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Increased soil moisture intensifies the impacts of forest-to-pasture conversion on methane emissions and methane-cycling communities in the Eastern Amazon

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ABSTRACT

Climatic changes are altering precipitation patterns in the Amazon and may influence soil methane (CH_4) fluxes due to the differential responses of methanogenic and methanotrophic microorganisms. However, it remains unclear if these climate feedbacks can amplify land-use-related impacts on the CH_4 cycle. To better predict the responses of soil CH_4-cycling microorganisms and emissions under altered moisture levels in the Eastern Brazilian Amazon, we performed a 30-day microcosm experiment manipulating the moisture content (original moisture; 60%, 80%, and 100% of field capacity - FC) of forest and pasture soils. Gas samples were collected periodically for gas chromatography analysis, and methanogenic archaeal and methanotrophic bacterial communities were assessed using quantitative PCR and metagenomics. Positive and negative daily CH_4 fluxes were observed for forest and pasture, indicating that these soils can act as both CH_4 sources and sinks. Cumulative emissions and the abundance of methanogenesis-related genes and taxonomic groups were affected by land use, moisture, and their interaction. Pasture soils at 100% FC had the highest abundance of methanogens and CH_4 emissions, 22 times higher than forest soils under the same treatment. Higher ratios of methanogens to methanotrophs were found in pasture than in forest soils, even at field capacity conditions. Land use and moisture were significant factors influencing the composition of methanogenic and methanotrophic communities. The diversity and evenness of methanogens did not change throughout the experiment. In contrast, methanotrophs exhibited the highest diversity and evenness in pasture soils at 100% FC. Taken together, our results suggest that increased moisture exacerbates soil CH_4 emissions and microbial responses driven by land-use change in the Amazon. This is the first report on the microbial CH_4 cycle in Amazonian upland soils that combined one-month gas measurements with advanced molecular methods.

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1. Introduction

The Amazon rainforest is considered one of the Earth's most important reservoirs of biodiversity (Heckenberger et al., 2007). In addition, it provides crucial ecosystem services at local, regional, and global scales, such as water and nutrient recycling, watershed protection, erosion control, climate regulation, and carbon storage (Foley et al., 2007; Andersen, 2015). Nevertheless, the Amazonian biodiversity, together with the stability of the ecosystem services provided, is under increasing threat due to land-use and climate change, processes that are occurring simultaneously in the region and can interact with each other (Nobre et al., 2016). Deforestation and changes in land use in the Brazilian Amazon have been intensified since the 1970s mainly due to cattle ranching, but also as a consequence of the expansion of agriculture, logging, fires, and population growth (Fearnside, 2005; Arraes et al., 2012). It is estimated that 20% of the original forest cover has been cleared (Da Cruz et al., 2021), with more than 60% of this area converted into pastures (Almeida et al., 2016). Despite a decline in deforestation rates after 2004, these rates have increased sharply in recent years, reaching the highest values of the decade in 2020 (10,851 km^2) (INPE, 2021).

Land-use change in the Amazon is known to impact the overall soil bacterial and archaeal communities (e.g., Rodrigues et al., 2013; Paula et al., 2014; Mendes et al., 2015; Navarrete et al., 2015; Khan et al., 2019) and to influence nutrient cycling processes (Townsend et al., 2002; Asner et al., 2004; Cerri et al., 2004; Soltangheisi et al., 2019) and greenhouse gas fluxes (Garcia-Montiel et al., 2001; Melillo et al., 2001; Neill et al., 2005). In particular, forest-to-pasture conversion has been associated with an inversion of the soil methane (CH_4) fluxes from a sink to a source (Steudler et al., 1996; Verchot et al., 2000; Fernandes et al., 2002; Meyer et al., 2020) with several impacts on the soil CH_4-cycling communities in both Western and Eastern Amazon (Meyer et al., 2017, 2020; Kroeger et al., 2021).

As deforestation in the Amazon proceeds at high rates, major concerns are raised regarding future effects on the CH_4 cycle and their consequences at different scales. Moreover, land-use change impacts on this cycle could even be amplified by climate change. The Amazon region is already experiencing climatic changes in several ways. For instance, warming has reached 0.6–0.7 °C in the past 40 years, and according to recent climate models, an increase of about 6 °C may be expected by 2100, with even higher values for the Eastern Amazon (Marengo et al., 2018). Rainfall patterns in the Amazon have also undergone alterations over the last decades. Some studies indicated that annual and rainy season precipitation has increased in some parts of the basin while dry season precipitation has decreased, driving a stronger contrast between seasons (Gloor et al., 2013, 2015; Almeida et al., 2017). On the other hand, Haghtalab et al. (2020) revealed that, in general, wet areas exhibit a wetter trend (Western region), and dry areas, a drier trend (Eastern and Southern regions). Extreme weather events, such as floods and droughts, which are part of the region's natural climate (Marengo et al., 2013), have also become more frequent (Gloor et al., 2015). For example, in the Central Amazon, eight extreme flood events occurred from 1903 to 1998, while nine events have already been observed between 1999 and 2021 (Espinoza et al., 2022). It is also projected, considering a global warming of 2 °C and above, an increase in the frequency and intensity of extreme precipitation events and pluvial floods (Intergovernmental Panel On Climate Change - Ipcc, 2021).

Changes in the precipitation regime and, consequently, in the soil moisture content are known to impact CH_4-cycling microbial communities in soils, favoring CH_4-producing metabolisms under higher moisture conditions (Nazaries et al., 2013). Therefore, the combination of land-use and climate change effects could lead to unforeseen consequences to the balance of CH_4 in Amazonian soils. However, the combination of both factors is yet to be tested for the Amazon region. To date, the few studies available about these critical microbial communities focused on the impacts of the forest-to-pasture conversion on the soil CH_4-producing and -consuming microorganisms (Meyer et al., 2017, 2020; Kroeger et al., 2021), but not their interaction with altered environmental factors related to the ongoing climatic changes in the region.

Soil CH_4 fluxes result from the net balance between the activities of microbial producers (methanogens) and consumers (methanotrophs) (Zhang et al., 2018), which differ in their taxonomic, functional, and ecological characteristics. Methanogenic archaea are strictly anaerobic microorganisms that produce CH_4 as the final product of their metabolism using as main substrates carbon dioxide (CO_2) and hydrogen (H_2) (or formate and few alcohols), acetate, and methylated compounds. Therefore, according to the substrate used, they are classified as hydrogenotrophic, acetoclastic, and methylotrophic/methyl-reducing, respectively (Sorokin et al., 2017; Lyu and Liu, 2018). The production of CH_4 from methoxylated aromatic compounds was also recently described (Mayumi et al., 2016). Regardless of the metabolic pathway, all methanogenic archaea known to date share the methyl-coenzyme M reductase (MCR), the final enzyme in CH_4 formation; and the gene that encodes its alpha subunit (*mcrA*) is commonly used to detect and quantify these organisms in the environment (Luton et al., 2002; Lyu and Liu, 2018). All traditionally known methanogens belong to the phylum *Euryarchaeota* (Lyu and Liu, 2018), but recent culture-independent studies have revealed the presence of *mcr* genes in members of the candidate phyla *Bathyarchaeota* (Evans et al., 2015) and *Verstraetearchaeota* (Vanwonterghem et al., 2016).

In aerated soils, large fractions of the CH_4 produced are oxidized by aerobic methanotrophic bacteria, which can use CH_4 as their sole source of carbon and energy (Hanson and Hanson, 1996). These methanotrophs belong to the phyla *Proteobacteria* (Hanson and Hanson, 1996) and *Verrucomicrobia* (Dunfield et al., 2007; Pol et al., 2007; Islam et al., 2008). The former is frequently divided into type I and type II methanotrophs (classes *Gammaproteobacteria* and *Alphaproteobacteria*, respectively), distinguished by their main carbon fixation pathway (Knief, 2015). Similar to methanogens, these microorganisms can be studied by targeting the genes for methane monooxygenase (MMO), the enzyme responsible for the initial oxidation of CH_4 to methanol. The *pmoA* gene encodes the beta subunit of the particulate methane monooxygenase (pMMO) and is present in all aerobic methanotrophic bacteria except in the genera *Methylocella* and *Methyloferula*; whereas *mmoX* encodes the alpha subunit of the soluble methane monooxygenase (sMMO), which has a variable presence among methanotrophs even within the same genus (Knief, 2015; Dedysh and Knief, 2018). Methanotrophy can also occur under anoxic conditions by different processes associated with archaeal and bacterial taxa distinct from the previously described methanotrophs – sulfate-, nitrate/nitrite-, and metal ion- (iron – Fe^{3+} – and manganese – Mn^{4+}) dependent anaerobic CH_4 oxidation (Cui et al., 2015).

This study aimed to determine the effects of increased soil moisture on CH_4-cycling communities in rainforest and pasture soils in the Eastern Brazilian Amazon. Through a microcosm experiment with four moisture levels (original moisture, as found in the field; 60%, 80%, and 100% of moisture at field capacity), we investigated how the combination of land-use legacy and moisture content could affect the abundance, taxonomic and functional diversity of those microbial communities. Soil microbial communities were assessed using quantitative PCR (qPCR) targeting 16S rRNA and CH_4 metabolism genes and by metagenomic shotgun sequencing. Molecular data were also integrated with soil gas measurements to assess how the balance between methanogens and methanotrophs may relate to the emissions of this greenhouse gas.

2. Materials and methods

2.1. Site description, soil sampling, and physicochemical analysis

The two study sites are located in the Eastern Amazon Basin, in the Tapajós National Forest and surrounding areas, in the state of Pará, Brazil. This region has a tropical monsoon climate (Am) according to the Köppen's classification, characterized by the average temperature of the coldest month equal or higher than 18 °C (Alvares et al., 2013) and two well-defined seasons (rainy and dry), with a mean annual air temperature of 25.5 °C (minimum of 21.0 °C and maximum of 30.6 °C) and precipitation of 1,820 mm (Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis - IBAMA, 2004). The precipitation is irregularly distributed between seasons, and about 70% of the annual precipitation occurs in the rainy season, between December and June (De Oliveira Junior et al., 2015). In July 2015, soil samples from 0 to 10 cm depth (after the removal of the litter layer) were collected from a pristine forest (3°17′44.4″S 54°57′46.7″W) and an adjacent active cattle pasture (3°18′46.7″S 54°54′34.8″W) in three sampling points, separated by 50 m, per land-use type. According to satellite data and the pasture owners, the site was cleared more than 25 years before the sampling date for agriculture purposes and converted into pasture in 2007 (Supplementary Fig. S1). Soil samples from each site were transported to the laboratory, combined, and sieved through a 5 mm mesh sieve to remove litter material. Three subsamples of each representative sample were collected for physicochemical analysis and kept refrigerated until processing at the Department of Soil Science of the Luiz de Queiroz College of Agriculture (ESALQ/USP), Piracicaba, São Paulo, Brazil.

The following chemical properties were determined, according to standard procedures (Raij et al., 2001): pH determined in 0.01 M calcium chloride ($CaCl_2$); soil organic matter (SOM) determined by colorimetry; total nitrogen (N) determined by the Kjeldahl method; phosphorus (P) extracted with ion exchange resin and determined by the colorimetric method; sulfur (S) extracted with 0.01 M calcium phosphate ($Ca_3(PO_4)_2$) and determined by turbidimetry; potassium (K) extracted with ion exchange resin and determined by atomic emission spectrophotometry; calcium (Ca) and magnesium (Mg) extracted with ion exchange resin and determined by atomic absorption spectrophotometry; exchangeable aluminum (Al) extracted with 1 M potassium chloride (KCl) and determined by the colorimetric method; potential acidity (H + Al) determined with the Shoemaker-McLean-Pratt (SMP) buffer; boron (B) extracted with hot water and determined by colorimetry; copper (Cu), iron (Fe), manganese (Mn), and zinc (Zn) extracted with DTPA and determined by atomic absorption spectrophotometry. The calculations of the sum of exchangeable bases (SB), cation-exchange capacity (CEC), base saturation (V), and aluminum saturation (m) were made based on these previous results. Sand, silt, and clay contents were determined by the hydrometer method and classified according to the United States Department of Agriculture textural classification system (USDA, 2019). The gravimetric moisture of each representative sample was determined in triplicate by its weighing before and after drying at 105 °C for 72h (Claessen, 1997); while the gravimetric field capacity (FC) was determined in triplicate as described by Heavenrich and Hall (2016) by its saturation and drainage undercover at room temperature, followed by its weighing before and after drying at 105 °C for 72h (Claessen, 1997).

The physicochemical properties of the forest and pasture soils were compared by two-sample Student's t-test in R studio 1.0.153 (Rstudio Team, 2020). The assumptions of normality and homoscedasticity of these data were checked using the Anderson-Darling's test in SAS 9.3 (SAS Institute, Inc., Cary, NC, USA) and the Levene's test using the car package 3.0–0 (Fox and Weisberg, 2019) in R studio 1.0.153 (Rstudio Team, 2020), respectively.

2.2. Microcosm experimental design

The microcosm experiment had a 2 × 4 factorial design whereby soil samples from each land use (forest and pasture) were subjected to four soil moisture levels. The moisture gradients were defined as follows: one treatment with the original soil moisture of each site, as found in the field (22% for forest and 24% for pasture); and three treatments with increasing soil moisture based on their FC – defined as the amount of water held in a soil after gravitational drainage (Rai et al., 2017) – 60%, 80% and 100% of moisture at FC (determined as 50% for forest and 63% for pasture) (Supplementary Fig. S2). The microcosm experiment was also briefly described by Lemos et al. (2020). Each treatment was established in triplicate in 1.5 L glass jars filled with 350 g of fresh soil each. Jar dimensions, considering its base, were approximately 10.5 × 10.5 × 20 cm. The lids of the jars were previously altered to allow gas sampling after their closing (Supplementary Fig. S3).

The experiment was carried out in a Biochemical Oxygen Demand (B.O.D.) incubator for 30 days at 25 °C, which is the mean annual temperature of the Tapajós National Forest region (Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis - IBAMA, 2004). The jars were left open during incubation. The soil moisture in each microcosm was monitored daily by weighing and, when needed, corrected with sterile Milli-Q water using a spray bottle, followed by homogenization. Gas samples were collected from each microcosm at time zero and days 1, 2, 3, 6, 9, 12, 15, 18, 21, 24, 27, and 30. The gas sampling procedure was performed for 30 min (1, 10, 20, and 30 min after closing the jars) using 20-mL nylon syringes during the morning period, before moisture checking and correction. At the end of the experiment, after the last gas sampling (T30), soil samples from each microcosm were frozen in liquid N_2 and stored at −80 °C.

2.3. Measurement of CH_4 and CO_2 fluxes by gas chromatography

The concentrations of the gas samples were measured using a SRI 8610C gas chromatograph (SRI Instruments, Torrance, CA, USA). CH_4 and CO_2 were detected with a flame ionization detector and analyzed as previously described (Cerri et al., 2013). In summary, gas fluxes from each jar were calculated according to their concentration as a function of the incubation time (1, 10, 20, and 30 min), considering the jar volume, amount of dry soil in each jar, atmospheric pressure, and air temperature. For each treatment, daily CH_4 and CO_2 fluxes were determined by the average of the fluxes (in triplicate), and total cumulative emissions were determined through the linear interpolation of the daily fluxes between two successive samplings and the sum of the results obtained throughout the experimental period. The cumulative emissions of both gases were aligned rank-transformed and analyzed by two-way factorial analysis of variance (ANOVA) using the ARTool package 0.10.5 (Kay and Wobbrock, 2018) in R studio 1.0.153 (Rstudio Team, 2020). Post-hoc tests (Holm-adjusted) were carried out whenever necessary, using the lsmeans package 2.27–62 (Lenth, 2016). If a significant interaction was found, post-hoc tests examined the differences of differences as described by Kay and Wobbrock (2018). Plots were generated using ggplot2 3.0.0 (Wickham, 2016) and gridExtra 2.3 (Auguie and Antonov, 2017). The daily fluxes were plotted using Origin 7 software (OriginLab Corporation, Northampton, MA, USA).

2.4. DNA extraction and quantification

Total DNA was extracted from soil samples collected on day 30 (T30) from all treatments. Extractions were carried out in duplicate from 0.25 g of soil each using the PowerLyzer PowerSoil DNA Isolation Kit (QIAGEN, Hilden, North Rhine Westphalia, Germany), following a modified version of the manufacturer's protocol optimized for Amazon soils (Venturini et al., 2020): at the initial stage, after adding the solution C1, the samples were vortexed for 15 min at maximum speed and centrifuged for 3 min at 10,000×*g*; and following the addition of the solutions

C2 and C3, the incubations were made at −20 °C instead of 4 °C. The concentration and purity of the DNA samples were evaluated by 1% agarose gel electrophoresis and using a Nanodrop 2000c spectrophotometer (Thermo Fisher Scientific, Inc., Waltham, MA, USA). The DNA samples were stored at −20 °C until processed.

2.5. Quantitative PCR

The absolute abundances of methanogens and methanotrophs in the soils were investigated by qPCR using the standard-curve method, targeting the genes for the methyl-coenzyme M reductase (*mcrA*) and methane monooxygenase (particulate – *pmoA*, and soluble – *mmoX*) enzymes, respectively. In addition, the archaeal and bacterial 16S rRNA genes were quantified to estimate the overall abundances of both groups. The primer sets employed are listed in Supplementary Table S1. The standard curves were prepared using serial dilutions from 10^8 to 10^0 copies of gene fragments amplified from the following strains from the German Collection of Microorganisms and Cell Cultures (Braunschweig, Lower Saxony, Germany): *Methanolinea mesophila* (DSM 23604) for the archaeal 16S rRNA and *mcrA*, *Gordonia* sp. (DSM 11192) for the bacterial 16S rRNA, and *Methylosinus sporium* (DSM 17706) for *pmoA* and *mmoX*. For all genes, 10-μL qPCR reactions were carried out in triplicate for each DNA sample, containing 5 μL of SYBR Green ROX qPCR Master Mix (Thermo Fisher Scientific, Inc., Waltham, MA, USA), 0.2 μl of bovine serum albumin (BSA) (20 mg mL^{-1}) (Thermo Fisher Scientific, Inc., Waltham, MA, USA), 1 μl of each primer (5 pmols), 1 μl of DNA (approximately 10 ng), and ultra-pure H_2O.

The reactions were performed using a StepOnePlus instrument (Thermo Fisher Scientific, Inc., Waltham, MA, USA). The amplification conditions for the archaeal 16S rRNA gene consisted of 95 °C for 10 min, 40 cycles of 95 °C for 45 s, 57 °C for 45 s, and 72 °C for 45 s; for the bacterial 16S rRNA gene, 95 °C for 10 min, 40 cycles of 95 °C for 45 s, 60 °C for 15 s, and 72 °C for 20 s; for *mcrA*, 95 °C for 10 min, 45 cycles of 95 °C for 30 s, 60 °C for 45 s, and 72 °C for 30 s; for *pmoA*, 95 °C for 10 min, 45 cycles of 95 °C for 30 s, 58 °C for 30 s, and 72 °C for 45 s; and for *mmoX*, 95 °C for 10 min, 45 cycles of 95 °C for 10 s, 66 °C for 20 s, and 72 °C for 30 s (Supplementary Table S1). The amplification cycles were followed by a melting curve of 95 °C for 15 s, the annealing temperature of each gene for 1 min, and 95 °C for 15 s. The results were analyzed in StepOne Software v2.3 (Thermo Fisher Scientific, Inc., Waltham, MA, USA) and expressed as the number of gene copies per ng of DNA, considering that approximately 10 ng of DNA was used as the input material for each reaction.

The difference in abundance of each gene, as well as of the ratio of *mcrA* by the sum of *pmoA* and *mmoX*, were analyzed by non-parametric two-way factorial ANOVA, as previously described. Plots were generated using ggplot2 3.0.0 (Wickham, 2016) with tagger 0.0.0.9000 (Campitelli, 2021) in R studio 1.0.153 (Rstudio Team, 2020). The Spearman's rank correlation coefficient (Holm-adjusted) was used to determine the correlations among the abundances of CH_4 metabolism genes, cumulative CH_4 emissions, and moisture, using the psych package 2.0.9 (Revelle, 2020). The correlation plot was generated using the corrplot package 0.84 (Wei and Simko, 2017).

2.6. Metagenomic shotgun sequencing and bioinformatics

Shotgun metagenomic sequencing was carried out to analyze the microbial communities of the 12 microcosms under the treatments of original moisture and 100% FC at day 30 (T30). The DNA samples were used to construct libraries using the NEBNext Ultra II DNA Library Prep Kit for Illumina (New England Biolabs, Inc., Ipswich, MA, USA), followed by paired-end shotgun sequencing (2 × 150 bp) on an Illumina HiSeq platform (Illumina, Inc., San Diego, CA, USA) at Novogene Co., Ltd. (Beijing, China), with an expected raw-data output of 12 GB per sample. The forward reads were analyzed using the MG-Rast server 4.0.3 (Meyer et al., 2008). applying default parameters, and are available under the project "Metagenomic sequencing of forest and pasture soils of Eastern Amazon under different treatments - Forward reads" (mgp83208).

Taxonomic and functional assignments were performed against RefSeq (O'Leary et al., 2016) and SEED (Overbeek et al., 2005) databases, respectively, using the default settings, except for the alignment length, modified from 15 to 30 bp. The taxonomic hits of each sample were filtered according to the RefSeq taxonomy for the following groups: *Archaea*, *Bacteria*, methanogenic archaea, and methanotrophic bacteria. Within those last two groups, the functional hits for the methanogenic and methanotrophic communities were further filtered to obtain the reads related to methanogenesis – genes involved in the methanogenesis pathway (Subsystems - Level 3 - Methanogenesis; the details of this subsystem can be found at: https://pubseed.theseed.org/SubsysEditor.cgi?page=ShowSubsystem&subsystem=Methanogenesis) and encoding the methyl-coenzyme M reductase enzyme (MCR, Subsystems - Functional level) – and methanotrophy – genes encoding the particulate (pMMO, Subsystems - Functional level) and the soluble methane monooxygenase (sMMO, Subsystems - Functional level) – according to the SEED subsystems, respectively. The functional hits of the *Archaea* and *Bacteria* communities as a whole were also analyzed. For each database, the relative abundance of each individual hit was calculated relative to the total number of hits for each sample.

Significant differences in the composition of the communities at the genus and functional group levels were tested by permutational multivariate ANOVA (PERMANOVA) on Bray-Curtis dissimilarities using the vegan package 2.5–1 (Oksanen et al., 2018) in R studio 1.0.153 (Rstudio Team, 2020). Alpha diversity of the methanogenic and methanotrophic communities at the genus level, including richness (number of different taxa), Pielou's evenness (distribution of abundance among taxa), and Shannon's and Simpson's diversity (indices that combine both richness and evenness), were also calculated for each sample using the vegan package. The effects of land use and moisture on these indices, as well as on the relative abundances of the taxonomic and functional categories of interest, were analyzed by non-parametric two-way factorial ANOVA, as described above. Plots were generated using ggplot2 3.0.0 (Wickham, 2016) with tagger 0.0.0.9000 (Campitelli, 2021).

3. Results

3.1. Soil gas emissions

Throughout the experiment, positive (net emission) and negative (net uptake) daily CH_4 fluxes were observed for both forest and pasture soils, demonstrating their potential to act as a source and sink for this gas (Supplementary Fig. S4B). Cumulative CH_4 emissions throughout the experimental period were affected by land use, moisture level, and their interaction, with average emissions ranging from −1,421 to 6,816 ng CH_4–C g^{-1} dry soil (Table 1 and Fig. 1B). Post-hoc analysis showed that the difference between forest and pasture CH_4 emissions in the 100% FC treatment was significantly different ($p < 0.05$) compared to the difference in the original, 60%, and 80% FC treatments. Therefore, the microcosms with pasture soils under 100% FC had the highest emission values of all treatments (6,816 ng CH_4–C g^{-1} dry soil). In contrast, forest soils under the same moisture conditions emitted 306 ng CH_4–C g^{-1} dry soil, thus being 96% lower (average emission). Considering the average cumulative CH_4 emissions for each treatment, the forest and pasture microcosms exposed to the other moisture levels (original, 60%, and 80% FC) exhibited negative fluxes, i.e., acted as CH_4 sinks. Cumulative CO_2 emissions were similar among land uses and moisture treatments, with average emissions ranging from 131 to 515 μg CO_2–C g^{-1} dry soil (Supplementary Fig. S4A and Fig. 1A).

3.2. Quantification of 16S rRNA and CH_4 metabolism genes

The qPCR assays presented R^2 values above 0.98 and amplification

Table 1
Non-parametric two-way factorial ANOVA (degrees of freedom, F-ratios, and p-values) of cumulative gas emissions, gene quantification by qPCR, and taxonomic and functional profiling of the metagenomes from forest and pasture soils after a 30-day incubation period.

Data	Land use			Moisture			Land use × Moisture		
	df	F	p	df	F	p	df	F	p
Cumulative gas emissions									
CO_2	1, 16	0.194	0.665	3, 16	1.147	0.360	3, 16	0.914	0.456
CH_4	1, 16	**30.537**	**< 0.001**	3, 16	**15.558**	**< 0.001**	3, 16	**26.596**	**< 0.001**
Gene quantification									
16S rRNA of *Archaea*	1, 16	0.421	0.525	3, 16	**6.437**	**0.005**	3, 16	2.406	0.105
16S rRNA of *Bacteria*	1, 16	**44.317**	**< 0.001**	3, 16	1.646	0.218	3, 16	1.365	0.289
mcrA	1, 16	**50.330**	**< 0.001**	3, 16	**8.400**	**0.001**	3, 16	**69.819**	**< 0.001**
pmoA	1, 16	**21.022**	**< 0.001**	3, 16	**6.812**	**0.004**	3, 16	**20.355**	**< 0.001**
mmoX	1, 16	**41.350**	**< 0.001**	3, 16	**16.233**	**< 0.001**	3, 16	**32.121**	**< 0.001**
mcrA:(*pmoA* + *mmoX*) ratio	1, 16	**13.444**	**0.002**	3, 16	2.512	0.096	3, 16	2.490	0.097
Taxonomic profiling									
Methanogenic taxa									
Shannon's diversity	1, 8	1.029	0.340	1, 8	3.125	0.115	1, 8	3.125	0.115
Simpson's diversity	1, 8	1.044	0.337	1, 8	1.910	0.204	1, 8	2.512	0.152
Pielou's evenness	1, 8	1.029	0.340	1, 8	3.125	0.115	1, 8	3.125	0.115
Relative abundance	1, 8	**27.000**	**< 0.001**	1, 8	**25.920**	**< 0.001**	1, 8	**7.539**	**0.025**
Methanotrophic taxa									
Shannon's diversity	1, 8	**24.923**	**0.001**	1, 8	**24.923**	**0.001**	1, 8	1.873	0.208
Simpson's diversity	1, 8	**24.923**	**0.001**	1, 8	**24.923**	**0.001**	1, 8	**12.190**	**0.008**
Pielou's evenness	1, 8	**24.923**	**0.001**	1, 8	**24.923**	**0.001**	1, 8	1.873	0.208
Relative abundance	1, 8	**25.920**	**< 0.001**	1, 8	**17.000**	**0.003**	1, 8	**9.278**	**0.016**
Methanogens:methanotrophs ratio									
Relative abundance	1, 8	**27.000**	**< 0.001**	1, 8	**17.785**	**0.003**	1, 8	**9.677**	**0.014**
Functional profiling									
Methanogenesis	1, 8	**27.000**	**< 0.001**	1, 8	**25.920**	**< 0.001**	1, 8	**17.785**	**0.003**
MCR	1, 8	**17.190**	**0.003**	1, 8	**5.669**	**0.044**	1, 8	4.751	0.061
pMMO	1, 8	3.366	0.104	1, 8	**25.920**	**< 0.001**	1, 8	0.742	0.414
sMMO	1, 8	**26.722**	**< 0.001**	1, 8	1.418	0.268	1, 8	4.100	0.077
MCR:(pMMO + sMMO) ratio	1, 8	**26.315**	**< 0.001**	1, 8	**6.022**	**0.040**	1, 8	4.830	0.059

Bold values are significant at $p < 0.05$.

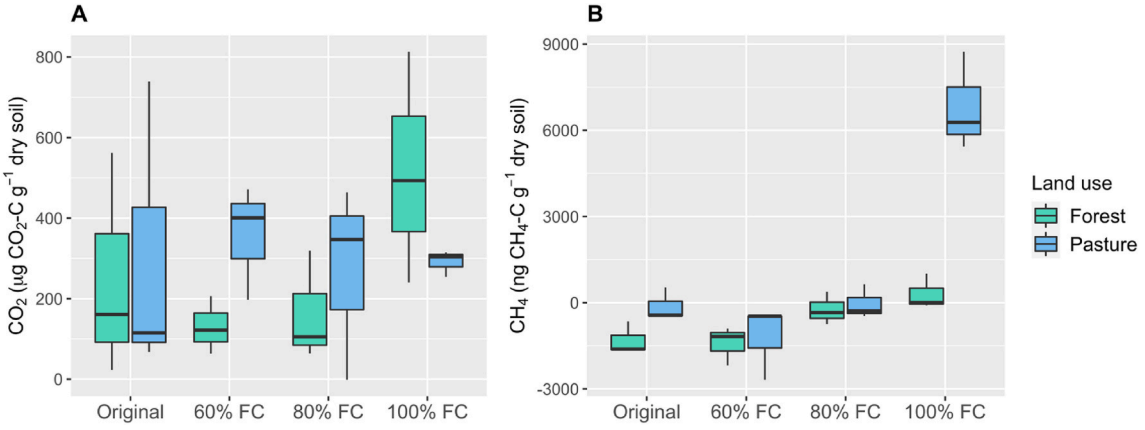

Fig. 1. Cumulative (A) CO_2 (µg CO_2–C g^{-1} dry soil) and (B) CH_4 (ng CH_4–C g^{-1} dry soil) emissions from forest and pasture soil microcosms over a 30-day period. The results expressed per gram of soil were calculated from the amount of dry soil in each jar. FC, field capacity.

efficiencies between 75% and 120%, calculated based on the regression lines of each standard curve. At the end of the experiment, the average number of copies per ng of DNA (copies ng^{-1} DNA) ranged from 4.38×10^3 to 6.78×10^3 for the archaeal 16S rRNA gene; 1.38×10^6 to 3.77×10^6 for the bacterial 16S rRNA gene; 2.19×10^0 to 9.91×10^2 for the *mcrA* gene; 6.38×10^0 to 2.69×10^2 for the *pmoA* gene; and 2.16×10^{-1} to 1.70×10^0 for the *mmoX* gene (Fig. 2).

The abundance of the archaeal 16S rRNA gene was affected by the soil moisture levels, found in post-hoc tests (Holm-adjusted) to be significantly lower ($p < 0.05$) under original moisture than in the other treatments, while the abundance of the bacterial 16S rRNA gene was higher in pasture than in forest soils (Table 1). The three functional genes analyzed – *mcrA*, *pmoA*, and *mmoX* -– were influenced by land-use legacy, soil moisture level, and their interaction. According to post-hoc analysis (in the form of differences of differences and Holm-adjusted), the differences between their abundances in the 100% FC treatment were different ($p < 0.05$) compared to the differences in the original, 60%, and 80% FC treatments. For the *mmoX* gene, this was also true for the comparison between the original and 80% FC treatment. Therefore, the microcosms with pasture soils, especially under 100% FC, presented the highest number of genes related to CH_4-cycling. The ratio between the abundance of methanogenesis- (*mcrA*) and methanotrophy- (*pmoA* plus *mmoX*) related genes was significantly higher in pasture than in forest soils. In fact, the abundance ratio showed that pasture soils under all moisture treatments, as well as forest soils under 100% FC, exhibited a greater abundance of methanogenesis-over methanotrophy-related genes (ratio higher than one).

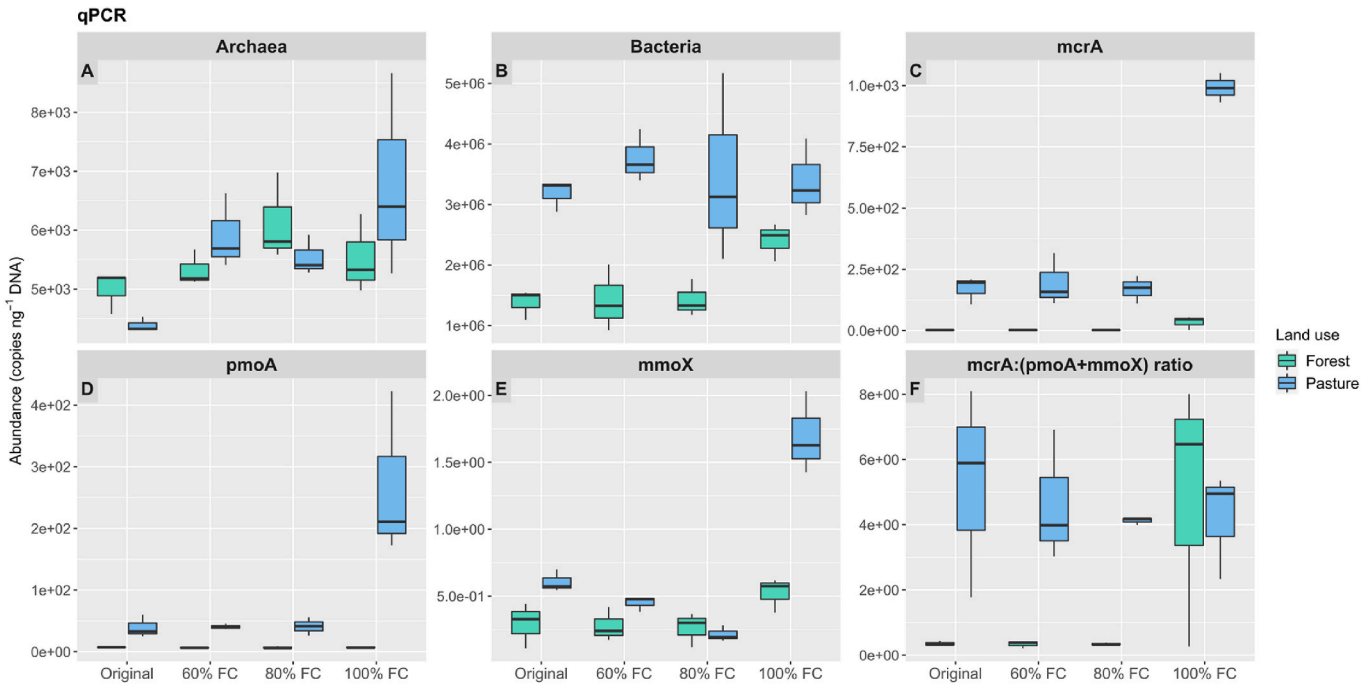

Fig. 2. Number of gene copies per ng of DNA (copies ng^{-1} DNA) of the (A) archaeal 16S rRNA, (B) bacterial 16S rRNA, (C) *mcrA*, (D) *pmoA*, (E) *mmoX*, and (F) *mcrA:(pmoA + mmoX)* ratio obtained by qPCR from forest and pasture soils after a 30-day incubation period. FC, field capacity. Note the different ranges of the y-axes throughout.

3.2.1. *Correlations among CH_4 traits and soil moisture*

We evaluated the relationship among the abundances of CH_4 metabolism genes – *mcrA*, *pmoA*, and *mmoX*, cumulative CH_4 emissions, and soil moisture using Spearman's rank correlations (Holm-adjusted). Positive significant correlations ($p < 0.05$) were observed between the abundances of *mcrA* and methanotrophy-related genes (Fig. 3). CH_4 emissions were positively correlated with the soil moisture content.

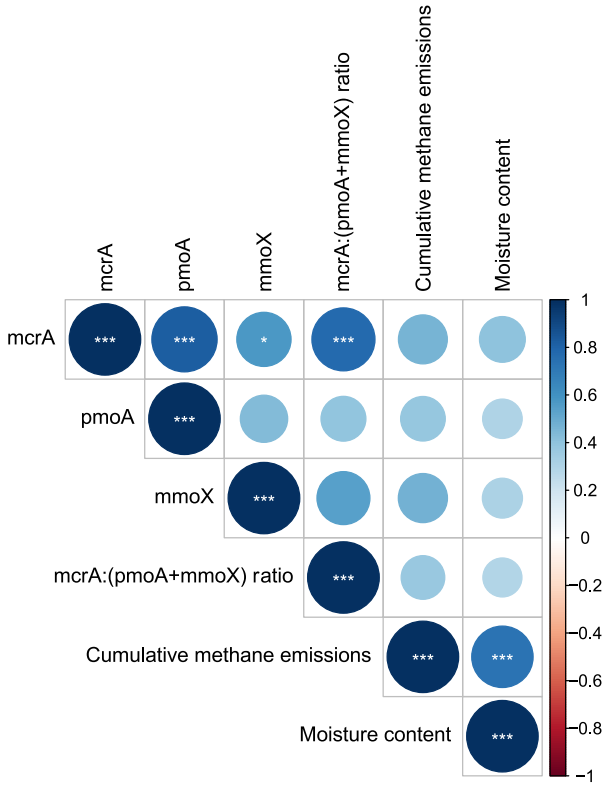

Fig. 3. Spearman's correlations (Holm-adjusted) among the qPCR abundances of CH_4 metabolism genes, cumulative CH_4 emissions, and soil moisture of forest and pasture soils after a 30-day incubation period. The circle areas represent the absolute values of the corresponding correlation coefficients. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

3.3. *Metagenomic sequencing results*

The metagenomic sequencing resulted in 594.9 million reads for 12 samples, with an average of 49.6 million per sample (Supplementary Table S3). Approximately 97% and 92% of the sequenced bases had a Phred quality score above 20 and 30, respectively. A total of 520.4 million reads passed the MG-Rast quality control (Meyer et al., 2008), averaging 43.4 million per sample and 150 bp in length (Supplementary Table S4). Less than 0.1% of the reads were assigned to rRNA, and 38.6% and 61.3%, predicted proteins with known and unknown functions, respectively (Supplementary Table S5).

3.3.1. *Taxonomic profiling of the metagenomes*

According to the taxonomic classification carried out using the RefSeq database (O'Leary et al., 2016), the metagenomic libraries were dominated by *Bacteria* (98.23%) but also contained sequences matching *Archaea* (0.93%), *Eukaryota* (0.82%), and viruses (0.03%). Less than 0.001% of the hits were classified as belonging to other groups. All *Archaea* found in the soil samples belonged to five phyla, while *Bacteria* belonged to 27 phyla (Supplementary Fig. S5). Based on Bray-Curtis dissimilarities, the compositions of the archaeal and bacterial communities were significantly affected by land use, moisture, and their interaction at the genus level (Table 2). Similar metagenomic relative abundances could be observed among treatments for both communities. Interestingly, their abundance results reveal a distinct but a predominant effect of the forest-to-pasture conversion (for Archaea, land use: $F_{1,8} = 24.923$, $p = 0.001$; moisture: $F_{1,8} = 2.564$, $p = 0.148$; land use × moisture: $F_{1,8} = 2.632$, $p = 0.143$; and for Bacteria, land use: $F_{1,8} =$

Table 2
Permutational multivariate ANOVA (degrees of freedom, F-ratios, R^2, and p-values) on Bray-Curtis dissimilarities of the taxonomic (at the genus level) and functional (at the function level) profiling of the metagenomes from forest and pasture soils after a 30-day incubation period.

Data	Land use				Moisture				Land use × Moisture			
	df	F	R^2	p	df	F	R^2	p	df	F	R^2	p
Taxonomic profiling												
Archaea	1, 8	**59.609**	**0.690**	**< 0.001**	1, 8	**12.741**	**0.148**	**0.004**	1, 8	**5.999**	**0.069**	**0.039**
Bacteria	1, 8	**180.775**	**0.713**	**< 0.001**	1, 8	**49.769**	**0.196**	**< 0.001**	1, 8	**14.917**	**0.059**	**0.002**
Methanogenic taxa	1, 8	**37.320**	**0.586**	**< 0.001**	1, 8	**14.307**	**0.224**	**0.003**	1, 8	4.105	0.064	0.072
Methanotrophic taxa	1, 8	**479.730**	**0.947**	**< 0.001**	1, 8	**18.749**	**0.037**	**0.002**	1, 8	0.247	<0.001	0.580
Functional profiling												
Archaea	1, 8	**26.435**	**0.621**	**< 0.001**	1, 8	**5.913**	**0.139**	**0.006**	1, 8	2.223	0.052	0.099
Bacteria	1, 8	**91.881**	**0.719**	**< 0.001**	1, 8	**23.247**	**0.182**	**0.001**	1, 8	4.601	0.036	0.054

Bold values are significant at $p < 0.05$.

24.923, p = 0.001; moisture: $F_{1,8} = 0.498$, p = 0.501; land use × moisture: $F_{1,8} = 2.740$, p = 0.136) (Supplementary Fig. S5).

The methanogenic archaea detected in the soils were assigned exclusively to the phylum *Euryarchaeota*, comprising six orders and 12 families from the classes *Methanomicrobia* (78.7%), *Methanococci* (9.8%), *Methanobacteria* (9.1%), and *Methanopyri* (2.5%) (Supplementary Table S6). The most abundant genus was *Methanosarcina*, followed by *Methanoregula* and *Methanoculleus* (Fig. 4D). Land-use legacy and moisture level were significant factors influencing the composition of the methanogenic archaeal community at the genus level (Table 2), but its richness, diversity, and evenness were not affected by those factors (Tables 1 and 3). The relative abundance of methanogens differed according to the land use, moisture, and their interaction (Fig. 4A). Similar to the qPCR quantification, pasture soils, particularly of the 100% FC treatment, had the highest relative abundance for this group. Regarding the most dominant methanogenic genus, *Methanosarcina* presented a significant higher relative abundance in pasture soils and under 100% FC (land use: $F_{1,8} = 25.920$, $p < 0.001$; moisture: $F_{1,8} = 12.800$, p = 0.007; land use × moisture: $F_{1,8} = 3.781$, p = 0.088).

The methanotrophic bacterial community was composed mainly of taxa from the proteobacterial classes *Alphaproteobacteria* (72.1%) and *Gammaproteobacteria* (20.7%). In addition, 7.3% of this community was composed of *Methylacidiphilae*, a class from the *Verrucomicrobia* phylum. The diversity of methanotrophic bacteria comprised three orders and four families (Supplementary Table S7), with the genus *Methylocella* presenting the highest relative abundance across all samples, followed by *Methylosinus* and *Methylococcus* (Fig. 4E). As observed for methanogens, the composition of the methanotrophic community was affected by land use and moisture at the genus level (Table 2) and, despite similar richness values, the soils from the pasture site and of the 100% FC treatment exhibited significantly higher Shannon's diversity and Pielou's evenness (Table 3). Land-use legacy and soil moisture also

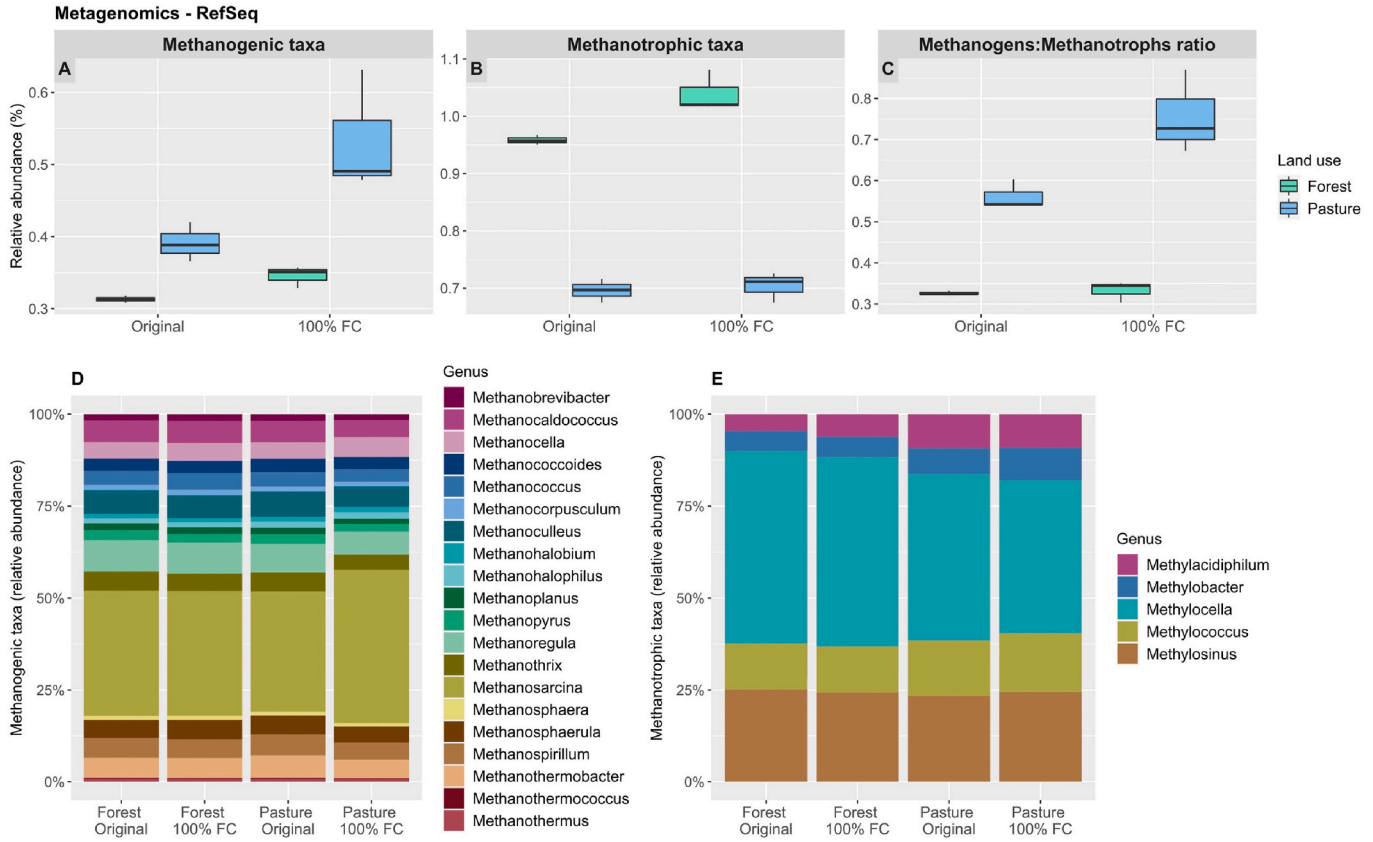

Fig. 4. Relative abundance (%) of (A) methanogenic taxa, (B) methanotrophic taxa, and (C) methanogens:methanotrophs ratio; and mean composition (%) of the (D) methanogenic archaeal and (E) methanotrophic bacterial communities at the genus level obtained by shotgun metagenomics from forest and pasture soils after a 30-day incubation period. FC, field capacity. Note the different ranges of the y-axes throughout.

Table 3
Richness, Shannon's diversity, Simpson's diversity, and Pielou's evenness (mean and standard deviation) for all taxa from the methanogenic archaeal and methanotrophic bacterial communities at the genus level obtained by shotgun metagenomics from forest and pasture soils after a 30-day incubation period.

Land use	Moisture	Methanogenic taxa				Methanotrophic taxa			
		Richness	Shannon	Simpson	Pielou	Richness	Shannon	Simpson	Pielou
Forest	Original	20 ± 0	2.432 ± 0.003	0.851 ± 0.001	0.812 ± 0.001	5 ± 0	1.244 ± 0.003	0.641 ± 0.002	0.773 ± 0.002
	100% FC	20 ± 0	2.434 ± 0.003	0.852 ± 0.000	0.813 ± 0.001	5 ± 0	1.277 ± 0.009	0.653 ± 0.003	0.794 ± 0.005
Pasture	Original	20 ± 0	2.466 ± 0.035	0.859 ± 0.011	0.823 ± 0.012	5 ± 0	1.390 ± 0.007	0.704 ± 0.003	0.863 ± 0.004
	100% FC	20 ± 0	2.269 ± 0.178	0.798 ± 0.058	0.758 ± 0.059	5 ± 0	1.434 ± 0.006	0.725 ± 0.002	0.891 ± 0.004

FC, field capacity.

displayed a significant interaction for Simpson's diversity and the overall relative abundance (Fig. 4B). Interestingly, while these three diversity indices were higher for pasture soils, the methanotrophic abundance was higher in forest soils, but with an increase of all these attributes (diversity, evenness, and relative abundance) under 100% FC. The relative abundance of *Methylocella*, the most dominant methanotrophic genus, differed according to the land use and its interaction with soil moisture (land use: $F_{1,8} = 25.920$, $p < 0.001$; moisture: $F_{1,8} = 0.758$, $p = 0.409$; land use ^{x} moisture: $F_{1,8} = 25.920$, $p < 0.001$).

Lastly, when the methanotrophic community was analyzed with a higher taxonomic resolution, its attributes were also influenced by both factors and their interaction (Supplementary Tables S8 and S9, and Fig. S6). The relative abundances of methanotrophs belonging to *Alpha-* and *Gammaproteobacteria* were not affected by moisture levels and the factors' interaction, respectively. The former class presented the highest values in forest soils, and the latter, in soils of the 100% FC treatment.

The ratio of methanogens to methanotrophs, assessed by the relative abundances of those taxonomic groups, was influenced by land-use legacy, soil moisture, and their interaction (Fig. 4C). Even though distinct results were found regarding the methanotrophic abundance through both molecular techniques used in our work (qPCR and shotgun metagenomics), similar trends were observed, with pasture soils presenting the highest ratio values, closer to one.

3.3.2. *Functional profiling of the metagenomes*

The distribution of the archaeal and bacterial functional categories (at Subsystems level 1) among treatments, using the SEED database (Overbeek et al., 2005), is displayed in Supplementary Fig. S7. Based on Bray-Curtis dissimilarities from the functional assignments, the gene contents of both archaeal and bacterial communities were significantly different across land-use legacies and moisture treatments at the functional level (Table 2). The relative abundance of the genes of the methanogenic community related to methanogenesis and, specifically, the relative abundance of the genes encoding the MCR enzyme was significantly influenced by land use and moisture, and the former, also by their interaction (Table 1, Fig. 5A and B). Again, the highest relative abundance values were found for pasture soils, especially at 100% FC. Regarding the methane monooxygenase enzyme, different results were observed for the genes encoding its particulate and soluble forms. The relative abundances of the genes encoding pMMO (particulate) and sMMO (soluble) were, respectively, higher in the 100% FC treatment and in pasture soils (Fig. 5C and D). Interestingly, the moisture treatments caused contrasting alterations on the relative abundance of sMMO in the soils: it tended to increase in the forest under higher moisture conditions but to decrease in the pasture. The MCR:(pMMO + sMMO) ratio also increased in both conditions (Fig. 5E), but only the pasture microcosms exhibited values above one.

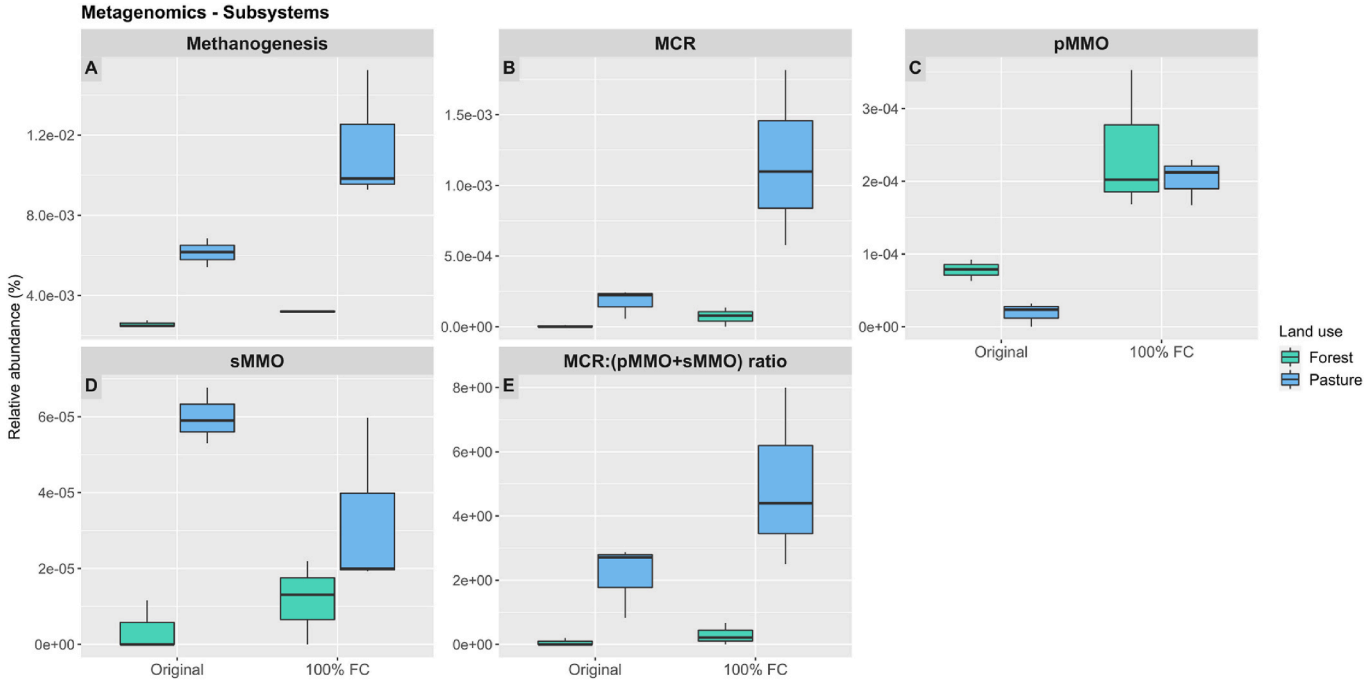

Fig. 5. Relative abundance of genes (A) involved in methanogenesis, (B) encoding the methyl-coenzyme M reductase enzyme (MCR), (C) encoding the particulate methane monooxygenase (pMMO), (D) encoding the soluble methane monooxygenase (sMMO), and (E) MCR:(pMMO + sMMO) ratio obtained by shotgun metagenomics from forest and pasture soils after a 30-day incubation period. FC, field capacity. Note the different ranges of the y-axes throughout.

4. Discussion

The Amazon basin has been experiencing climatic changes over the last decades, which have altered its rainfall patterns and the frequency of extreme weather events, such as droughts and floods (Gloor et al., 2013, 2015; Almeida et al., 2017; Haghtalab et al., 2020), likely impacting soil moisture patterns. We asked how increasing soil moisture changes might impact the cycling of CH_4 and its related microbial communities using soils that represent two of the most common land uses in the Amazon: upland rainforest and cattle pasture established on previously forested land.

The conversion of forest areas into agricultural and pasture lands in the Amazon is typically carried out by burning the downed woody vegetation, which increases soil pH and nutrient content and availability (Juo and Manu, 1996; Giardina et al., 2000). Considering only the land-use legacy, our results showed similar trends, indicating a significant impact on soil properties (except P, K, Mg, B, and Cu), as well as on the taxonomic and functional composition of archaeal and bacterial communities. In agreement with previous studies indicating that soil moisture has a strong influence on the activity and diversity of soil microorganisms (Lennon et al., 2012), both archaeal and bacterial communities responded to our moisture treatments. The interaction between land use and moisture was also found to have a significant impact on the taxonomic composition of these communities. Therefore, in light of the climatic changes in the Amazon basin, land use may cause even higher impacts on the soil microbial communities. Our microbial abundance data (assessed by qPCR) also indicated that *Archaea* and *Bacteria* responded differently to the treatments: while soil moisture had a pronounced effect on the archaeal community abundance, which increased with increasing moisture (at 60%, 80%, and 100% FC), the bacterial community seems to respond more strongly to land-use legacy and presented a higher abundance in pasture than in forest soils.

When we looked at the taxonomic composition of the CH_4-cycling communities, we also observed an effect of both land-use legacy and soil moisture. Among the methanogens, the genus *Methanosarcina*, which was the most abundant across all treatments, exhibited a significantly higher relative abundance in pasture soils and at 100% FC. This dominance was also recently noticed in soils of the Eastern and Western Amazon, considering the active methanogenic communities obtained through DNA stable-isotope probing (Kroeger et al., 2021). Unlike most CH_4-producing organisms, members of this metabolically versatile genus can use up to nine substrates and have all three major methanogenic pathways (hydrogenotrophic, acetoclastic, and methylotrophic), which together with the ability to form complex multicellular structures in response to the environment, may provide an advantage to this group in soils under changing conditions (Galagan et al., 2002).

The abundance of genes and microbial groups involved in methanogenesis, as observed in the qPCR and the metagenomic data, was also influenced by the land-use legacy, soil moisture, and their interaction, presenting higher values in pasture soils than in forest soils, and further exacerbated under high moisture levels. Several studies have demonstrated that the use of heavy machinery for forest clearing and cattle trampling in pasture sites leads to soil compaction, resulting in a decrease of soil porosity and an increase of anaerobic sites, suitable for the growth of methanogenic microorganisms (Radl et al., 2007; Frey et al., 2011; Bradley et al., 2012; Koubová et al., 2012; Chroňáková et al., 2015; Wang et al., 2016). Radl et al. (2007) and Frey et al. (2011) have shown that such impacts may persist in temperate soils for several months after removing the cause of the compaction so that the soil still sustains a high abundance of methanogens and thus increased potential to emit CH_4. Our microcosm data support this legacy effect since pasture soils tended to exhibit a higher abundance of methanogens and cumulative CH_4 emissions than forest soils even after sampling and a 30-day period of incubation under controlled conditions, also differently responding to the soil moisture treatments.

In addition, pH increases following fire, intensified by the deposition of cattle urine and manure, make pasture soils a more suitable environment for a methanogenic metabolism (Radl et al., 2007; Chroňáková et al., 2015). Cattle manure is also a nutrient-rich organic source, containing larger fractions of easily degradable compounds than the forest litter (Lammel et al., 2015a), besides serving as an inoculum of methanogens into the soil (Gattinger et al., 2007; Radl et al., 2007; Prem et al., 2014; Chroňáková et al., 2015). Similar to our results, Kroeger et al. (2021) and Meyer et al. (2017, 2020) also found that soils from pasture sites in the Eastern and Western Amazon have a higher abundance of methanogens and methanogenesis genes than forests soils, suggesting that this result, in particular, may represent a general microbial response to land-use change in the Amazon. Additionally, the forest soils under original moisture and at 60% and 80% FC consumed in average more CH_4 (negative cumulative fluxes) during the experiment than the respective pasture soils under these same conditions.

Our soil moisture treatments suggest that increased precipitation in parts of the Amazon basin could drive further soil CH_4 emissions beyond those seen due to cattle pasture establishment. In samples at 100% FC, both molecular methods used in our study showed a clear increase in the abundance of methanogenesis-related genes and taxa due to the lower oxygen availability in the soils (Nazaries et al., 2013). This response was observed for both land uses, although the abundance of methanogens continued to remain higher in pasture than in forest soils. Supporting our results, the cumulative CH_4 emissions were strongly correlated with the moisture content and influenced by land use, moisture, and their interaction. Therefore, our results indicate that the Amazon soils that act as CH_4 sinks can become considerable sources of this gas under high saturation conditions, which is particularly true for pastures. Considering only the 100% FC treatment, the mean of the cumulative CH_4 emissions of the pasture soils was about 22-fold higher than the forest soils. Other long-term biogeochemical studies in the Amazon region have revealed a sink-to-source shift after forest-to-pasture conversion so that pasture soils often consume CH_4 in the dry season but emit it in the wet season with increasing soil moisture (Steudler et al., 1996; Verchot et al., 2000; Fernandes et al., 2002).

The taxonomic composition of the methanotrophic bacterial community, composed of *Proteobacteria* of the *Alpha* and *Gamma* classes and *Verrucomicrobia*, was also influenced by land use and moisture. *Methylocella* was the dominant methanotrophic genus in all soils of the experiment, particularly those from the forest site. This group from the *Beijerinckiaceae* family is composed of facultative methanotrophic species that can utilize several multicarbon compounds (and often prefer acetate over CH_4) (Dedysh and Dunfield, 2016). This versatility confers an advantage in environments under oscillation of CH_4 emissions (Dedysh et al., 2005). In our study, the relative abundance of this genus was higher in forest soils, as observed by Meyer et al. (2017, 2020), but its response to moisture changed for each land use since a significant interaction between both factors was found. *Methylocella* species contain only the soluble form of the MMO enzyme (Dedysh and Dunfield, 2016), while all other taxa found in our forest and pasture soils possess its particulate form (Knief, 2015; Dedysh and Knief, 2018). While pMMO is more selective regarding alternative substrates, the sMMO enzyme can act on linear and branched alkanes and alkenes of up to eight carbons, besides aromatic, heterocyclic, and halogenated compounds (as reviewed by Sazinsky and Lippard, 2015). Since the Amazon soils are known to have high weathering rates (Souza et al., 2018), the

predominance of *Methylocella*, and consequently sMMO, in these soils may be related to its capacity of growing on different and complex carbon sources.

Methanotrophic diversity and evenness also varied according to differences in land use and moisture, and a significant interaction between both factors was found for Simpson's diversity (as well as for the results of *Alpha-* and *Gammaproteobacteria*, as shown in the Supplementary Tables S8 and S9), presenting the highest values for pasture soils, especially of the 100% FC treatment. Other studies carried out in temperate forests, agricultural and pasture lands have shown that managed environments have more diverse methanotrophic communities (Kravchenko et al., 2005; Kizilova et al., 2013; Kravchenko and Sukhacheva, 2017; Sengupta and Dick, 2017).

Unlike for methanogens, we did not observe an agreement between qPCR and metagenomic datasets regarding the methanotrophic community abundance. Most of the available studies have found a decrease in the quantification of methanotrophs and genes encoding pMMO after cattle grazing (Lammel et al., 2015a; Wang et al., 2016; Meyer et al., 2017, 2020), and the association of *pmoA* with Amazon forest soils was also reported (Paula et al., 2014). However, other studies detected no significant changes (Zheng et al., 2012; Yang et al., 2013) and even an opposite tendency in soils of the Southern Amazon rainforest (Lammel et al., 2015b). In our data, the contrasting results obtained from qPCR and metagenomic approaches could be associated with several causes, including the high abundance of methanotrophs found in our samples that do not possess the pMMO enzyme (*Methylocella* species) (Dedysh and Dunfield, 2016); the bias of the *pmoA* primers used for qPCR, which do not capture *Verrucomicrobia* methanotrophs (Dedysh and Dunfield, 2011; Ghashghavi et al., 2017) and have lower amplification efficiency for some *Alphaproteobacteria* (Deng et al., 2013); and the limitations of the annotation of short metagenomic reads. Furthermore, the primers used for the detection of the *mmoX* gene were designed and evaluated based on strains of *Methylococcus* and *Methylosinus* (Miguez et al., 1997) and, according to results obtained using the FunGene pipeline (Fish et al., 2013), they do not capture all the *Methylocella* diversity (data not shown). Despite the smaller number of data available, several land-use studies could not detect the *mmoX* gene in upland soils (Knief et al., 2003; Knief et al., 2005; Dörr et al., 2010; Kizilova et al., 2013; Kravchenko and Sukhacheva, 2017). Finally, it is also important to mention that all *Methylacidiphilum* species so far were isolated from hot and acidic geothermal environments (Schmitz et al., 2021). Even being annotated as such in our work by the MG-Rast server 4.0.3. (Meyer et al., 2008), as also found by Meyer et al. (2017), further studies are needed to evaluate their presence in these tropical soils.

Regardless of the dataset used, the methanotrophic community tended to increase in our soil samples of the 100% FC treatment, probably due to the greater amount of CH_4 available. However, clearer patterns emerged when we considered the most prevailing methanotrophic taxa found in our soils separately. The relative abundance of *Alphaproteobacteria* was affected by the factor's interaction and land use (62.3% higher in forest than in pasture soils), which is in accordance with several studies that described its dominance in forests and other natural environments and decrease in agricultural and pasture sites (Kravchenko et al., 2005; Singh et al., 2007, 2009; Malghani et al., 2016; Meyer et al., 2017). The life strategies of some organisms of this group may allow for an advantage in low-nutrient habitats (Steenbergh et al., 2009; Meyer et al., 2017), such as our forest soils, besides having several members with the capability of fixing nitrogen (Dedysh and Knief, 2018). *Gammaproteobacteria* showed significantly higher values in soils from forest and under 100% FC, corroborating findings that suggest these organisms rapidly respond to disturbances (Steenbergh et al., 2009; Ho et al., 2013) and can be favored under increasing moisture (Siljanen et al., 2011, 2012).

Despite the different results obtained for the methanotrophic community, all datasets indicate that the ratio of methanogens to methanotrophs changed with the land use, with higher values in pasture than in forest soils, which agrees with the results presented by Meyer et al. (2017) for the Western Amazon. The taxonomic and functional ratios based on the metagenomic data were also influenced by soil moisture levels (and a significant interaction between both studied factors was found for the former). These values remained higher in pasture than in forest, even under field capacity conditions. Taken together, our experimental results showed that soil CH_4 emissions and methanogenic and methanotrophic communities responded to changes in land use and soil moisture. The pasture soil exhibited a greater potential to produce CH_4 than the forest soil, which was enabled by increasing soil moisture.

It is also worth mentioning that, although soils contribute to a small percentage of the CH_4 emissions related to pasture establishment and use in the Brazilian Amazon, dominated by emissions from livestock and biomass burning, the transformation of forests into pastures is estimated to result in a net source of about 1 g CH_4 m^{-2} $year^{-1}$ from the soil (Steudler et al., 1996). Hereafter, this contribution may increase with the deforestation process and changes in precipitation patterns, as we previously described. Our data exhibited patterns similar to those obtained in the field in previous studies and go beyond by showing that increases in soil moisture could further exacerbate CH_4 emissions and related microbial responses resulting from the forest-to-pasture conversion. The development of this work on a microcosmic scale allowed the isolation of moisture as a study factor and the homogenization of the other environmental conditions. To our knowledge, this is the first report on the microbial CH_4 cycle in upland soils of the Brazilian Amazon that combined one-month gas measurements with advanced molecular methods.

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Data availability statement

The metagenomic data that support the findings of this study are available in the MG-Rast server 4.0.3 (Meyer et al., 2008) at https://www.mg-rast.org under the project "Metagenomic sequencing of forest and pasture soils of Eastern Amazon under different treatments - Forward reads" (mgp83208).

Author contributions

AMV and SMT designed the research with contributions from CAY and AGF. The study sites are part of the ECOFOR project of EB and JB. AMV, JBG, FMN, and CDB collected the samples for the experiment. AMV conducted the experiment, chromatographic, and molecular analysis with the help of JBG, CAY, and AGF. AMV analyzed soil properties and microbial data. NMSD analyzed gas data. AMV and NMSD discussed the results with all other authors. SMT contributed with reagents, materials, and analytic tools. AMV wrote the article with contributions from NMSD and FSP. All authors critically revised the manuscript.

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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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envres.2022.113139.

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