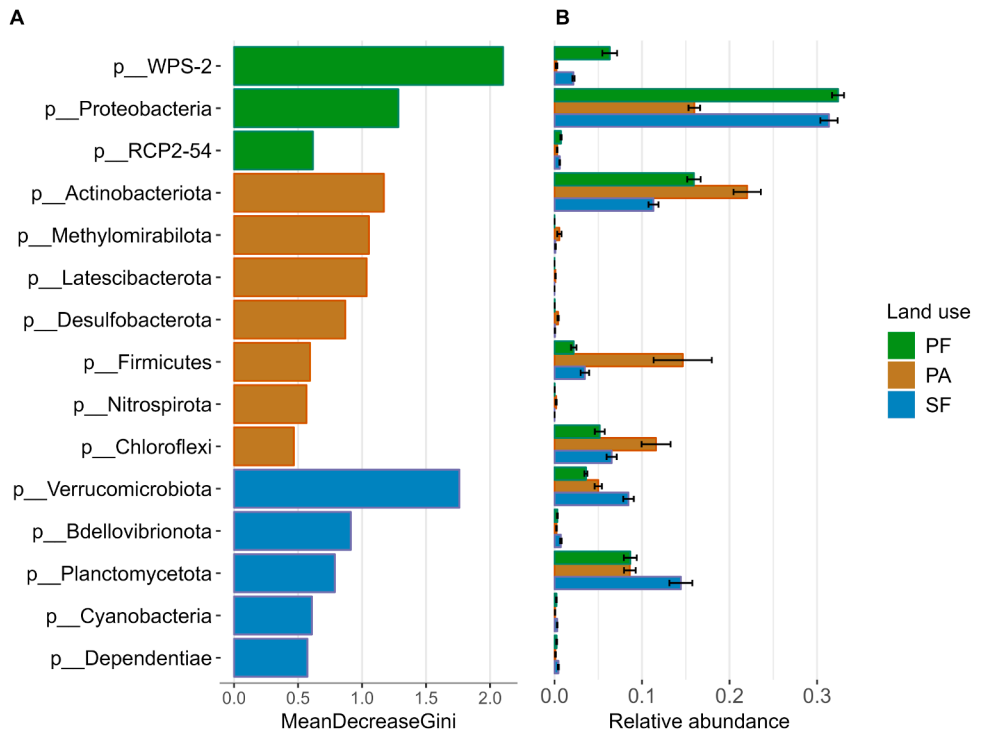

Fig. 5. Random forest analysis identifying microbial phyla that discriminate between primary forest (PF), pasture (PA), and secondary forest (SF) soils. (A) The top 15 phyla ranked by importance based on the Mean decrease in Gini index. (B) Relative abundance (mean ± standard deviation) of these phyla across the three land-use types.

Interestingly, we also observed an effect of seasonality on different land uses. We compared the primary forest results between seasons and noticed that most groups were generalists (48.4%). Pasture had the highest proportion of specialists when comparing the wet and dry seasons, with 3.4% and 4.3%, respectively, and 46% of generalists, while the secondary forest had 2.2% and 0.7% of specialists in the wet and dry seasons, and 48.4% of generalists.

3.6. Microbial community abundance

Quantitative PCR was used to assess the abundance of archaea and bacteria using the 16S rRNA gene, as well as functional genes associated with methanogenesis (*mcrA*) and methanotrophy (*pmoA* and *mmoX*) (Fig. 7). Forest-to-pasture conversion had a notable impact on the abundance of both taxonomic and functional genes. Overall, the abundance of archaea and bacteria was higher in pastures, particularly during the dry season ($p < 0.05$) (Table 2 and Fig. 7a, b). In secondary forests, the abundance of these genes exhibited an interaction effect between land use and season, showing distinct patterns compared to other areas.

Seasonality and its interaction with land use were also significant factors influencing the abundance of *mcrA* and *pmoA*; additionally, *mcrA* was influenced by land use (Table 2 and Fig. 7c, d). The highest abundance of *mcrA* was observed in pasture soils, particularly during the wet season. Conversely, *pmoA* exhibited distinct interaction effects across all land uses, with the highest abundance during the wet season and an increasing trend after conversion. These results support our sequencing data that showed an increase in methanogenic and methanotrophic groups in pasture soils, mainly in the wet season (Supplementary Figure S2). The abundance of *mmoX* was also significantly higher during the wet season (Fig. 7e). The ratio between CH_4 marker genes (*mcrA*/*pmoA*+*mmoX*) indicated that in both seasons pasture had a higher proportion of *mcrA*, while primary and secondary forests displayed an increase in *pmoA* and *mmoX* (Fig. 7f). Considering the correlation of CH_4 genes and soil parameters (Supplementary Figure S3), we noticed that *mcrA* abundance showed a strong and positive correlation with pH, as well as with some parameters related to fertility, including Ca, Mg, V%, and was negatively correlated with m% and Al content ($p < 0.05$). The *pmoA* gene showed the same pattern but with weaker correlations, while the *mmoX* gene showed no significant correlation with any of the parameters analyzed.

3.7. Functional profile of the CH_4-related microbial communities

The functional prediction of the microbial communities was performed using PICRUSt2, and functions associated with the CH_4 cycle were subsequently filtered (Fig. 8a, b). Although many functions did not show significance (Table 2), there was an increasing trend in functions associated with methanogenesis in pasture soils, mainly concerning the production of CH_4 from acetate, especially during the wet season (Fig. 8a). Also, CH_4 production from methylamine and dimethylamine was only present in pastures. On the other hand, functions associated with methanotrophy were more abundant in the forests, especially in primary forests during the wet season. Specifically, when we analyzed the abundance of sequences related to CH_4 oxidation and methanol formation, we noted the effects of the interaction between land use and seasonality differentiating forests from pastures (Fig. 8b). Finally, we found a similar pattern of CH_4 production and consumption functions when comparing secondary and primary forests across seasons, although the abundance of methanotrophic sequences tended to decrease in this land use in comparison to the primary forests.

4. Discussion

4.1. Soil chemical properties

Similar to previous studies (Jesus et al., 2009; Rodrigues et al., 2013; Lammel et al., 2015a), land-use change significantly impacted soil chemical and physical properties, particularly following the conversion from forest to pasture. However, secondary forests showed values

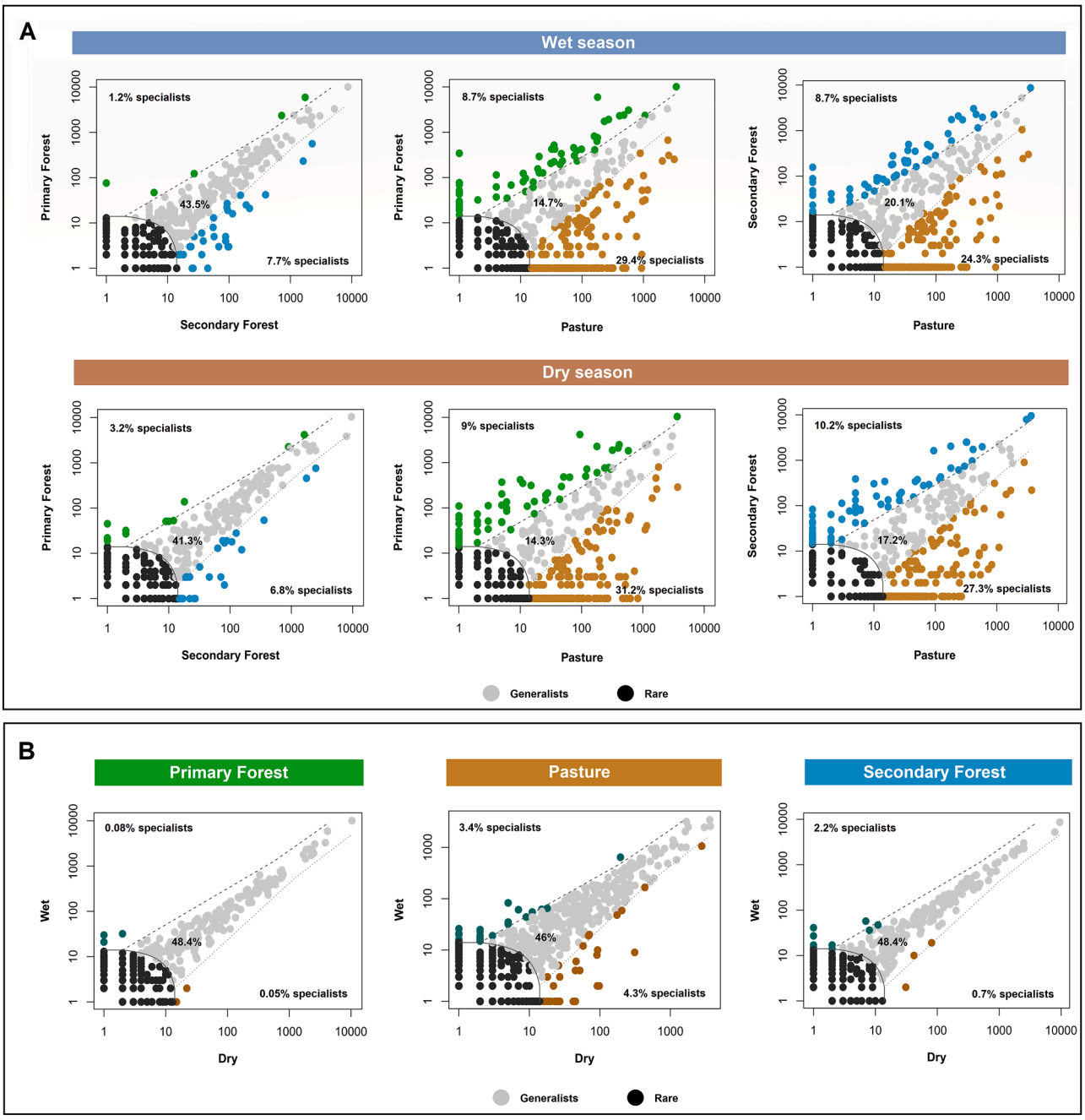

Fig. 6. Multinomial species classification (CLAM) analysis of ASVs showing the proportion of generalists, specialists, and rare taxa. Panel A shows comparisons between land-use types (primary forest, pasture, and secondary forest) within each season (wet and dry). Panel B shows comparisons between seasons (wet vs. dry) within each land-use type. The percentage of ASVs classified into each category (generalists, specialists, and rare taxa) are indicated in each plot.

similar to those of primary forests for most soil chemical parameters, a finding supported by our NMDS and pairwise analyses. Primary forest soils had low pH and Ca and V levels while showing elevated levels of Al, H+Al, and m (Lammel et al., 2018; Merloti et al., 2019; Pedrinho et al., 2019).

In our study, we observed an increase in pH after forest-to-pasture conversion, which can be attributed to soil alkalinization caused by ash generated from burning, a common practice after forest cutting and management (Fernandes et al., 2002; Rittl et al., 2017). Numerous studies on tropical soils have reported changes in pH due to land-use changes (Petersen et al., 2019), including those conducted in the region (Lammel et al., 2015a; Pedrinho et al., 2019; Khan et al., 2019; Pedrinho et al., 2020; Venturini et al., 2022). Secondary forest soils did not exhibit significant differences in pH compared to primary forests, indicating the soil's potential to restore this attribute to pre-conversion levels. In addition, most soil chemical properties were restored in secondary forests after 15 years of abandonment and recovery. The influence of seasonal variation was also observed, accentuating these chemical differences between forests and pasture.

4.2. *Archaeal and bacterial communities in different land uses*

Forest-to-pasture conversion affected the structure of soil microbial communities, which was associated with changes in soil chemical properties. Microbial communities in secondary forests were more similar to those in primary forests. In pastures, archaeal and bacterial

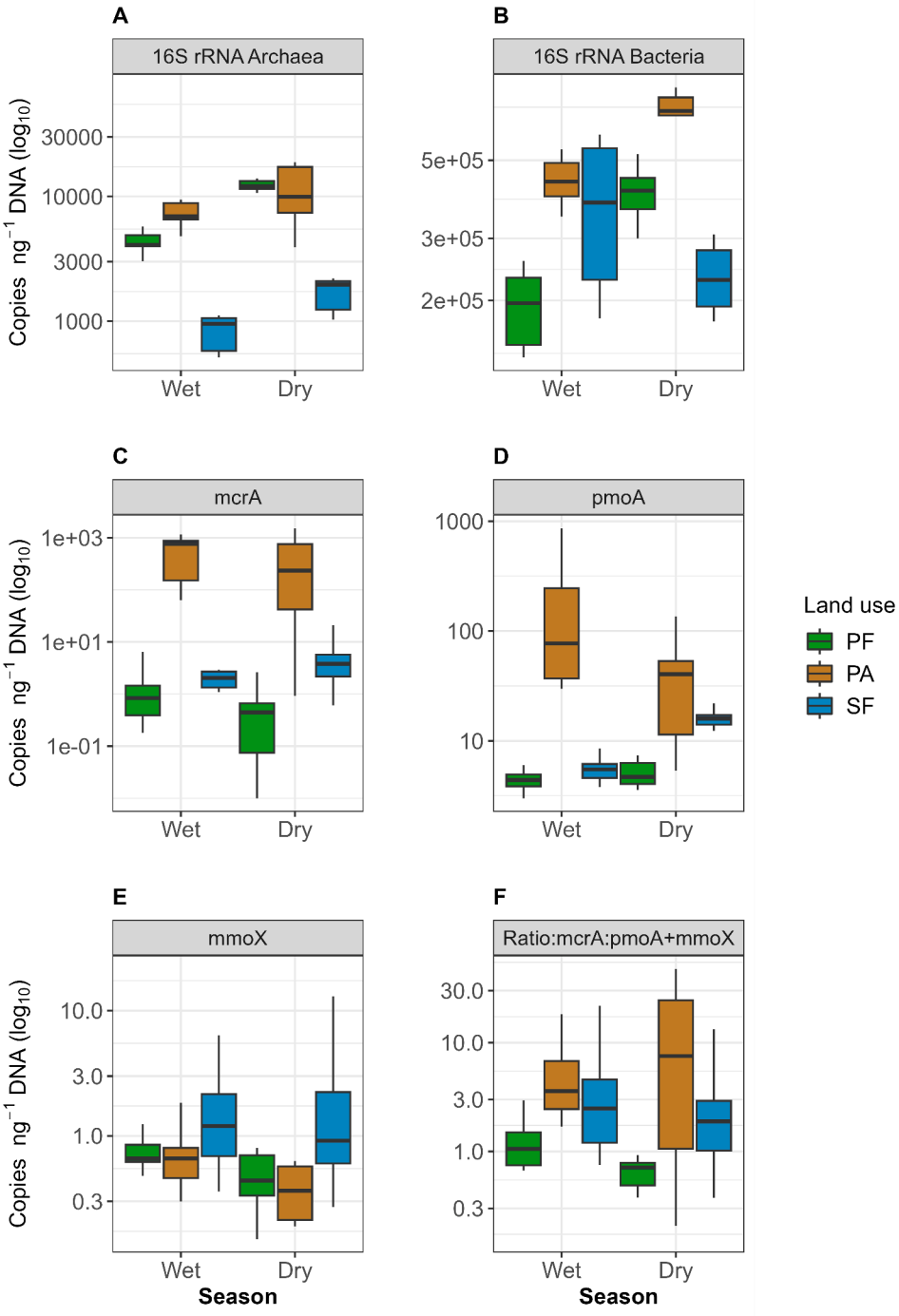

Fig. 7. Number of copies per ng of DNA (copies ng^{-1} DNA) of functional marker genes associated with the CH_4 cycle in primary forest (PF), pasture (PA), and secondary forest (SF) soils during the wet and dry seasons, quantified by real-time PCR (qPCR). Values are displayed on a $\log_{10}$-scaled axis. The panels show the abundance of archaeal 16S rRNA genes (A) and bacterial 16S rRNA genes (B); the functional genes *mcrA, pmoA*, and *mmoX* (C, D, and E, respectively); and, in panel F, the ratio between methanogenic and methanotrophic genes (*mcrA: pmoA* + *mmoX*).

communities were correlated with higher levels of pH, Ca, Mg, SEB, and V, and low levels of m and Al. The increase in pH related to pasture establishment and management tends to increase the dissimilarity of factors related to acidity, such as m% and Al, and to fertility itself, such as SEB and V (Rocha et al., 2023). These changes modify the composition and microbial biomass of the soil (Rousk et al., 2009), in addition to having "spillover effects", which are indirect effects that are still little known, especially in tropical soils. Such effects include changes in nutrient availability, as well as microbial physiology and activity (Lammel et al., 2018).

Regardless of the sampling period, the richness, Shannon diversity, and Pielou evenness of bacterial communities increased in pasture compared to forests, as also observed by Rodrigues et al. (2013). The changes in vegetation cover and organic matter input after forest-to-pasture conversion may favor decomposer groups, leading to an increase in bacterial diversity (Franco et al., 2019). In fact, Mendes et al. (2017) demonstrated that this reflects an increase in functional diversity in response to the stress caused by anthropogenic disturbances. Furthermore, Venturini et al. (2025b) demonstrated, through metagenomic analysis of bacterial and archaeal genomes, that

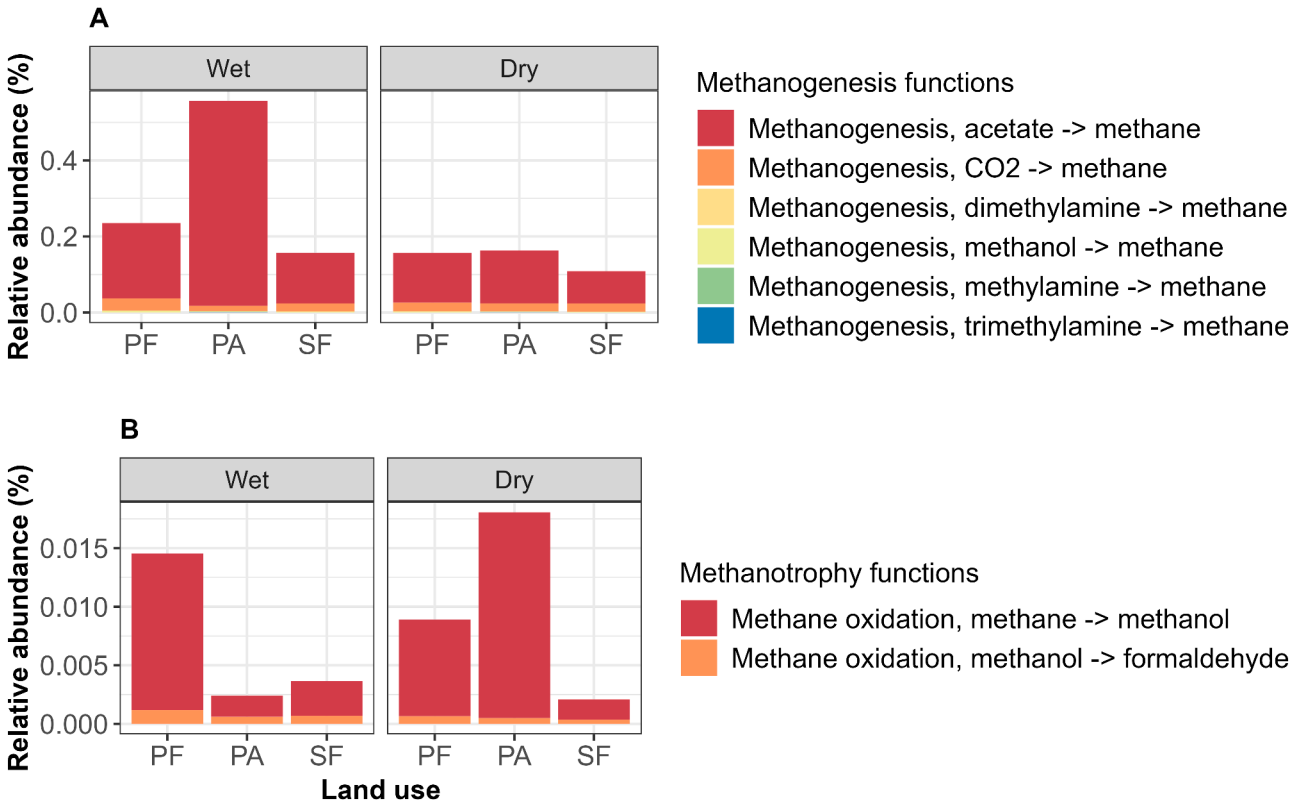

Fig. 8. PICRUSt2 predicted functional pathways associated with the CH_4 cycle in primary forest (PF), pasture (PA), and secondary forest (SF) soils during the wet and dry seasons, inferred from ASV-level data. Panel A shows predicted methanogenesis pathways, and panel B shows predicted methanotrophy pathways. Values represent the relative abundance (%) of predicted functions within each land-use type and season.

forest-to-pasture conversion in Eastern Amazonia significantly alters the genomic characteristics of soil microbial communities. Microorganisms from pasture soils exhibited larger genome sizes, higher GC content, and a greater number of genes involved in biogeochemical cycling and carbohydrate metabolism. These soil changes likely select for microorganisms with genomic features associated with greater functional potential and adaptive capacity in pasture soils, corroborating our observations of increased bacterial diversity and highlighting the importance of examining microbial functional traits to better understand responses to land-use change. For archaea, diversity was higher in pastures during the wet season, while the opposite pattern was observed in the dry season. Although the distribution of this group has been associated with soil moisture, in addition to the C:N ratio in more arid climates (Shi et al., 2016), in tropical Amazonian soils, the decrease in diversity may be associated with variations in soil acidity (Taketani and Tsai, 2010). For both groups, an increase in abundance (quantification of 16S rRNA genes) was observed in pastures. This reinforces that alterations in the structure and composition of these communities are a strategy for adapting to new environmental conditions.

Certain groups of soil microorganisms can act as indicators of environmental change, allowing the behavior of these communities to be inferred based on them (Joos et al., 2023). The phylum Firmicutes, consistent with several other studies in the Amazon (Mendes et al., 2015a; Kroeger et al., 2018; Rodrigues et al., 2013; Ranjan et al., 2015; Petersen et al., 2019; Danielson and Mazza Rodrigues, 2022), had a higher abundance in pastures. Interestingly, this group was characterized, in our random forest analysis, as one of the main biomarkers of this land use. Firmicutes taxa have been cited as capable of establishing survival strategies under adverse conditions (including spore formation) (Ramos et al., 2019), a common scenario in disturbed environments such as pastures, which may have benefited this group. In primary forests, some of the main biomarker phyla were WPS-2 (Candidatus Eremiobacterota) and Proteobacteria, and we noticed that both had their abundance reduced in pastures. Proteobacteria are ubiquitous in soil and play several important roles in carbon and nitrogen cycling (Mhete et al., 2020), while WPS-2 was previously observed to be higher in forest soils (Rocha et al., 2021). Apparently, these organisms prefer more acidic and oxygen-rich environments, which are more common in forests (Ward et al., 2019). Interestingly, the abundance of both groups increased again in secondary forests, suggesting the reestablishment of soil microbial communities.

Ranjan et al. (2015) revealed that Verrucomicrobiota communities showed signs of recovery in secondary forests, which was consistent with our results. Using random forest analysis, we demonstrated that not only does this phylum increase again in abundance in secondary forests, but it is also an important biomarker of this land use. Verrucomicrobiota is known for its roles in the degradation of polysaccharides and the metabolism of carbohydrates, especially in places where the quality and quantity of matter do not favor copiotrophic taxa (Fierer et al., 2013). Furthermore, other groups within the phylum participate in important ecological processes, such as methane oxidation, nitrogen fixation, and organic matter degradation. Our limited knowledge about these organisms may be causing an underestimation of their abundance as well as their real ecological importance (Shen et al., 2017), especially in soils of secondary forests that may have different carbon inputs. Planctomycetota also increased in secondary forests, being one of the main bioindicators of this land use. This phylum is still poorly studied in Amazonian soils, but its members are associated with the degradation of different sources of organic matter, which could be an important adaptation strategy for an environment in the process of recovery (Rocha et al., 2021).

Changes in the taxonomic composition of microbial groups can be relevant to functional inferences (de Carvalho et al., 2016), and an interesting aspect observed in our data was that forest-to-pasture

conversion also influenced niche occupation of soil microbial communities, increasing the presence of specialist groups in pastures. Niche breadth generally decreases towards environmental extremes since more specialized taxonomic groups are required in selective environments (Malard et al., 2022). Thus, different environments tend to host different microbial communities, and this process is mainly due to different selection pressures from abiotic factors, including the quantity and quality of carbon available in the soil, pH, and salinity (Ferrenberg et al., 2013). Bacilli (phylum Firmicutes) and Thermoleophilia (phylum Actinobacteriota) were the most common specialist classes in pasture, regardless of the season. Several Bacillus taxa can form endospores that allow them to resist radiation, desiccation, UV light, and heat, in addition to being commonly found in acidic soils (Liu et al., 2019), while members of Thermoleophilia can be found in warmer environments and are important for the decomposition of a wide range of carbon sources (Hu et al., 2019).

Although the most common specialist classes in secondary forests (Verrucomicrobiae from Verrucomicrobiota, and Alpha and Gammaproteobacteria from Proteobacteria) were distinct from primary forests (Nitrososphaeria from Crenarchaeota, Acidimicrobiia from Actinobacteriota, Alphaproteobacteria from Proteobacteria, and an uncultured class of WPS-2), the presence of specialists between these two land uses was drastically reduced. This reinforces that niche occupation tends to recover when the area is abandoned, and the forest starts to regrow. Finally, when we compared the same land use throughout the year, we noticed that the primary forest showed the greatest resilience between seasons, with very few specialist groups, while the presence of specialists was intermediate in the secondary forest and increased in pasture, showing once again that, together with the land use, seasonality is an important factor that must be considered in studies in the Amazon.

4.3. *Effect of forest-to-pasture conversion and forest regeneration on the CH_4 cycle*

The abundance of the *mcrA* gene, related to methanogenesis, increased in pasture soils. This result has been a consistent response of the CH_4-associated microbiota to the impacts of forest-to-pasture conversion (Meyer et al., 2017; Meyer et al., 2020; Kroeger et al., 2018; Venturini et al., 2022; Alves et al., 2022). The enrichment of methanogenic groups, as well as *mcrA*, which may contribute to increased CH_4 emissions in pastures, has been associated with soil compaction and increased bulk density that occurs with cattle grazing and the use of heavy agricultural machinery (Meyer et al., 2017; Meyer et al., 2020; Venturini et al., 2022). In addition, pasture management influences the CH_4 dynamics in these soils (Fonseca de Souza et al., 2022). Burning is a common practice for pasture establishment and management in the Amazon region, which can lead to soil degradation (de Lima et al., 2022). This is already a problem in Brazil, as around 57% of Amazonian pastures are currently at some stage of degradation (Projeto MapBiomas, 2023). In addition to land use, another important factor that impacted *mcrA* abundance was seasonality. In a microcosm experiment, Venturini et al. (2022) showed an increase in *mcrA* when simulating an increase in moisture in pasture soils of the Amazon, one of the environmental factors that can impact and modulate methanogenic communities. Our study validates these results in the field, showing that a higher number of *mcrA* gene copies were also found in pasture soils during the wet season.

Soil pH was an important environmental factor modulating microbial CH_4-cycling responses to forest-to-pasture conversion. More alkaline conditions in pastures tend to favor methanogenic archaea adapted to less acidic environments, thereby increasing methanogenic potential (Le Mer and Roger, 2001; Hofmann et al., 2016; Hao et al., 2019). At the same time, pH may also act as an ecological filter for certain methanotrophs, particularly promoting those harboring pMMO in more alkaline soils (Knief et al., 2015; Deng et al., 2019; Liu et al., 2022). These mechanisms are aligned with our findings of higher *mcrA* and *pmoA* abundances and increased *mcrA:pmoA* ratios in pasture soils, as well as their positive linear correlations with pH. It is important to highlight that field-based evidence from Amazonian soils indicates that liming in pastures can modulate methanotrophic activity, suggesting that a latent methanotrophic community may become more active after soil pH correction (Lammel et al., 2018; Fonseca de Souza et al., 2025). Since *pmoA*-containing methanotrophs include both low- and high-affinity groups, such a response may reflect changes in community composition or activation of different methanotrophic groups rather than solely the stimulation of low-affinity types. Thus, the results indicate that shifts in soil chemistry, particularly increses in pH, represent an important mechanism through which land-use change alters CH_4-related microbial communities in Amazonian soils.

We also expected that the land-use effects on methanotrophic communities would be consistent with previous studies in which higher *pmoA* gene abundance was observed in primary forests (Lammel et al., 2015b; Wang et al., 2016; Meyer et al., 2017, 2020). However, our data revealed a concomitant increase in methanotrophs in pastures, contrasting with previously reported results for the Amazon, which indicated reductions in these groups after forest conversion (Meyer et al., 2020; Kroeger et al., 2020; Paula et al., 2014). More recent studies, however, have shown that *pmoA* abundance can indeed increase in pasture areas (Venturini et al., 2022; Obregón Alvarez et al., 2023), indicating that the methanotrophic response to forest conversion may be more variable than initially proposed. Despite this increase, these studies consistently point out that the *mcrA:pmoA* ratio tends to be higher in pastures, suggesting that the methanogenic potential exceeds the oxidative capacity, which may favor net CH_4 emissions in this land-use type.

These results may be related to methodological issues associated with primer design, for example, which could limit the quantification of certain groups of methanotrophs carrying *pmoA* (Venturini et al., 2022). The increase in methanotrophs in pasture soils could also reflect an increase in soil pH (Obregón Alvarez et al., 2023), favoring specific groups within the methanotrophic community. In fact, pH can influence methanotrophic groups through its action on particulate and soluble methane monooxygenase enzymes (pMMO and sMMO, respectively), favoring some alkalophilic groups with pMMO over those with sMMO (Yao et al., 2023). These associations among gene abundances and soil chemical parameters are further supported by correlation analyses (Supplementary Figure S1). Interestingly, when we evaluated the correlation of genes associated with MMO coding (Supplementary Figure S2), we saw that *pmoA* had a positive correlation with the increase in pH and parameters linked to soil fertility, as well as *mcrA*, while *mmoX* showed a negative correlation with increasing pH.

We also quantified the *mmoX* gene by qPCR and found that its abundance was higher in the secondary forest, especially in the wet season. Although sMMO (encoded by *mmoX*) is less frequent in the environment because it is restricted to only a subset of methanotrophic groups, it exhibits greater catalytic versatility than pMMO (encoded by *pmoA*), being able to oxidize a broader range of reduced compounds (Nazaries et al., 2013; Knief et al., 2015). No previous study has quantified *mmoX* in the context of forest regeneration, although metagenomic analyses by Kroeger et al. (2018) have already identified a higher prevalence of sMMO-related sequences in secondary forests. From a physiological perspective, the greater abundance of *mmoX* observed in our secondary forests may indicate a methanotrophic community more reliant on sMMO, whose expression is favored under low copper availability, in contrast to pMMO. This enzymatic shift suggests that secondary forests may harbor methanotrophs that are functionally distinct from those present in primary forests, potentially modulating CH_4 oxidation through different metabolic pathways.

Although our results for secondary forests may be indicative of the recovery of the forest as a CH_4 sink, as suggested by Meyer et al. (2020), they also suggest that this may be related to changes in the structure of CH_4 communities and that the long-term impacts in these areas are still

not fully understood. Another interesting point is that while the qPCR results showed an increase in methanogenic and methanotrophic genes in pasture, when we evaluated the ratio between them, we found that pastures had the highest ratio of *mcrA* in relation to *pmoA* and *mmoX,* and that the opposite was observed in the forests. This reinforces that pasture areas can act as a source of CH_4, while forests act as sinks.

Predicted functions (based on our sequencing data) associated with CH_4 production remained high in the pasture, mainly associated with the methylotrophic methanogenesis pathway (Alves et al., 2022). Until a few years ago, little importance was given to methylotrophic methanogenesis, as it was believed to be much less abundant in the environment when compared to the hydrogenotrophic and acetoclastic pathways. However, more recent studies have changed this view, showing that it also plays an important role in CH_4 production and may be more common in soils than previously thought (Söllinger and Urich, 2019). On the other hand, we also found an increase in CH_4 oxidation and methanol formation genes in the pasture during the dry season, while primary forests maintained higher abundances of CH_4 oxidation and lower abundances of CH_4 production genes in both seasons. A similar result was seen in secondary forests, showing us a recovery process in these areas regarding CH_4 consumption. This result is very promising, since not only can methanotrophic pathways be recovered in secondary forests, as suggested by Kroeger et al. (2020), but the low abundance pattern of methanogenesis genes from primary forests can also be achieved. However, interpretations based on predictive functional data should be considered with caution, given that these tools have inherent limitations (Langille et al., 2013), particularly in highly diverse and taxonomically underrepresented environments, such as Amazonian soils (Venturini et al., 2022, 2025a, 2025b). The high proportion of novel or poorly represented taxa in reference databases may affect the accuracy of methane-related metabolic pathways designation, meaning that these predictions should be viewed as potential functional trends rather than direct measurements of metabolic activity.

Additionally, our findings reinforce the influence of seasonality on microbial communities associated with the CH_4 cycle, especially in pastures. This indicates that pasture microbial communities may be more vulnerable to environmental fluctuations than previously assumed, reflecting a reduced functional resilience in disturbed ecosystems or even a decline in ecological functions (Paula et al., 2014). In this sense, Venturini et al. (2023) reported that microorganisms are sensitive indicators of land-use and climate alterations in the Amazon, indicating shifts in important processes, such as CH_4 cycling, that have long been overlooked in conservation and management initiatives.

4.4. Ecological implications for methane emissions and soil health

Our results reinforce previous findings on the effects of forest-to-pasture conversion in the Amazon and provide important implications for ecosystem functioning. The increased methanogenic potential in pasture soils, reflected by the higher abundance of *mcrA* and the elevated *mcrA:pmoA* ratios, indicates a potential shift from methane uptake in forests to methane production in pasture areas. This transition highlights the role of pasture soils as a relevant atmospheric source of CH_4 in the region, contributing to greenhouse gas emissions. In contrast, secondary forests exhibited a partial recovery of methanotrophic functions, emphasizing their potential to restore methane uptake capacity over time. However, the degree of resilience strongly depends on the intensity of the previous land use, as intensive disturbances have been associated with reduced recovery capacity in secondary forests (Jakovac et al., 2015). Therefore, this recovery may depend on time since abandonment and regeneration processes, and there is still no consensus regarding the full restoration of methane-cycling functions in these areas. Evidence indicates that although secondary forests can have multiple ecosystem functions restored during regeneration, these gains remain vulnerable to new disturbance cycles (Bieluczyk et al., 2025a, 2025b), further reinforcing the need for protection policies to prevent them from being subjected again to deforestation cycles.

Other microbial changes beyond methane-cycling communities also point to consequences for soil health. Pasture soils exhibited reduced functional resilience to seasonal fluctuations. Moreover, the enrichment of specialist taxa with stress-tolerance traits in pastures may indicate a potential reduction in functional redundancy, which may compromise carbon cycling, nutrient storage, and long-term soil fertility. In recovering forests, the reestablishment of some methanotrophic groups, including those capable of alternative methane oxidation pathways, may support the restoration of CH_4 sink capacity and improve the efficiency of soil carbon cycling, contributing to the recovery of ecological functioning.

In summary, these results demonstrate that microbial changes resulting from land-use change can lead to persistent functional consequences at the ecosystem scale. The conservation of primary forests and the promotion of natural regeneration are therefore crucial for sustaining methane-regulation services and mitigating climate change. Finally, we emphasize that the spatial heterogeneity of Amazonian landscapes may not be fully captured by our sampling design, and that seasonal variability beyond the sampled periods may also influence microbial dynamics. Nevertheless, the study encompasses land-use systems commonly investigated in the region and reveals consistent patterns of soil microbial responses to forest-to-pasture conversion and regeneration.

5. Conclusion

Although the effects of forest-to-pasture conversion on soil microbial communities associated with CH_4 cycling in Amazonian soils are well known, it is crucial to understand the dynamics of these communities in areas undergoing regeneration. Our study shows that archaeal and bacterial communities, as well as those associated with the CH_4 cycle, are altered by land-use change and that these effects are mainly due to alterations in soil chemical properties. We also found that seasonal variation influences these soil communities and amplifies the effects of land-use change.

We further emphasize that although methanogens are favored in pastures and methanotrophs in primary forests, our secondary forest results indicate a recovery process and some degree of resilience in the CH_4cycling microbiota. However, the structure and function of the overall community may not fully recover in the short term, but instead exhibit partial recovery, reinforcing the importance of maintaining primary forests. Thus, although secondary forests may represent an important strategy for climate change mitigation, we emphasize the need for further studies in forest recovery areas, covering a broader spatial-temporal scale.

CRediT authorship contribution statement

Jéssica A. Mandro: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis. **Júlia B. Gontijo:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Data curation. **Fernanda M. Nakamura:** Writing – review & editing, Writing – original draft, Methodology. **Clovis D. Borges:** Writing – review & editing, Methodology. **Raimundo Cosme de Oliveira Jr:** Writing – review & editing, Methodology. **Erika Berenguer:** Writing – review & editing, Methodology. **Brendan J.M. Bohannan:** Writing – review & editing, Funding acquisition, Conceptualization. **Klaus Nüsslein:** Writing – review & editing, Funding acquisition, Conceptualization. **Jorge L. Mazza Rodrigues:** Writing – review & editing, Funding acquisition, Conceptualization. **Siu M. Tsai:** Writing – review & editing, Supervision, Resources, Methodology, Funding acquisition, Conceptualization. **Andressa M. Venturini:** Writing – review & editing, Supervision, Methodology, Investigation, Data curation.

Declaration of competing interest

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

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.temicr.2026.100069.

Data availability

16S rRNA sequence data were deposited on NCBI's Sequence Read Archive (SRA) under the accession number PRJNA1191494.

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