Received: 15 September 2022 | Revised: 24 January 2023 | Accepted: 27 February 2023

DOI: 10.1111/mec.16912

ORIGINAL ARTICLE

MOLECULAR ECOLOGY WILEY

Shifts in functional traits and interactions patterns of soil methane-cycling communities following forest-to-pasture conversion in the Amazon Basin

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Funding information
Canada First Research Excellence Fund; Conselho Nacional de Desenvolvimento Científico e Tecnológico, Grant/Award Number: CNPq - 311008/2016-0; 140953/2017-5; 307670/2021-0; Coordenação de Aperfeiçoamento de Pessoal de Nível Superior, Grant/Award Number: CAPES- 001; 88887.185941/2018-00; Fundação de Amparo à Pesquisa do Estado de São Paulo, Grant/Award Number: FAPESP- 2014/50320-4; 2015/13546-7; 2016/24695-6;, 2015/13546-7 and 2016/24695-6;; National Science Foundation - Dimensions of Biodiversity, Grant/Award Number: DEB 1442214; Natural Sciences and Engineering Research Council of Canada

Handling Editor: Aurélie Bonin

Abstract

Deforestation threatens the integrity of the Amazon biome and the ecosystem services it provides, including greenhouse gas mitigation. Forest-to-pasture conversion has been shown to alter the flux of methane gas (CH_4) in Amazonian soils, driving a switch from acting as a sink to a source of atmospheric CH_4. This study aimed to better understand this phenomenon by investigating soil microbial metagenomes, focusing on the taxonomic and functional structure of methane-cycling communities. Metagenomic data from forest and pasture soils were combined with measurements of in situ CH_4 fluxes and soil edaphic factors and analysed using multivariate statistical approaches. We found a significantly higher abundance and diversity of methanogens in pasture soils. As inferred by co-occurrence networks, these microorganisms seem to be less interconnected within the soil microbiota in pasture soils. Metabolic traits were also different between land uses, with increased hydrogenotrophic and methylotrophic pathways of methanogenesis in pasture soils. Land-use change also induced shifts in taxonomic and functional traits of methanotrophs, with bacteria harbouring genes encoding the soluble form of methane monooxygenase enzyme (sMMO) depleted in pasture soils. Redundancy analysis and multimodel inference revealed that the shift in methane-cycling communities was associated with high pH, organic matter, soil porosity and micronutrients in pasture soils. These results comprehensively

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characterize the effect of forest-to-pasture conversion on the microbial communities driving the methane-cycling microorganisms in the Amazon rainforest, which will contribute to the efforts to preserve this important biome.

KEYWORDS
land-use change, metagenomics, methanogens, methanotrophs, microbial networks, soil functioning

1 | INTRODUCTION

The Amazon basin, with an area of ~5.3 million km^2, constitutes 40% of the world's rainforests. This biome provides important local, regional and global ecosystem services, including climate regulation, carbon sequestration, hydrological cycling and greenhouse gas (GHG) uptake (Aragão et al., 2014; Strand et al., 2018). However, despite enormous efforts to preserve the Amazon biome over the last decades, deforestation and agribusiness continue to be a significant threat (Artaxo, 2019; Rajão et al., 2020). It is estimated that ~20% of the Brazilian Amazon forest has already been converted into agricultural areas (Artaxo, 2019), with the predominant use (~89%) being cattle ranching (Amazonía, 2020). Amazon deforestation and cattle ranching are closely intertwined with GHG emissions in Brazil (Lapola et al., 2014; Malhi et al., 2008), and has led to a significant decline in the carbon sink capacity of the Amazon (Gatti et al., 2021).

Methane (CH_4) is one of the most affected GHGs due to land-use change (LUC) in the Amazon, including an increasing production (+11%) by cattle enteric fermentation (Basso et al., 2021). In addition, studies conducted 30 years ago revealed that Amazonian forest soils, which normally are sinks of atmospheric CH_4, shift from sinks to sources of CH_4 emission following conversion from forest to pasture (Fernandes et al., 2002; Steudler et al., 1996). Methane cycling in upland soils is driven by microbial community dynamics in which CH_4 is produced by strictly anaerobic archaea (methanogens), and then oxidized by aerobic bacteria (methanotrophs) that use CH_4 as a carbon and energy source. It is estimated that 50%–90% of the CH_4 produced below ground is oxidized by methanotrophs before reaching the atmosphere (Conrad, 2009; Kotsyurbenko et al., 2004).

Methane production or methanogenesis is the final step in the anaerobic degradation of organic matter (Conrad, 2020; Serrano-Silva et al., 2014). Depending on the available substrates, three main metabolic pathways are used: (i) hydrogenotrophic, (ii) acetoclastic and (iii) methylotrophic methanogenesis (Evans et al., 2019). Acetoclastic and hydrogenotrophic pathways are the most common methanogenic pathways and contribute around 67% and 33% of CH_4 biosynthesis, respectively (Conrad, 2009; Kotsyurbenko et al., 2004). The role of methylotrophic methanogenesis is less clear (Conrad, 2020). However, the recent discovery of hydrogen-dependent methylotrophic methanogens in the order Methanomassiliicoccales suggests they may be common in soils (Söllinger & Urich, 2019). The last step of the three methanogenesis pathways consists of the reduction of methyl-coenzyme M to CH_4, which is catalysed by the methyl-coenzyme M reductase (MCR) enzyme complex (Evans et al., 2019). Two forms of MCR exist: the MCR-I which is present in all methanogens, while the isoenzyme MCR-II is only present in members of the classes Methanococci and Methanobacteria (Rospert et al., 1990).

Methane oxidation or methanotrophy involves two pathways, the Type I methanotrophs (γ-Proteobacteria) use the ribulose monophosphate (RuMP) pathway, while Type II (α-Proteobacteria) use the serine pathway (Knief, 2015). The first step in both methanotrophy pathways consists of the oxidation of CH_4 to methanol (CH_3OH), catalysed by the enzyme methane monooxygenase (MMO; Knief, 2015; McDonald et al., 2008). Two forms of MMO have been identified, the most common being a copper-containing membrane-bound enzyme particulate MMO (pMMO) found in nearly all methanotrophs, while the cytosolic iron-containing soluble (sMMO) form is present in just a few methanotrophs (Banerjee et al., 2019).

Studies in the Amazon Basin have demonstrated the impacts of LUC on soil microbiota (de Carvalho et al., 2016; Mendes et al., 2015; Navarrete et al., 2011; Paula et al., 2014; Rodrigues et al., 2013). Ground-breaking work at the Amazon Rainforest Microbial Observatory (AMRO) in the Brazilian state of Rondonia used a combined approach of quantifying methanotrophs and methanogens using functional genes, and metagenomics to characterize methane-cycling organisms (Meyer et al., 2017). Their study found that forest-to-pasture conversion at ARMO had few impacts on methanogen community, and suggested that the shift to methane emission may be due to altered methane consumption rates caused by changes to the methanotroph community. However, the study was limited to one unique site. More recently, an expanded study looking at multiple regions in Amazonia found that while all converted pastures emitted CH_4, the Rondonian pastures showed the highest CH_4 emissions. Using an amplicon sequencing approach, it was evident that the Rondonian pastures had the highest microbial richness, and contrary to Meyer et al. (2017), higher methanogen and lower methanotroph relative abundance (Meyer et al., 2020). Using undisturbed soil columns from the same sites, Kroeger et al. (2020) used a stable isotope probing (SIP)-DNA approach and determined that active methanogenesis was higher in pastures compared to rainforest soil cores. While these studies shed light on possible microbial processes driving the shift to methane emission, they were limited by the amplicon sequencing approaches, or laboratory-based studies. Gaps remain regarding the environmental factors driving increased

methanogenesis in pasture soils, and the enzymatic pathways that may be involved.

LUC affects several soil properties, including organic matter, carbon and nutrient cycling, pH, and soil structure and porosity (Oguike & Mbagwu, 2009) which are known to affect methanogenesis and methanotrophy (Serrano-Silva et al., 2014; Tate, 2015). A better understanding of the abiotic and biological processes regulating soil CH_4 flux will allow the prediction of conditions (e.g., land-use and management practices) that favour higher soil CH_4 oxidation rates and lower net CH_4 emissions (Fonseca De Souza et al., 2021; Tate, 2015). Furthermore, microbe–microbe interactions probably play a key role in these systems. Methanogens are known to be syntrophically related and cannot exist independently (Fowler et al., 2016; Kotsyurbenko, 2005; Zhang et al., 2020), while studies on methanotrophs suggest that their interaction with nonmethanotrophic microbes can modulate CH_4 oxidation (Ho et al., 2016, 2020). The analysis of microbial networks constitutes a useful tool to explore microbial community structure, enabling the predictions of community states, patterns of species interactions and the identification of hub species (Morriën et al., 2017; Röttjers & Faust, 2018).

In this study, we use metagenomic sequencing from field-collected soil samples to assess functional gene content and high-resolution taxonomic structure, as well as to analyse predicted microbe–microbe interaction networks in forest and pasture soils. The aims were to: (i) determine the community composition of methanogens and methanotrophs, and their relative abundance within the soil microbiota; (ii) determine the interaction range and "keystoneness" of CH_4-cycling microorganisms; and (iii) identify the shift in metabolic traits from CH_4-cycling communities in forest and pasture soils. Lastly, we use a multimodel inference approach to determine the relative importance of soil physicochemical properties and shifts in CH_4-cycling microbial community traits in predicting CH_4 fluxes (in situ measurements). Specifically, the analyses aimed to (i) identify the changes in soil abiotic factors underlying shifts in CH_4-cycling communities, and (ii) identify the best predictive variables of CH_4 fluxes, using integrated analysis of soil abiotic factors and microbial functional traits.

2 | METHODS

2.1 | Study area and sampling

This study was carried out at "Agropecuaria Nova Vida" (09°54′58″S, 63°02′27″W), a 20,000-ha cattle ranch in the central region of Rondônia state, Brazil (Figure 1). Habitats in this area belong to the Amazon biome, where the local climate is humid tropical and classified as Af (Koppen's classification), with a mean annual temperature of 25.5°C and precipitation of 2200 mm (Alvares et al., 2013). The soil is classified as Ultisol (Santos et al., 2018). Forest soil comprises areas of primary (undisturbed) forest. The pasture areas were established in 1972, following slash-and-burn soil preparation with no mechanical interventions or chemical fertilization (Feigl et al., 2006). Pastures are managed by cattle rotation, fire applied only to control eventual pests, mechanical removal of invasive trees, and at least one record of liming 15 years before the sampling.

Soil and gas samples were taken in April 2017 at the end of the rainy season, which occurs from October to May. The samples were

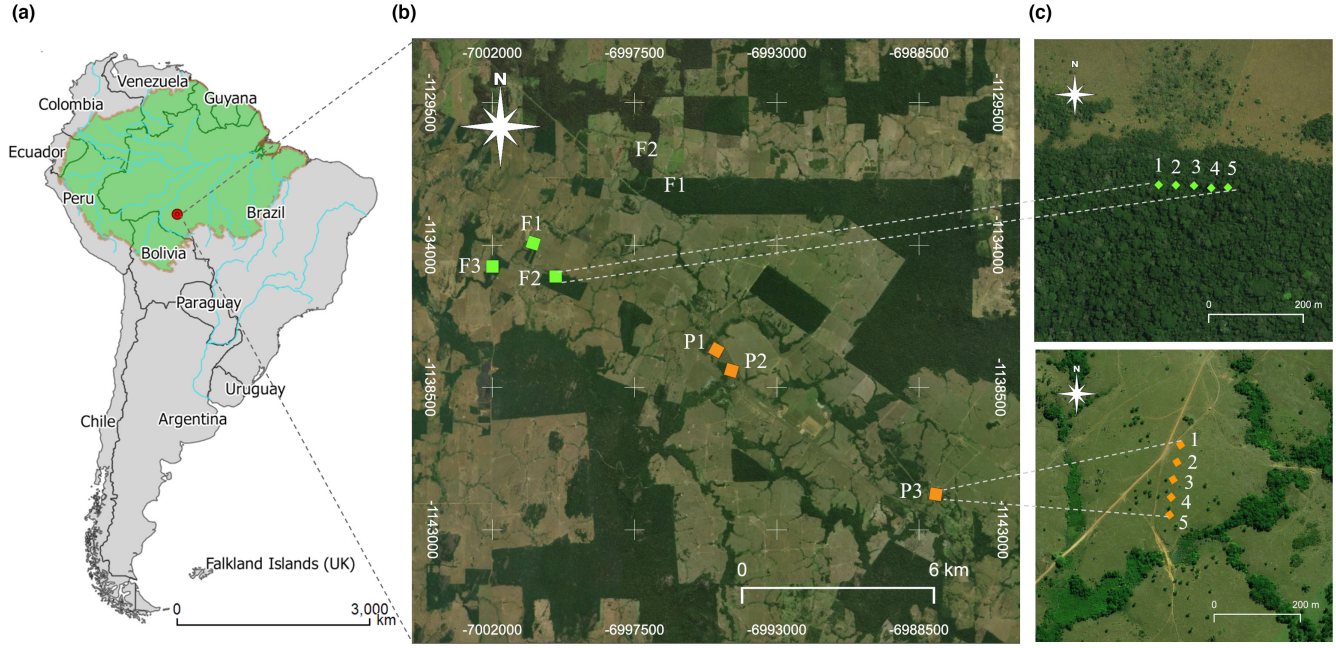

FIGURE 1 Geographical location of the study area. (a) Map of South America showing the Amazon Basin (green). The red dot indicates the location of the "Agropecuaria Nova Vida" Ranch wherein the study was conducted, situated in the Brazilian state of Rondônia. (b) Aerial view of the sampled sites. Dots indicate the three sampling sites in primary forests (F1, 2, 3), and pasture (P1, 2, 3) soils. (c) At each site, a transect (200 m) with five sampling points 50 m apart was delineated.

collected from three sites in each land use (forest and pasture). Within each site, a transect with five points 50 m distant was established (Figure 1), totalling 15 sampling points ($n = 15$) per land use. At each point, surface litter material was removed and static cylindrical chambers (with removable lid) were used for gas sampling. The dimensions of the chambers were 12 × 30 cm (total volume of 6.3 L) and were inserted 3 cm deep in the soil. Gas samples were taken four times after closing chambers (0, 10, 20 and 30 min), using plastic syringes and pre-evacuated (<0.05 kPa) 20-mL glass vials as gas sample containers. The vials were crimp sealed with a 20-mm blue butyl rubber stopper (Chemglass Life Sciences). The measurements of gas concentration were made in the laboratory within 1 week, as detailed below.

After gas sampling, soil samples were collected from the top layer (0–10 cm) and kept on ice. Once in the laboratory, one part of the soil sample was kept at −20°C for DNA extraction, and the other part was kept at 4°C for the measurement of chemical properties. Additional undisturbed soil cores (5.0 × 5.0 cm) were collected in the centre of the topsoil layer (0–10 cm) for soil physical analyses.

2.2 | Methane flux measurement and processing

The CH_4 concentration in the gas samples was measured using an SRI8610C gas chromatograph (GC; SRI Instruments), with a flame ionization detector (FID). Nitrogen was used as a carrier gas at a flow rate of 25 cm^3 min^{-1}. Oven and detector temperatures were 50 and 150°C, respectively. Standard CH_4 samples (White Martins) were used for GC calibration. CH_4 flux calculations were performed using a simple linear regression model of change in the concentration as a function of the incubation time within the chamber. Daily CH_4 flux (F, μg CH_4-C m^{-2} h^{-1}) was calculated according to Equation 1, proposed by Ussiri and Lal (2009):

$$F = \left(\frac{\Delta g}{\Delta t}\right)\left(\frac{V}{A}\right)k \quad (1)$$

where $\Delta g/\Delta t$ is the linear change in CH_4 concentration inside the chamber (i.e., μg CH_4-C chamber min^{-1}), V is the chamber volume (m^3), A is the surface area circumscribed by the chamber (m^2) and k is the time conversion factor (60 min). Chamber CH_4 concentration was previously converted from a molar mixing ratio (ppm), determined by GC analysis, to volumetric mass density by assuming an ideal gas law.

2.3 | Soil physical–chemical properties

Soil chemical properties were analysed at the Laboratory of Soil Fertility, Luiz de Queiroz College of Agriculture, USP, Brazil, as described by van Raij et al. (2001). Briefly, soil pH was measured from $CaCl_2$ suspension. Organic matter (OM) was measured by the colorimetric method. Total nitrogen (TN) was extracted and determined by the combustion catalytic oxidation method. Available phosphorus (P) and potassium (K^+) were determined by colorimetry and atomic emission spectroscopy, respectively. Calcium (Ca) and magnesium (Mg) were determined by atomic absorption spectrometry, and aluminium (Al) was determined by acid–base titration. The total exchangeable bases (EB) was calculated as the sum of Ca^{2+}, Mg^{2+} and K^+. Potential acidity (H) is the sum of Al^{3+} and H^+. The potential cation exchange capacity ($CEC_{pH7.0}$) was calculated as the sum of EB and potential acidity. The percentage of base saturation (v) was calculated as the relationship between EB and CEC. The aluminium saturation (m) was calculated as the relationship between Al^{3+} and effective cation exchange capacity (ECEC), where ECEC is the total amount of exchangeable cations plus Al^{3+}. Available micronutrients (Fe^{2+}, Mn^{2+}, Zn^{2+} and Cu^{2+}) were extracted with diethylenetriaminepentaacetic acid (DTPA).

To determine macro- and microporosity, soil cores were saturated and subjected to drainage at −6 kPa in the tensile table, as described in Teixeira et al. (2017). Soil macroporosity (Mac) comprised pores larger than 50 μm, which drained at −6 kPa. Microporosity (Mic) included pores smaller than 50 μm that retained water at −6 kPa. Soil bulk density (BD) was calculated from the relationship between dry soil mass (oven-dried at 105°C for 24 h) and cylinder volume. Total porosity (TP) was calculated using two methods: (i) soil saturation (TPs), and (ii) calculated (TPc) from the relationship between BD and particle density (PD) measured with a pycnometer.

2.4 | DNA extraction and quantitative PCR analyses

DNA extraction was carried out from 250 mg of each soil sample ($n = 15$ in each land use) using a PowerLyzer PowerSoil DNA Isolation Kit (Qiagen), according to the manufacturer's protocol with modifications. Briefly, after adding C1 solution, the stirring and centrifugation times were extended to 15 and 3 min respectively (Venturini et al., 2020). DNA quality and concentration were measured using a NanoDrop 1000 spectrophotometer (Thermo Scientific).

The abundance of methanogens and methanotrophs was assessed in each sample through quantitative polymerase chain reaction (PCR) assays targeting the marker genes *mcr*A, *pmoA* and *mmoX*, using referenced primer sets (Table S1), which were purchased from Sigma-Aldrich Chemical. For each qPCR assay, standard curves were constructed based on purified PCR products, performed with DNA extracted from strains of *Methanolinea mesophila* (DSMZ 23604), used for the *mcr*A gene, and *Methylosinus sporium* (DSMZ 17706) used to construct the *pmoA* and *mmoX* standard curves. Both strains were obtained from the German Collection of Microorganisms and Cell Cultures (DSMZ; Leibniz Institut DSMZ). The starting gene copy number was calculated with the DNA copy number calculator at the URI Genomics and Sequencing Center website (http://cels.uri.edu/gsc/cndna.html). The standard curves included six DNA concentrations in a range of 10^1–10^7 DNA copies μl^{-1}.

The qPCRs were made in a 10-μl final volume, containing 5 μL of 2× SYBR Green ROX qPCR Master Mix (Thermo Scientific), 0.5 μM of

each primer, 0.5 μM bovine serum albumin (BSA; Thermo Scientific), 1 μL of total DNA (10–30 ng μl^{-1}) and nuclease-free water. Each DNA sample and each dilution in the standard curve were analysed in triplicate. The qPCR amplifications were carried out as follows: 95°C for 30 s; 42 cycles of 94°C for 15 s, 56°C for 30 s and 72°C for 30 s. Also, a melting curve was included, between 65 and 95°C with increments of 0.5°C at 5 s. The reactions were carried out in a StepOne Plus thermocycler (Applied Biosystems), and the results were analysed using STEPONE Software version 2.3 (Applied Biosystems). The R^2 values remained between 96% and 101% in all the trials, and the slope remained at −3.28 ± 0.15, −3.27 ± 0.10 and − 3.30 ± 0.14 in the *pmoA*, *mmoX* and *mcr*A assays, respectively. Results were expressed as gene copy numbers per gram of dry soil (log copy number g^{-1} soil).

2.5 | Whole metagenome shotgun sequencing and annotation

Three out of five sampling points per site were selected for whole metagenomic sequencing (WGS). In total, 18 DNA metagenome samples (n = 9 in each land use) were processed using the TruSeq kit (Illumina) for library preparation, according to the manufacturer's protocol for WGS in an Illumina HiSeq 2500 platform (2×100-bp paired-end). The WGS sequences were preprocessed and annotated with the web application server Metagenomic Rapid Annotations using Subsystems Technology (MG-RAST), pipeline version 4 (Meyer et al., 2008). Briefly, the raw sequences were processed by quality control (QC) using SOLEXAQA software by removing low-quality segments using the "Dynamic Trim" method (Cox et al., 2010), according to the lowest Phred score of 15 and a maximum of five bases below the Phred score. Subsequently, artificial replicate sequences and singletons were removed (Gomez-Alvarez et al., 2009).

The identification of protein-coding regions (features) was based on the protein database M5nr for a nonredundant integration of many protein databases (Wilke et al., 2012). The taxonomic origins of the features were determined using the RefSeq database (O'Leary et al., 2016), and the functional profiles were analysed according to the SEED subsystems database. Annotation parameters were those recommended by Randle-Boggis et al. (2016) using MG-RAST: maximum *e*-value cut-off of 1e-5, and minimum alignment length of 15 bp, while the minimal identity cut-off was 60% and 80% for taxonomic and functional profiling respectively, allowing a trade-off between sensitivity and accuracy. Abundance profiles were determined using the "Representative Hit Classification" method. During the analysis, features annotated as eukaryotic and viral sequences were removed.

2.6 | Microbial co-occurrence networks

The taxonomic profiling from each metagenomic data set (land use) was used to infer co-occurrence networks of interacting bacteria and archaea at the species level. We used the SparCC method from the package "SpiecEasi" (Friedman & Alm, 2012) implemented in R version 3.6.3 (R Core Team, 2021) to build the correlation matrix. All bacteria and archaea were considered in the input tables, but only significant positive correlations (SparCC > 0.75; $p < .01$) were included in the networks. Several metrics were used to explore the topology and strength of the networks (i.e., number of nodes and edges, weighted degree, the diameter of the network, modularity and the clustering coefficient). We explored several centrality metrics (i.e., betweenness centrality, harmonic centrality and closeness centrality; Newman, 2008) to infer the "keystoneness" of each node, which is defined as the ability of a species to alter the abundance of other species and the structure of the community (Cagua et al., 2019). Keystone taxa are highly connected taxa (i.e., high network centrality) that individually or in a guild exert a considerable influence on microbiome structure and functioning, and thus its alterations can have knock-on effects on ecosystem functions and services (Banerjee et al., 2018). We selected eigenvector centrality for the analysis because it takes into account both the number of connections of a given node and its relevance in terms of influence within the network (Ruhnau, 2000). The calculations and network visualizations were done with the software GEPHI 0.9.2 (Bastian & Jacomy, 2009).

2.7 | Statistical analysis

Average values of CH_4 flux, as well as the abundance of marker genes (*pmoA*, *mmoX* and *mcr*A) and the gene ratios, were compared between the two land-use groups using the two-tailed Student's *t* test, with Welch's correction (95% confidence interval). Soil properties were compared using the Multiple *t* test methods (*t* test per row) with Holm–Sidak correction. Statistical analyses and graphs were performed and made using GraphPad PRISM software version 8.0.1 (GraphPad Software).

Differences in taxonomic and functional profiles between whole microbiomes from pasture and forest soils were tested using permutational multivariate analysis of variance (PERMANOVA) and visualized using nonmetric multidimensional scaling (NMDS), both based on the Bray–Curtis dissimilarity index. PERMANOVA and NMDS were performed using PAST software (Hammer et al., 2001).

The differential abundances of well-known methanogens (Evans et al., 2019) and methanotrophs (Knief, 2015) were tested in the context of the overall microbiome composition at the species level using the ALDEX2 method (Fernandes et al., 2014) in R version 1.26.0. ALDEX2 is recognized as a powerful method for differential taxonomic analysis because of its low false discovery rate (FDR; Nearing et al., 2022). In this approach, low-abundance (<10 counts) and low-prevalence (<10%) taxa were removed from the unrarefied taxonomic table, and then Monte Carlo samples of Dirichlet distributions were generated to account for the uncertainty in the proportions of each sample. The proportional data were then transformed using the centred log ratio (clr) transformation (Aitchison, 1986), which made the data more symmetrical and allowed standard statistical analysis. Wilcoxon and

Welch's tests were used, and the results were the median difference in clr values between groups, the median effect size and *p*-values adjusted according to the Benjamini–Hochberg FDR method to avoid the inflation of type-I error (Benjamini & Hochberg, 1995).

The functional microbiome profiles involved in CH_4-cycling were compared between forest and pasture soils. We compared the abundance of genes encoding the enzymes pMMO, sMMO and MCR, but also looked at the abundance of key enzymes involved in the three methanogenic pathways (i.e., acetoclastic, hydrogenotrophic and methylotrophic) according to the MetaCyc pathway mapping (Superpathway of methanogenesis; PWY-6830; Caspi et al., 2018). The differences between land uses were detected by comparing the $\log_2$ fold change (LFC), using Wald's test as implemented in the DESEQ2 method (Love et al., 2014) in the R package (version 1.38.2). The DESEQ2 pipeline is intended for differential gene analysis on smaller data sets (<20 samples per group) of RNA sequencing (RNA-seq) or shotgun sequencing data, which frequently have overdispersion (Quince et al., 2017; Weiss et al., 2017). The method assumes a negative binomial distribution of the count data and models it using the maximun-likelihood estimation (MLE) method. It includes shrinkage estimation of dispersions and fold-changes for each feature, yielding accurate FDRs and FDR-corrected *p*-values (Love et al., 2014). For consistency and improved data visualization, the relative abundance of taxa or functional genes was shown as clr-transformed, which shows how genes (or taxa) behave relative to the per-sample average, using the geometric mean in each sample as a reference (Quinn et al., 2019).

The relationship between gene abundance (log-transformed) and soil properties from pasture and forest sites was analysed using redundancy analysis (RDA) following Box–Cox transformation of the explanatory variables (soil properties). A similar analysis was performed to infer the relationship between taxon abundance and soil properties. The Hellinger transformation was applied to the taxonomic table (i.e., including only methanoges and methanotrophs) to generate the distance matrix suitable for RDA (Legendre & Gallagher, 2001). The selection of explanatory variables was performed by the forward-selection method, which has correct levels of type-I error and power (Legendre et al., 2011). The RDAs were performed using the CANOCO 5 software (ter Braak & Šmilauer, 2012).

We used a multimodel inference approach (Burnham & Anderson, 2002) to determine the contribution or importance of soil biotic (abundance of functional groups or genes) and abiotic (physicochemical properties) factors on the CH_4 emissions. Because we aimed to compare the suitability of different types of microbial data, we carried out separate analyses based on: (i) the abundance of genes *mcr*A, *pmoA* and *mmoX* as quantified by qPCR, and (ii) the abundance of key enzyme-coding genes involved in the methanotrophy and methanogenesis pathways obtained from WGS analysis. The two analyses were performed using the same set of physicochemical properties. To avoid multicollinearity, a set of predictor variables was chosen in each analysis, based on their collinearity with other properties (i.e., assessed using both Pearson correlation and variance inflation factor [VIF]). For each of the data sets (i.e., functional groups or genes), we first created a global model using linear mixed-effects models (lme) in the R package "nlme" (Pinheiro & Bates, 2019). This global model included all the selected explanatory variables as fixed effects, as well as "site" as a random effect. To validate the global model in terms of homoscedasticity and normality, residuals were analysed using graphical tools. Then, we compared models with different sets of predictors using the function "dredge" in the R package "MuMIn" (Barton, 2019). This function generates a set of models with all possible combinations of predictors and ranks them by second-order Akaike Information Criterion (AICc; Burnham & Anderson, 2002). In addition, we calculated the relative importance of the different predictor variables, which is based on the AIC weight of the models in which the variable appears.

3 | RESULTS

3.1 | Increase in soil CH_4 flux and CH_4-cycling microorganisms after forest-to-pasture transition

Methane fluxes were higher in pasture than forest soils ($t = 1.97$, $p < .05$; Figure 2a). As expected, forest soil acted as a CH_4 sink, with an average of $-67.14 \pm 34.40\,\mu g\,m^2\,h^{-1}$ (95% CI: −140.9 to 6.64). Pasture soils, on the other hand, exhibited net CH_4 emissions, with an average of $23.82 \pm 30.63\,\mu g\,m^2\,h^{-1}$ (95% CI: −41.88 to 89.53), although CH_4 consumption was observed at some sampling points.

A high abundance of methanogens (*mcrA* gene) was observed in pasture soils ($t = 13.96$, $p < .001$; Figure 2b). Different abundance patterns were observed among the methanotrophs based on the quantification of the *pmoA* and *mmoX* genes. While *mmoX*-harbouring methanotrophs were more abundant in forest soils ($t = 6.56$, $p < .001$), *pmoA*-harbouring methanotrophs were more abundant in pasture soils ($t = 8.55$, $p < .001$; Figure 2b). In addition, the gene ratios *pmoA*/*mcrA* and *mmoX*/*mcrA* were both higher in forest soils (Figure 2c), but the differences were more significant for *mmoX*/*mcrA* ($t = 7.39$, $p < .001$). Despite the observed imbalance in the abundance of methanogens and methanotrophs, the CH_4 flux was poorly explained by both *pmoA*/*mcrA* ($R^2 = .09$; $p = .10$) and *mmoX*/*mcrA* ($R^2 = .02$; $p = .51$) gene ratios (Figure 2d).

3.2 | Changes to the taxonomic composition of CH_4-cycling communities

From the 18 metagenomes, an average of $99{,}082{,}073 \pm 11{,}765{,}887$ sequences resulted from the quality control process (Post QC) from the pasture soil samples, while the average of sequences that passed the QC process in the forest soil samples was $106{,}355{,}652 \pm 14{,}815{,}574$. From then, an average of $37{,}827{,}472 \pm 4{,}458{,}689$ were annotated as bacteria and archaea (RefSeq database) in pasture soil samples, and $36{,}488{,}031 \pm 6{,}392{,}840$ in forest soil samples (Table S2). Overall, the taxonomic profiling of the whole metagenomic data set (Bacteria and Archaea) revealed significant differences (PERMANOVA; $F = 4.6$;

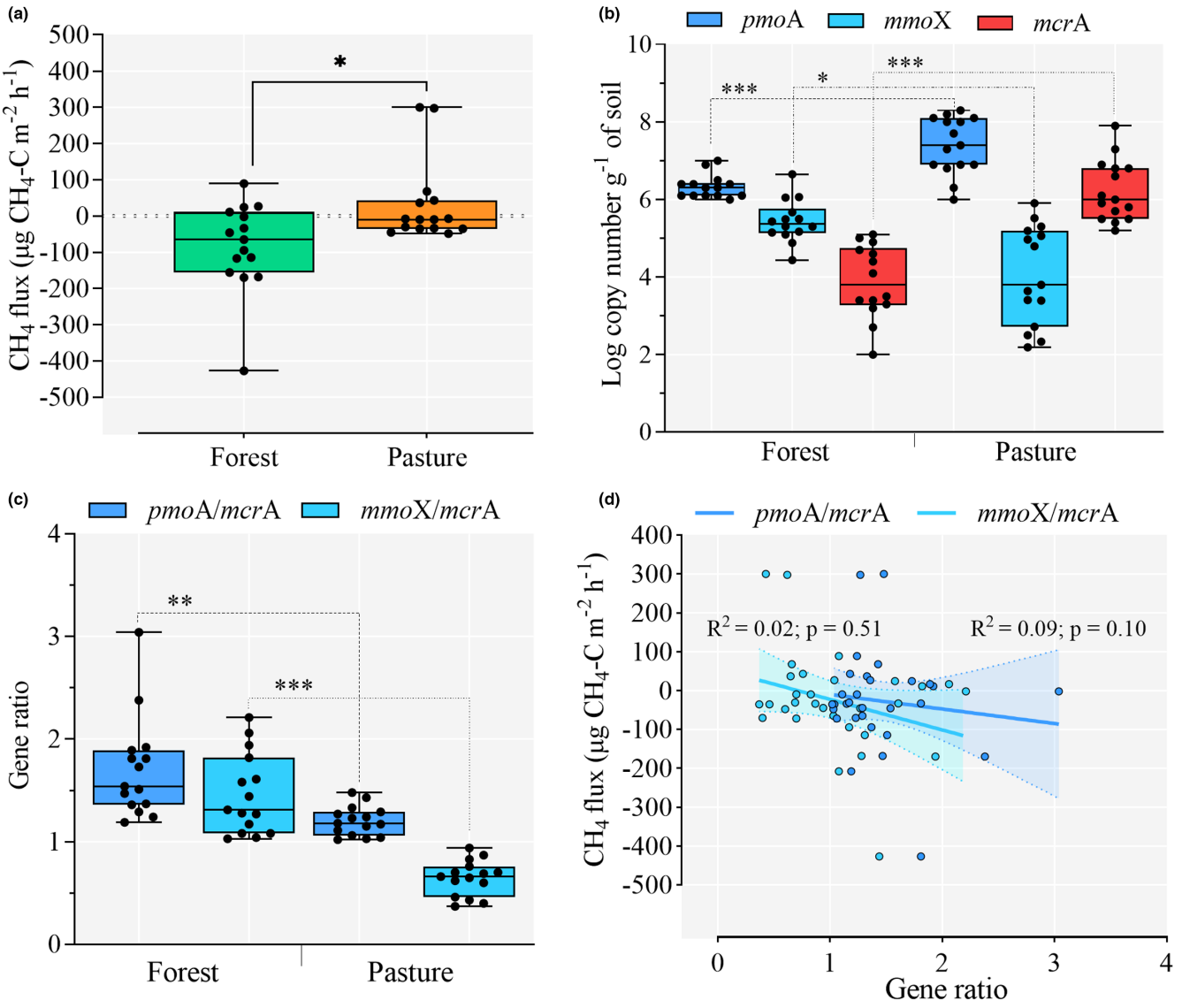

FIGURE 2 Methane flux and abundance of microbial functional genes associated with methanotrophy and methanogenesis in Amazon forest and pasture soils. (a) Methane flux in forest and pasture soils, measured in micrograms of CH_4-C (carbon contained in methane) per square metre per hour. (b) Abundance (gene copy number from DNA) of *mcrA* (methanogens) and *pmoA* and *mmoX* genes (methanotrophs). (c) Gene ratio indicates how many times the abundance of the functional genes of methanotrophy (*pmoA* and *mmoX*) contains the abundance of *mcrA*. Asterisks denote statistically significant differences (*$p < .05$; **$p < .01$; ***$p < .001$). (d) Relationships between the CH_4 flux and gene ratios; linear models were fitted for each ratio of genes (i.e., the ratio of each methanotrophic marker and *mcrA*).

$p < .001$) in community composition between forest and pasture soils (Figure S1). When exploring methanogenic and methanotrophic communities, it was found that methanotrophs had a higher relative abundance than methanogens, but were only represented by five bacterial taxa (Figure 3a). Yet, methanogens were more diverse, comprising 26 archaeal taxa (at the species level), belonging to seven orders among which the most frequent were Methanosarcinales (28%), Methanococcales (24%), Methanobacteriales (20%) and Methanomicrobiales (16%; Figure 3a). Methanotrophs were more abundant in forest soils than those observed in pastures, while methanogens were more abundant in pasture soils, except for three species of *Methanocaldococcus* (i.e., *M. jannaschii*; *M. fervens*, *M.* sp. FS406-22; *M. vulcanius*; Figure 3b).

3.3 | Keystoneness of methane-cycling taxa within microbial co-occurrence networks

Co-occurrence networks constructed from the taxonomic profiles from forest and pasture soils (Figure S2) revealed differences in the interaction patterns of microbial communities (Table 1). Although the number of nodes (connected taxa) was similar in both soils, the number of correlations (edges) in forest soils was twice as high as in pasture soils, indicating that the microbiota in the undisturbed forest soils is highly interconnected. The microbial network from forest soils consisted of fewer modules, a higher number of nodes per module and twice the average edges per node than the network from pasture soils (Table 1; Figure 4a,b).

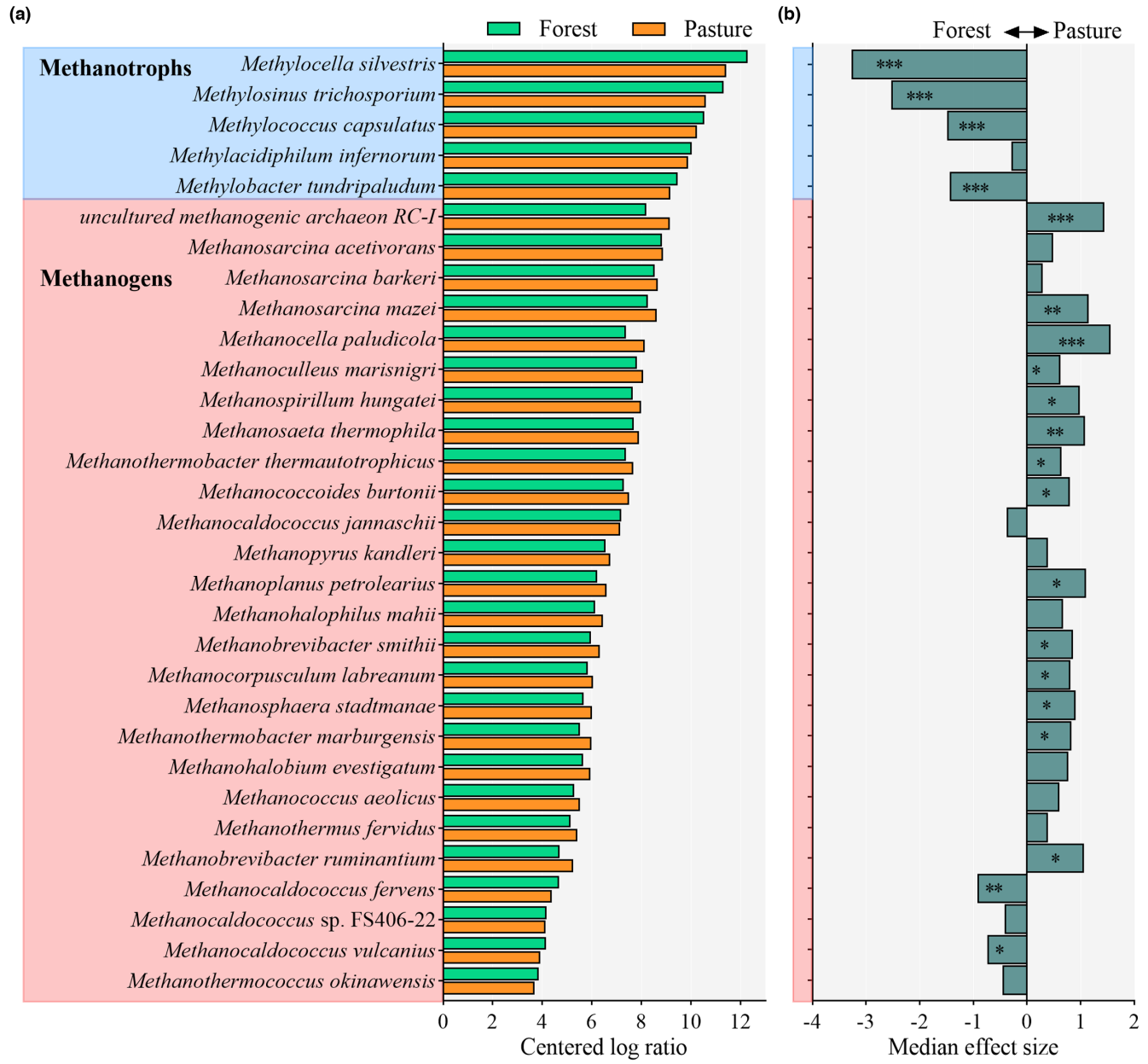

FIGURE 3 Changes to the relative abundance of methane-cycling taxa between forest and pasture soils, measured from metagenomic sequencing data. (a) Identity and relative abundance (centred log-ratio, clr) of methanogenic archaea and methanotrophic bacteria (species level). (b) Differential abundance of each taxon between forest and pasture soils as median effect size (diff.btw/max(largest difference)) and *p*-value. Asterisks denote statistically significant differences according to Benjamini–Hochberg adjusted *p*-value (***$p < .001$, **$p < .01$, *$p < .05$).

Among the nodes from both networks, 31 belonged to methane-cycling taxa, including 26 methanogens and five methanotrophs (Figure 4a,b). Methane-cycling taxa exhibited a greater degree of interactions within the microbial networks from forest soils, including interactions within methanogens but also between methanogens and methanotrophs and other microorganisms not directly related to CH_4 metabolism (Table 1). Overall, the average number of connections from methane-cycling taxa was three-fold higher in forests than in pasture soils. In addition to being more connected, they also showed a greater ecological relevance, exhibiting higher centrality within the microbial networks in forest soils (Figure 4c,d).

3.4 | Metagenomic analysis of functional markers of methanogenesis and methanotrophy

Annotated functional features corresponding to Bacteria and Archaea, based on the SEED subsystems database, resulted in an average of 38,627,142 ± 4,311,728 and 36,533,213 ± 6,549,490 hits from pasture and forest soil respectively (Table S2). Based on this, the functional profiles significantly differed ($F = 2.05$; $p = .05$) between land uses (Figure S3).

Genes encoding the subunits of the pMMO enzyme were slightly more abundant in pasture soils, while the subunits of sMMO were

TABLE 1 Topological parameters of co-occurrence networks of bacterial and archaeal species in forest and pasture soils and the interaction patterns of methane-cycling taxa

Network features and connectedness of CH_4-cycling	Forest	Pasture
Nodes	1472	1452
Methanogens	26	26
Methanotrophs	5	5
Edges	104,734	47,010
Network diameter	12	10
Average degree	142.3	64.7
Weighted degree	124.3	56.2
Average path length	4.2	4.2
Modularity	0.61	0.68
Number of modules	10	8
Average clustering coefficient	0.72	0.64
Edges from methane-cycling***	3588 (3.4%)	750 (1.6%)
Edges within methane-cycling*	178 (0.2%)	62 (0.1%)
Edges methanogens-methanotrophs	0	0
Edges from methanogens***	2988 (2.9%)	485 (1.0%)
Edges from methanotrophs*	776 (0.7%)	264 (0.6%)
Edges within methanogens*	176 (0.2%)	58 (0.1%)
Edges within methanotrophs	2 (0.0%)	4 (0.0%)

Note: Nodes represent taxa with at least a significant ($p < .01$) and positive (SparCC > 0.75) correlation. Edges represent the number of connections/correlations obtained by SparCC analysis. Modularity represents the strength of connections between the nodes within different communities. The number of modules is the number of communities in the network. Network diameter indicates the longest distance between nodes in the network. Average path-length is the average length of the shortest path between any possible pairs of network nodes. The average degree represents the average number of edges per node in the graph. Weighted degree is the sum of the weights of all edges attached to the node. The average clustering coefficient indicates how nodes are embedded in the network. Interactions of methane-cycling represent the percentage of edges connecting nodes from methanogens and methanotrophs. Significant differences between land uses were tested by the "two-proportion Z-test" (*$p < .05$; **$p < .01$; ***$p < .001$).

more abundant in forest soils (Figure 5). The analysis revealed that the two isoforms of methyl coenzyme M reductase (MCR-I and MCR-II) were more abundant in pasture soils. MCR-I was five-fold more abundant in pasture than in forest soils (Wald test, $p < .001$), while MCR-II was twice as high (Figure 5).

Acetoclastic methanogenesis was the most abundant pathway in both pasture and forest soils (Figure 6). A total of 11 out of 12 genes were more abundant in pasture soils in comparison to forest soils. The gene coding for the enzyme formylmethanofuran (formyl-MFR) dehydrogenase (EC 1.2.7.12) was more abundant in forest soils (Figure 6). This enzyme catalyses a reversible reaction in hydrogenotrophic methanogenesis by reducing carbon dioxide (CO_2) to form carboxy-MFR. However, the alternative forms of this enzyme, tungsten-containing and molybdenum-containing formyl-MFR dehydrogenases, were more abundant in pasture soils. The analysis also found an increased abundance of all genes encoding enzymes involved in methylotrophic methanogenesis in pasture soils (Figure 6).

3.5 | Influence of soil abiotic factors on the abundance of methanogens and methanotrophs

Pasture soils showed higher pH and consequently a reduced percentage of Al saturation, as well as higher available micronutrients (Fe, Mn, Zn and Cu). Other soil chemical properties showed no detectable differences between land uses (Table S3). We observed changes in soil structure, specifically in the ratio of micro- and macropores, with a predominance of micropores in pasture soils and macropores in forest soils.

RDA on the relationships between methane-cycling gene abundances and soil properties (Figure 7a) revealed a clear effect of soil properties on the differentiation between land uses. Soil porosity, pH and micronutrient content (i.e., Mn, Cu and Zn) were the main drivers influencing gene abundance profiles. The abundance of CH_4-cycling taxa was similarly associated with soil properties, but included significant effects only from soil macroporosity and Cu content (Figure 7b).

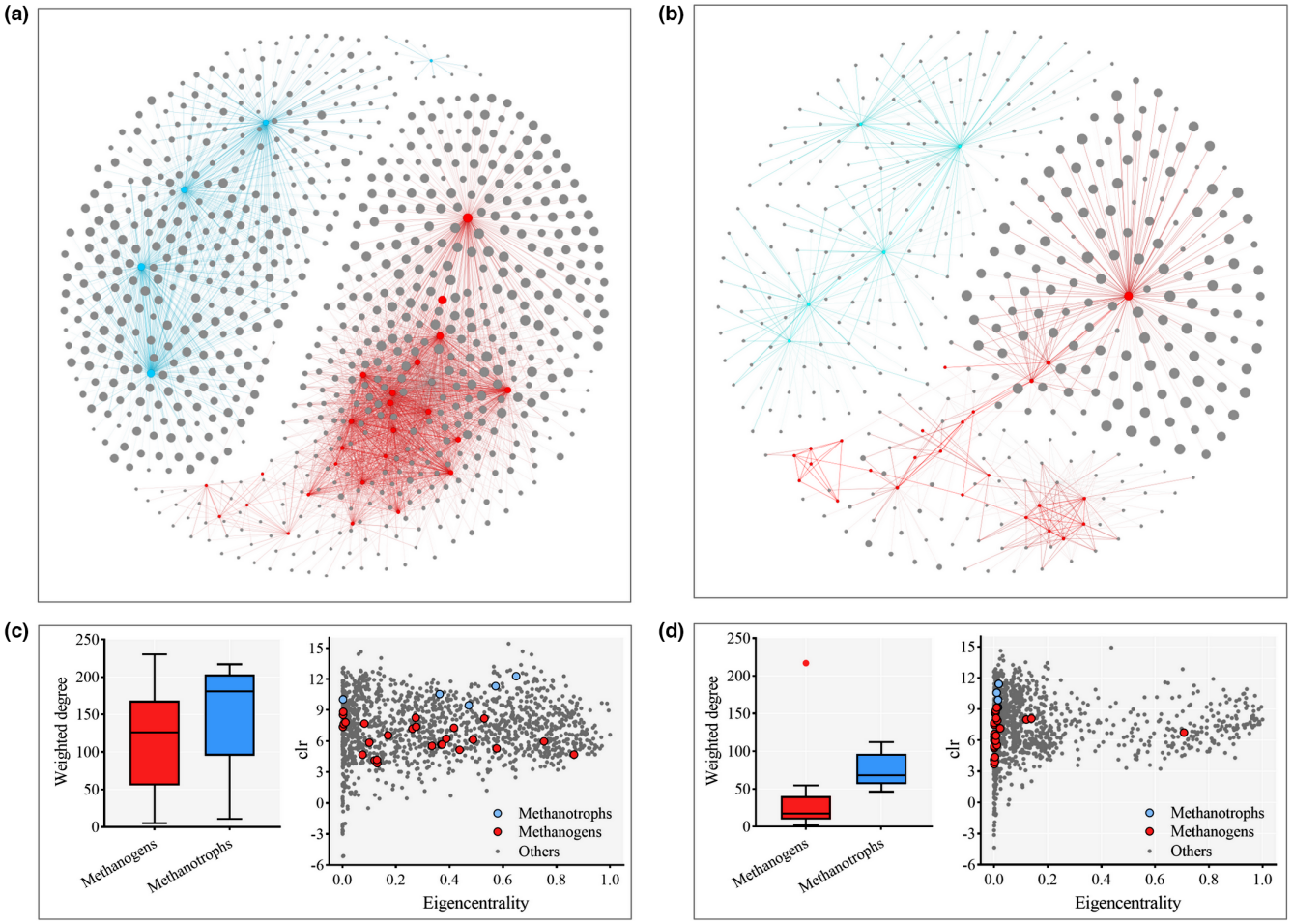

FIGURE 4 Co-occurrence networks of CH_4-cycling within the archaeal and bacterial communities from forest and pasture soils. Partial networks, including the interactions of the methane-cycling taxa (species level) in (a) forest and (b) pasture soils. In each plot, the nodes correspond to methanogens (red) and methanotrophs (blue), as well as all other taxa (grey) with which they co-occur. Connecting edges indicate significant and strong positive SparCC correlations ($r > .75$; $p < .01$). Only nodes with at least one significant correlation are represented. The colour of edges corresponds to its node of origin, and the size of the nodes is proportional to its eigencentrality value, which indicates the influence of a node in a network. Bottom figures depict the "keystoneness" of methane-cycling taxa within microbial interaction networks from (c) forest and (d) pasture soils, as its relationship with relative abundance (clr). Keystoneness was inferred in terms of connectedness (weighted degree) and eigencentrality in the networks.

3.6 | Identification of the best predictors of CH_4 flux based on soil factors and microbial functional traits

Only variables of low multicollinearity were included in the multimodel inference analyses to prevent overparameterization. Therefore, based on the correlations among the several edaphic factors initially considered, only pH, OM, Cu and the macropore/micropore ratio were included in further analysis (Figure S4). Similarly, for gene abundance data we included only *mcrA* and the ratio *mmoX*/*pmoA*, while from the metagenomic data set we selected the gene encoding the enzyme sMMO (methanotrophic) and four methanogenic enzymes (Figure S5).

The multimodel inference including gene abundance data produced a total of 63 models. The 10 best models, as ranked by AICc, included mostly edaphic properties and only 30% (3/10) included the gene ratio of methanotrophic genes (Figure 8a). In fact, the four best models did not include any microbial gene data and the R^2 did not improve significantly when including them in the model. Overall, R^2 values were relatively low, with only 16%–29% of the methane flux explained by the best models. The higher contribution of edaphic properties can also be seen in the importance of the predictors (Figure 8a), which is calculated as the sum of "Akaike weights" over all the models that include explanatory variables. The macropore/micropore ratio, Cu and OM content were the most important drivers of CH_4 flux, followed by the gene ratio *mmoX*/*pmoA* (Figure 8a).

Results were different when including metagenomic data instead of qPCR gene abundance. Of the 512 models produced by the analysis, the 10 models with the highest AICc included at least one methane-cycling enzyme, usually from methanogenesis pathways (Figure 8b). Soil OM was the only edaphic factor included in

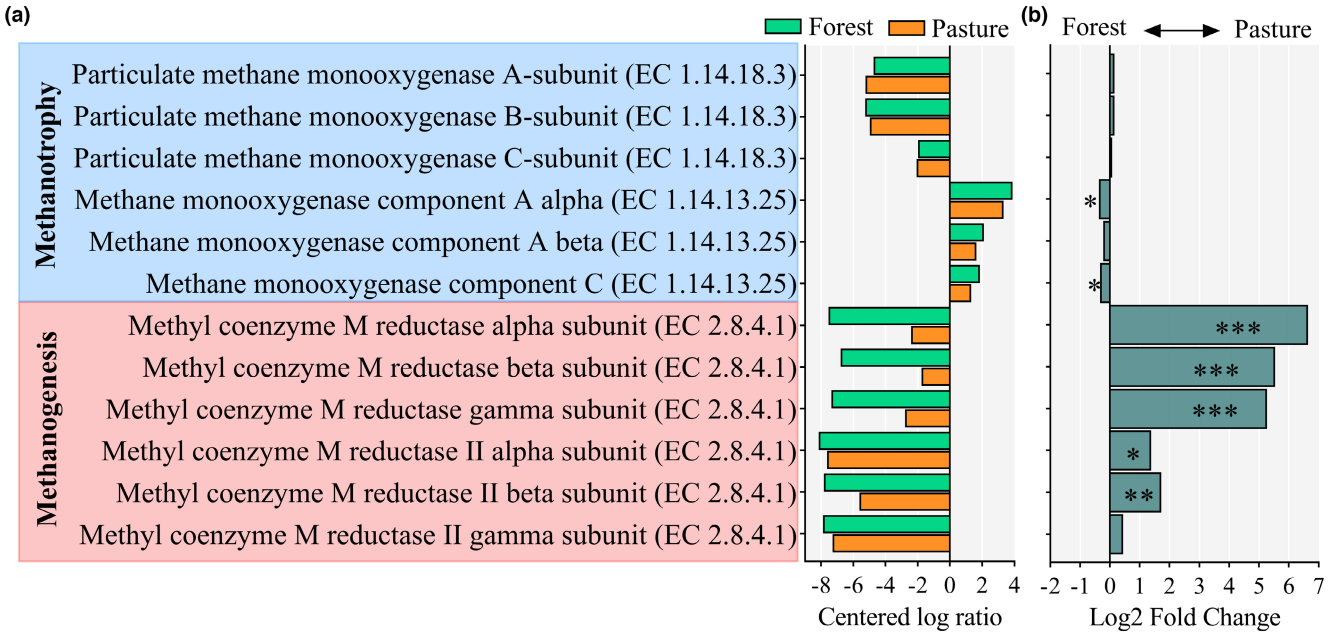

FIGURE 5 Differential abundance of the genes encoding key enzymes of methanotrophy (MMO) and methanogenesis (MCR) between forest and pasture soils, as measured by metagenomic sequencing. (a) The two types of MMO are shown: the copper-containing membrane-bound enzyme particulate MMO (pMMO), and the cytosolic iron-containing soluble form (sMMO). The two forms of MCR are presented: MCR-I, which is present in all methanogens, and MCR-II, which is present in only a few methanogenic taxa. The relative abundance (centred log-ratio transformed) of the main subunit is presented in each case. The centred log-ratio (clr) transformation uses the geometric mean (g) of the sample as the reference, and thus features have negative values when their abundance is less than the geometric mean. (b) Differential abundance of each enzyme (subunit) between forest and pasture soils as $\log_2$ fold change (a value of 1 indicates that it is twice as abundant). Asterisks denote statistically significant differences according to the Benjamini–Hochberg adjusted *p*-value (***$p < .001$, **$p < .01$, *$p < .05$).

the best models. Moreover, these models explained 38%–75% of methane fluxes, with most models exhibiting an $R^2 > .5$. The enzyme methanol–corrinoid protein methyltransferase (EC 2.1.1.90), implicated in methylotrophic methanogenesis, was the most important factor predicting CH_4 fluxes. Other enzyme-encoding genes following in importance were those from hydrogenotrophic and acetoclastic methanogenesis (Figure 6) and the enzyme sMMO from methanotrophy.

4 | DISCUSSION

The disruption of natural ecosystems associated with LUC often leads to increased emissions of CH_4 to the atmosphere (McDaniel et al., 2019). The results from this study support previous work showing that forest-to-pasture conversion in the Amazon rainforest reverts the role of soil from sink to source of CH_4 (Fernandes et al., 2002; Steudler et al., 1996; Verchot et al., 2000). These results also confirm recent studies indicating that this phenomenon is associated with alterations in the abundance and structure of methane-cycling communities (Kroeger et al., 2020; Meyer et al., 2017, 2020). Yet, beyond previous discoveries, our findings provide a comprehensive characterization of the transformations of methane-cycling communities in response to forest-to-pasture conversion in the Amazon. For instance, similar to Meyer et al. (2017, 2020), we used field-based measurements and samplings to measure the abundance and taxonomic structure of the communities, but we used species-level differential taxonomic analysis, and this is the first study to assess the microbe–microbe interaction patterns of methane-cycling in the region, and the first study to use constrained analysis (RDA) to identify the main abiotic drivers of methanogens and methanotrophs associated with LUC in the Amazon. Furthermore, Kroeger et al. (2020), using SIP incubations and metagenomics, detected an increased functional diversity and activity of methanogens in pasture soil. Extending their results, we use field-based measurements (i.e., environmental DNA) of metabolic traits of the communities, and we use that data to explain the differences in CH_4 fluxes between land use.

We found that type II methanotrophs *Methylosinus trichosporium* and *Methylocella silvestris*, and the type I *Methylococcus capsulatus*, are among the most abundant taxa in Amazon forest soils, consistent with findings by Meyer et al. (2017). However, these authors also found an overall high abundance of *Methylocystis rosea* (type II), which was not observed in our analyses. Interestingly, both studies found a greater abundance of *Methylacidiphilum infernorum* in forest soils, an acidophilic methanotrophic aerobic bacterium from the phylum Verrucomicrobiota. This bacterium grows optimally at pH ~2.5 (Dunfield et al., 2007), and thus could be well suited for the acidic conditions of Amazon soils. In concordance with Meyer et al. (2017), we also found a great diversity

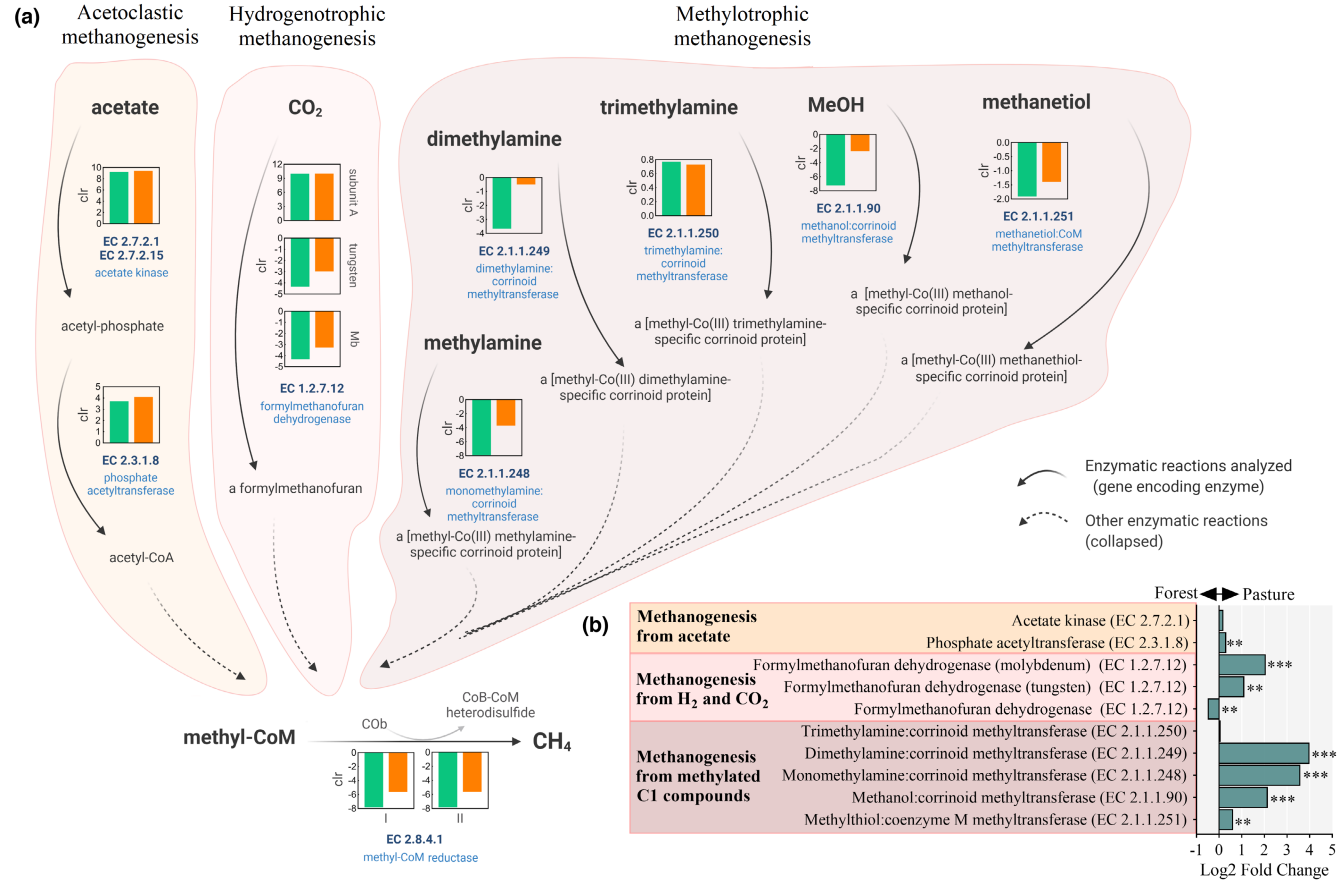

FIGURE 6 Differential abundance of the methanogenesis pathways from different substrates. (a) The abundance of the genes encoding enzymes from the first enzymatic reactions in each pathway are shown as their relative abundance (centred log-ratio, clr) in forest and pasture soils. The pathway maps are adapted from the "superpathway of methanogenesis" according to the MetaCyc database. Enzyme Commission (EC) numbers are provided according to MetaCyc and KEGG databases. (b) For a clearer view of the differences between land uses, the selected enzyme-encoding genes are represented as the magnitude of $\log_2$ fold change. Asterisks denote statistically significant changes according to the Benjamini–Hochberg adjusted *p*-value (***$p < .001$, **$p < .01$, *$p < .05$).

of methanogenic archaea inhabiting both forest and pasture soils, which belonged to seven out of the eight well-known orders from the classes Methanobacteria, Methanococci, Methanomicrobia and Methanopyri (Evans et al., 2019). Among the most abundant methanogens in pasture soils was the hydrogenotrophic Rice Cluster I, which is widely distributed and generally found living in rice roots (Conrad et al., 2006). Other abundant species were from the genera *Methanosarcina* and *Methanocella*, which seem to be ubiquitous in aerated soils and adapted to fluctuating oxic–anoxic conditions as soil water content changes (Angel et al., 2012).

The results of our network analyses suggested a loss of connectedness and influence of CH_4-cycling microbes within the soil microbiota following forest-to-pasture conversion. We hypothesize that changes in the interaction patterns of these microorganisms could be related to changes in nutrient availability. Nutrient availability can increase negative interactions between microbes by stimulating competition (Ratzke et al., 2020), thus affecting the stability of the microbial communities (Lozupone et al., 2012). Here, nutrient availability in pasture soils may have been increased by tree removal, changes in soil cover and slash-and-burn management practices (Feigl et al., 2006; Petersen et al., 2019). Slash-and-burn often leads to an increase in soil nutrient availability due to the input of nutrient-rich ash (De Souza Braz et al., 2013; Fujisaka et al., 1996). The nutrient input following slash-and-burn has been reported to shape bacterial community composition in Amazonian soils, dominated by fast-growing high-nutrient-requiring (i.e., copiotrophic) taxa (e.g., Actinomycetota) and fewer oligotrophic stress-tolerant taxa (e.g., Chlamydiota, Planctomycetota and Verrucomicrobiota) in deforested soils (Navarrete, Tsai, et al., 2015; Rodrigues et al., 2013).

Microbial interaction networks may become more complex in conditions limiting substrate availability since co-occurrence leads to greater metabolic exchange (Zelezniak et al., 2015). We assume that deforestation disrupts the microbial food web, in a way that CH_4-cycling microorganisms are less relevant. For instance, methanotrophs showed a significant reduction of connections between them and with other nonmethane-cycling microbes in pasture soils (Figure 4). Although not autotrophs, methanotrophs are the base of food webs acting as an accessible carbon source for heterotrophs (Hutchens et al., 2004; Kalyuzhnaya et al., 2013; Karwautz et al., 2018). In this regard, a network meta-analysis conducted by

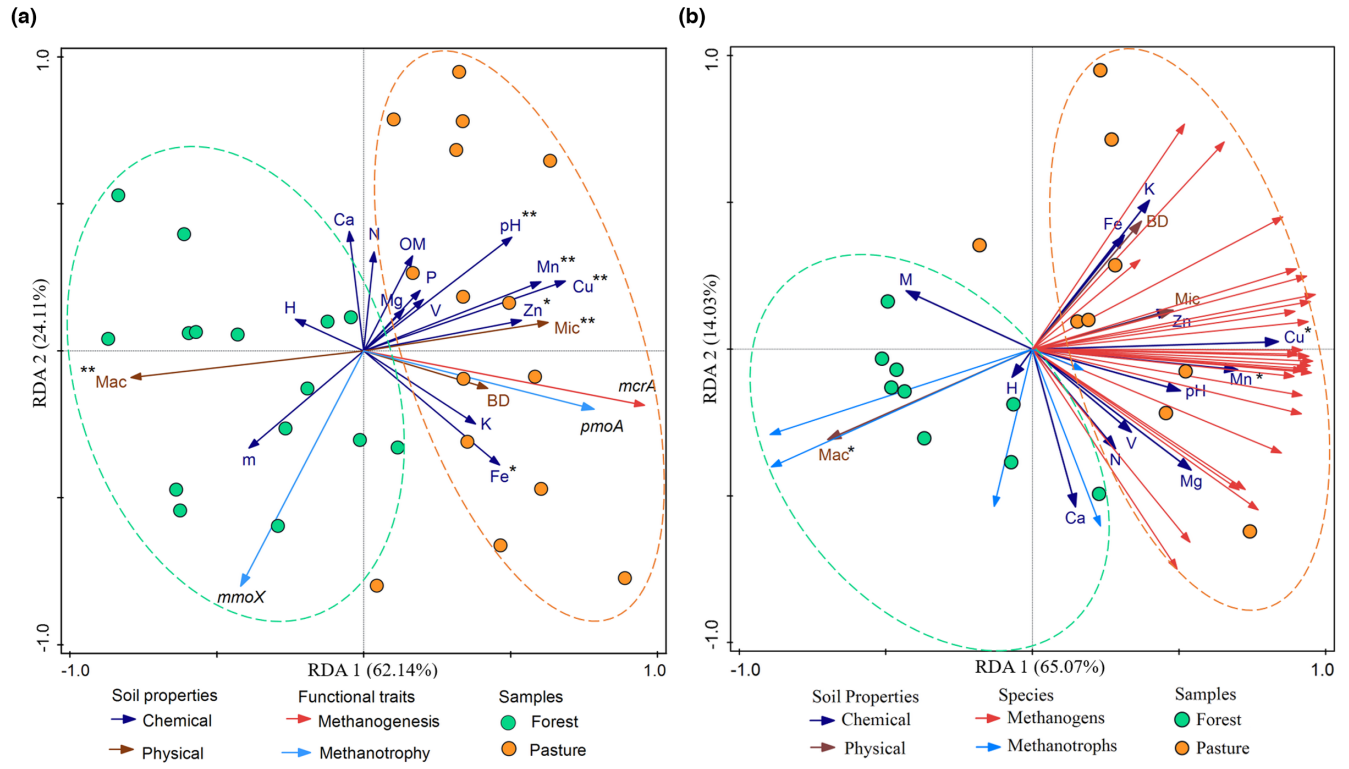

FIGURE 7 Redundancy analysis (RDA) links soil physicochemical properties to methane-cycling communities. (a) Analysis based on methane-cycling gene abundance profiles (*mcrA*, *pmoA* and *mmoX*) from qPCR. (b) Analysis based on the composition of methanogenic and methanotrophic communities as obtained by metagenomic sequencing (at the species level). Vector points toward an increase for a given soil property and gene abundance, and its length indicates the strength of between-variable correlation and ordination scores. Asterisks indicate explanatory variables with significant contributions (*$p < .05$, **$p < .01$, ***$p < .001$).

Ho et al. (2016) revealed a CH_4-derived food web in diverse ecosystems, in which methylotrophs were always present underlying the cross-feeding between methanotrophs and methylotrophs, whereas nonmethanotrophic communities were site-specific and not consistent among the diverse ecosystems. Consistent with our results, Ho et al. (2016) also found the lowest clustering around methanotrophs in pasture soils, suggesting that other microorganisms compatible with methanotrophs and more complex metabolic routes exist in pasture soils.

Methanotrophs that carry the soluble form of the enzyme MMO (sMMO) were depleted by LUC from forest to pasture, whereas those harbouring the particulate form of this enzyme (pMMO) were enhanced (Figures 2 and 5). These results provide strong evidence of functional diversification of the methanotrophic community due to the forest-to-pasture conversion. The selective process of *pmoA*- and *mmoX*-type methanotrophs is probably a consequence of changes in soil physical and chemical properties imposing a niche differentiation for these microorganisms (Ho et al., 2013, 2016). For instance, γ-proteobacterial methanotrophs (mainly *pmoA*-type) are predominant in neutral pH soils, while α-proteobacterial methanotrophs, comprising mostly *mmoX* type, are well adapted to acidic soils (Kolb, 2009). Consistently, the methanotrophic species with the highest reduction in abundance in pasture soils was *Methylocella silvestris* (Beijerinckiaceae), an *mmoX*-type with moderate acidophilic preference (Knief, 2015) usually found in forest soils (Dunfield et al., 2003).

Methanogenesis pathways also differentiated along with the forest-to-pasture conversion, with increased hydrogenotrophic and methylotrophic methanogenesis in pasture soils (Figure 6). Methylotrophic methanogens (mainly from the orders Methanosarcinales and Methanomassiliicoccales) are major contributors to CH_4 biosynthesis in marine sediments, where other methanogens are outcompeted by sulphate-reducing bacteria (Conrad, 2020). Yet, recent studies have shown that methylotrophic methanogens are much more diverse and widespread than expected (Borrel et al., 2013; Söllinger & Urich, 2019; Vanwonterghem et al., 2016). Our findings confirm results by Meyer et al. (2017), who reported that methylotrophic methanogenesis genes were significantly more abundant in pasture soils than in Amazon forest soils. These findings also support the idea that methylotrophy is an active methanogenesis pathway in Amazon soils that should be investigated further (Alves et al., 2022). Moreover, in microcosms supplemented with methanol, Alves et al. (2022) found that *Methanosarcina* members could be responsible for methylotrophic methanogenesis in Amazon pasture soils. *Methanosarcina* was consistently the only taxon found in our study that could include methylotrophic methanogens. Methyl-fermenting methanogens from Methanosarcinaceae

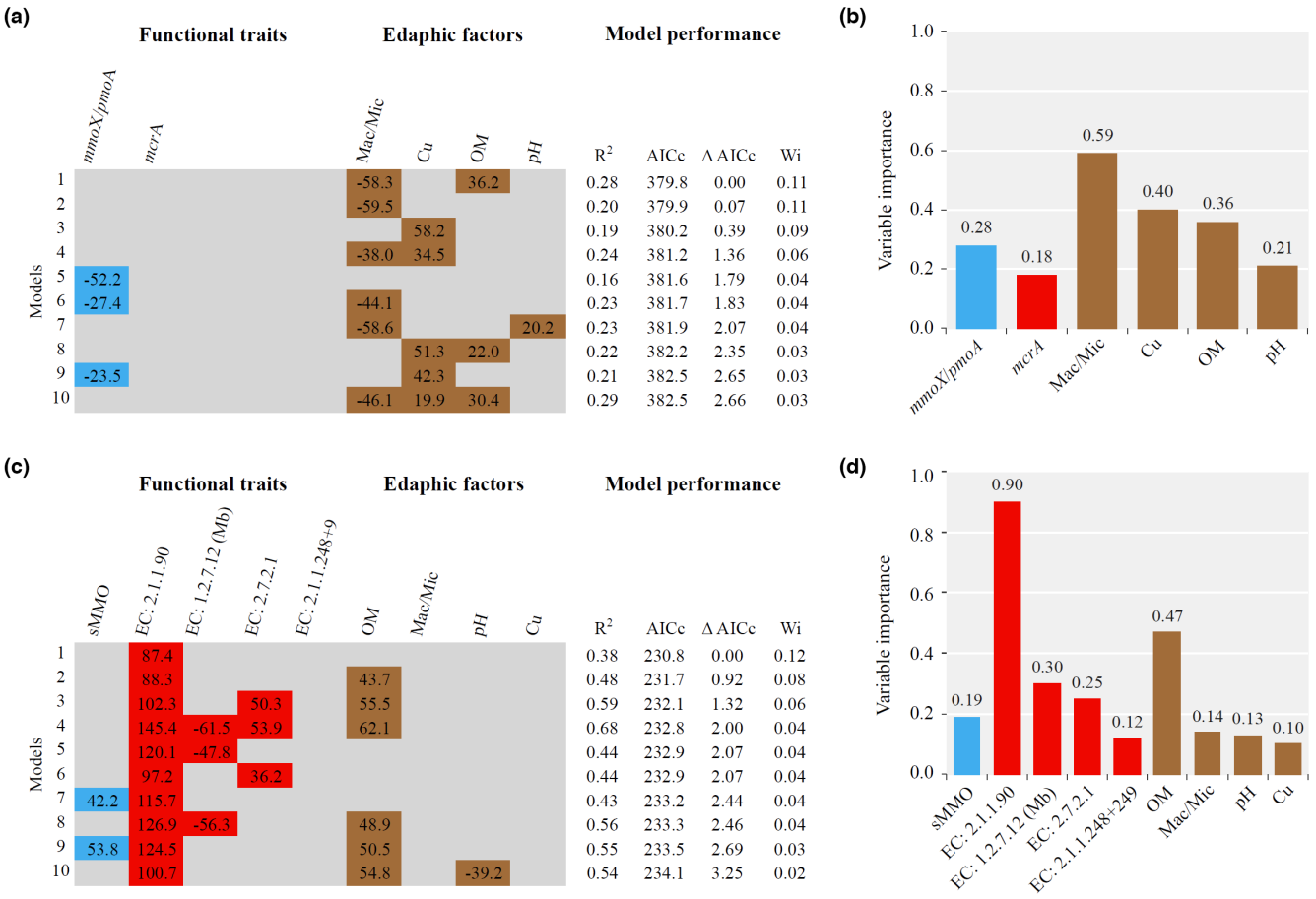

FIGURE 8 Best-fitting regression models explaining CH_4 flux in Amazonian soils along a forest-to-pasture conversion. (a) Multimodel analysis based on the abundance of functional genes (qPCR). (c) Multimodel analysis based on the abundance of key functional enzymes assessed through metagenomics. For each analysis, the 10 best-fitting models are presented in the tables; each row represents a model and each column is a different predictor variable (blue: functional markers of methanotrophy; red: functional markers of methanogenesis; brown: soil edaphic factors). Grey cells indicate variables that were not included in a particular model. The performance of the models is presented in terms of: R^2: likelihood-ratio based pseudo-R-squared; ΔAICc: difference between the AICc (corrected Akaike information criterion) of each model and the best model; and Wi: Akaike weights. (b, d) The relative importance of the different predictor variables (variable importance) was calculated based on the Wi of the models in which the variable appears.

possess a complete Wood–Ljungdahl pathway and can oxidize methyl groups at standard conditions of H_2, outcompeting methyl-reducing methanogens (Feldewert et al., 2020).

The observed microbial changes are likely to be linked to modifications in their abiotic environment. LUC induced modifications in soil abiotic factors, including pH, micronutrient content (i.e., Cu, Mn, Zn), and soil macro- and micropores (Table S3), consistently with previous reports in the Amazon region (Navarrete et al., 2010; Navarrete, Soares, et al., 2015; Paula et al., 2014; Ranjan et al., 2015). The increase in pH in pasture soils was positively associated with the abundance of methanogens and *pmoA*-type methanotrophs. Furthermore, the inversely proportional abundance of *pmoA* and *mmoX* methanotrophs also seemed to be associated with soil pH, since methanotrophs comprise taxa adapted to different pH (i.e., from alkaline to acidic soils; Ghashghavi et al., 2017; Knief, 2015; Kolb, 2009; Tate, 2015).

We found that the macro/micropore ratio was significantly reduced in pasture soils, probably due to compaction caused by animal trampling (Valladares et al., 2011); this was found to have significant impact on the abundance of methanogens and methanotrophs (Figure 7). Soil porosity is an important factor driving niche differentiation of both methanogens (strict anaerobes) and methanotrophs (Serrano-Silva et al., 2014; Tate, 2015). The low oxygen conditions may also impact methanotrophs, favouring the pMMO-containing methanotrophs that possess hemerythrin, an oxygen-binding protein that acts as an oxygen scavenger for the pMMO enzyme (Guerrero-Cruz et al., 2021). The higher Cu levels in pasture soils relative to forest soils also could be a key driver of the *mmoX*/*pmoA* ratio and the abundance of methanotrophs (Table S3; Figure 7). In laboratory experiments, Cu content has been shown to alter the physiology of methanotrophs. For example, high Cu conditions can suppress sMMO (*mmoX*) expression while increasing the expression of *pmoA*, a phenomenon known as the "copper switch" (DiSpirito et al., 2016; Semrau et al., 2010). Still, the physiological mechanism by which Cu regulates the expression and activity of MMO forms is still being investigated (Peng et al., 2022) and the Cu concentration threshold

for switching from sMMO to pMMO expression in soil has not been determined. We recommend further research on this topic along the forest-to-pasture conversion gradient in the Amazon region.

Besides characterizing changes in methane-cycling communities and their abiotic environment, we were also able to evaluate their capacity to predict methane emissions. As revealed by multimodel inference, the functional metagenomic profiling enabled a better prediction of CH_4 emissions than data derived from qPCR, possibly given the higher resolution of metagenomic data to split functional markers into different pathways. We also detected a slight change in the predictive value of different edaphic factors to explain CH_4 flux when combined with either functional gene quantification data (qPCR) or metagenomic functional profile data (Figure 8). For instance, soil porosity, followed by Cu and OM, were the most important predictors when qPCR data were used, while OM was the main abiotic predictor in models with functional metagenomic data. Such differences arise from the contrasting relationships between different biotic and abiotic variables (Figure 7). For example, Cu was associated with qPCR data, but it does not stand out when analysed in conjunction with metagenomic data because enzymes were better predictors of CH_4 fluxes. Interestingly, OM, which was not related to qPCR or taxonomic data in RDA, became the abiotic variable with the greatest importance in predicting CH_4 fluxes. This result suggests that OM, which shows a slight increase in pasture soils (+ ~8.75 g dm^{-3}), is influencing CH_4 fluxes possibly via the link of methanogenesis to the availability of substrates resulting from the degradation of OM (Conrad et al., 2009).

5 | CONCLUSION

While our study supports previous evidence that forest-to-pasture conversion in the Amazon impacts the abundance and taxonomic structure of soil methane-cycling communities, it also brings new insights into the changes affecting these communities. First, we found community assembly and microbe–microbe interaction patterns of methane-cycling taxa were also affected by LUC, resulting in a loss of connectedness of these microorganisms within the soil microbiota. Our results also revealed significant shifts in the potential functional capabilities of both methanogens and methanotroph communities, with a significantly increased abundance of marker genes of methylotrophic and hydrogenotrophic methanogenesis pathways in pasture soils. Methane uptake potential was also affected in pasture soils, with a significant reduction in sMMO-harbouring methanotrophs. Besides, we were able to link these shifts in methane-cycling communities to increased pH, compaction, OM and micronutrient content in pasture soils. Finally, we found that enzymes from the functional metagenomic profiling, with its higher resolution, enabled a better prediction of methane emissions than qPCR of marker genes. Overall, these results reinforce the importance of preserving the Amazon rainforest, which, in addition to maintaining biodiversity, contributes to atmospheric CH_4 sequestration, favouring the mitigation of this GHG on a global scale.

AUTHOR CONTRIBUTIONS

Dasiel Obregon Alvarez, Leandro Fonseca de Souza and Siu Mui Tsai designed the study with contributions from Lucas William Mendes and Plínio Barbosa de Camargo. The supporting research project was conceived and coordinated by Siu Mui Tsai, JLMR, Plínio Barbosa de Camargo and BJMB. Gas measurements and sample collection were performed by Leandro Fonseca de Souza, Plínio Barbosa de Camargo and Jorge L. Mazza Rodrigues. Leandro Fonseca de Souza performed gas chromatography and analysed gas data. Dasiel Obregon Alvarez and Leandro Fonseca de Souza performed the molecular analysis. Dasiel Obregon Alvarez and Leandro Fonseca de Souza analysed the metagenomic data. Moacir Tuzzin de Moraes analysed soil properties. Dasiel Obregon Alvarez conducted the statistical analyses with the support of Micaela Tosi. Dasiel Obregon Alvarez and Leandro Fonseca de Souza discussed the results with KM, Andressa Monteiro Venturini and Lucas William Mendes. Siu Mui Tsai funding. Siu Mui Tsai and KD advised. Dasiel Obregon Alvarez wrote the first draft with contributions from Leandro Fonseca de Souza, Micaela Tosi and Lucas William Mendes. Kari E. Dunfield, Kyle M. Meyer, Lucas William Mendes, Micaela Tosi and Jorge L. Mazza Rodrigues critically reviewed the manuscript. All the authors approved the final version of the manuscript.

ACKNOWLEDGEMENTS

We thank the Large Scale Biosphere-Atmosphere Program (LBA), coordinated by the National Institute for Amazon Researches (INPA), for the use and availability of data, for logistical support and infrastructure during field activities. We thank the owners and staff of Agropecuária Nova Vida for logistical support; and the owners Mr Aristeu, Dr Rigoberto and Mr Tocandira for permitting access to its lands. We thank Wagner Picinini and Alexandre Pedrinho for their assistance with field soil sampling. This research was supported by the São Paulo Research Foundation (FAPESP-2014/50320-4; 2015/13546-7; 2016/24695-6; 2017/09643-2; 2017/18327-7; 2018/09117-1; 2018/05223-1; 2020/12890-4), National Science Foundation – Dimensions of Biodiversity (DEB 1442214), Coordination for the Improvement of Higher Education Personnel – Brasil (CAPES- 001; 88887.185941/2018-00) and National Council for Scientific and Technological Development (CNPq – 311008/2016-0; 140953/2017-5; 307670/2021-0). MT was supported by Food from Thought, a programme funded by Canada First Research Excellence Fund (CFREF) and KD was supported by the Natural Sciences and Engineering Research Council (NSERC).

CONFLICTS OF INTEREST STATEMENT

The authors have no conflicts of interest.

DATA AVAILABILITY STATEMENT

Genetic data: The metagenome data set resulting from our QC and feature annotation are publicly available in the MG-RAST database (project mgp88746). Other data on soil properties and microbial analysis are included in this published article and its Supporting

Information. Sample metadata: Metadata are also stored in the MG-RAST repository (project mgp88746).

BENEFIT-SHARING STATEMENT

This research was developed with the collaboration of scientists from several institutions in three countries, creating a framework for the exchange of knowledge and know-how that strengthened the analysis and interpretation of the results. All data have been shared publicly via appropriate databases (MG-RAST project mgp88746).

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How to cite this article: Obregon Alvarez, D., Fonseca de Souza, L., Mendes, L. W., de Moraes, M. T., Tosi, M., Venturini, A. M., Meyer, K. M., Barbosa de Camargo, P., Bohannan, B. J. M., Mazza Rodrigues, J. L., Dunfield, K. E., & Tsai, S. M. (2023). Shifts in functional traits and interactions patterns of soil methane-cycling communities following forest-to-pasture conversion in the Amazon Basin. *Molecular Ecology*, *32*, 3257–3275. https://doi.org/10.1111/mec.16912