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Not just a methane source: Amazonian floodplain sediments harbour a high diversity of methanotrophs with different metabolic capabilities

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Abstract

The Amazonian floodplain forests are dynamic ecosystems of great importance for the regional hydrological and biogeochemical cycles and function as a significant CH_4 source contributing to the global carbon balance. Unique geochemical factors may drive the microbial community composition and, consequently, affect CH_4 emissions across floodplain areas. Here, we report the in situ composition of CH_4 cycling microbial communities in Amazonian floodplain sediments. We considered how abiotic factors may affect the microbial community composition and, more specifically, CH_4 cycling groups. We collected sediment samples during wet and dry seasons from three different types of floodplain forests, along with upland forest soil samples, from the Eastern Amazon, Brazil. We used high-resolution sequencing of archaeal and bacterial 16S rRNA genes combined with real-time PCR to quantify Archaea and Bacteria, as well as key functional genes indicative of the presence of methanogenic (*mcr*A) and methanotrophic (*pmo*A) microorganisms. Methanogens were found to be present in high abundance in floodplain sediments, and they seem to resist the dramatic environmental changes between flooded and nonflooded conditions. Methanotrophs known to use different pathways to oxidise CH_4 were detected, including anaerobic archaeal and bacterial taxa, indicating that a wide metabolic diversity may be harboured in this highly variable environment. The floodplain environmental variability, which is affected by the river origin, drives not only the sediment chemistry but also the composition of the microbial communities. These environmental changes seem also to affect the pools of methanotrophs occupying distinct niches. Understanding these shifts in the methanotrophic communities could improve our comprehension of the CH_4 emissions in the region.

KEYWORDS
16S rRNA sequencing, methanogens, methanotrophs, quantitative PCR, tropical wetlands

1 | INTRODUCTION

In the Amazon region, floodplain forests occupy about 800,000 Km^2 (Hess et al., 2015). These ecosystems comprise diversified and dynamic landscapes, which are exposed to seasonal flooding events by the expanding rivers as a consequence of the periodic excessive rainfalls. Floodplains seem to play a significant role in the regional and global C budget (Gedney et al., 2019; Junk, 1997; Moreira-Turcq et al., 2003; Pangala et al., 2017). While uplands forests are considered important tropical methane (CH_4) sinks (Meyer et al., 2020), floodplains represent one of the largest natural sources of CH_4 into the atmosphere (Conrad, 2009; Gedney et al., 2019), including the significant process of CH_4 transfer through trees (Pangala et al., 2017). Modelling studies have predicted that Amazonian floodplains may contribute up to 7% of total global CH_4 emissions (Potter et al., 2014; Wilson et al., 2016).

In anoxic environments, the CH_4 is generated as the final product of the anaerobic respiration by methanogenic archaea, which can use acetate, H_2/CO_2, formate, CO, or methylated compounds as substrates (Bridgham et al., 2013). Despite different pathways, all known methanogenic archaea share the methyl-coenzyme M reductase (MCR), the terminal enzyme in the CH_4 formation (Luton et al., 2002; Lyu et al., 2018). The ability to use one or more substrates varies across the different methanogenic archaeal taxa. Hence, substrate availability, along with other factors (biotic and abiotic), affects the diversity of these organisms in the environment (Barros et al., 2020).

In ecosystems that are sources of CH_4, methanotrophs are particularly important for oxidising it and, therefore, attenuate net fluxes of this greenhouse gas into the atmosphere (Conrad, 2009; Ho et al., 2013). CH_4-oxidising microbes can be divided into subgroups with distinct functional traits and ecological niches (Haque et al., 2020). Aerobic methanotrophic bacteria occur in terrestrial and aquatic environments, mainly at oxic/anoxic interfaces, where oxygen (O_2) is available as an electron acceptor and CH_4 as an energy and carbon source (Knief, 2015). Traditionally, aerobic methanotrophs are classified as type I (from Gammaproteobacteria class), type II (from Alphaproteobacteria class), and type III (from Verrucomicrobia phylum) (Ho et al., 2013; Knief, 2015). The key enzyme for CH_4 oxidation, the methane monooxygenase (MMO), exists in both particulate membrane (pMMO) and soluble (sMMO) forms. The former is present in all known aerobic methanotrophs, except for *Methylocella* and *Methyloferula*, while the presence of the latter seems to vary across taxa (Dedysh & Knief, 2018; Knief, 2015). By contrast, different types of anaerobic oxidation of CH_4 (AOM) metabolisms, using alternative electron acceptors, have been described for both archaeal and bacterial taxa (reviewed by Cui et al., 2015). Particularly, the archaeal family Methanoperedenaceae and the bacterial family Methylomicrobiaceae (former NC10) have been suggested to play an important role in AOM in both freshwater and marine environments (Gabriel et al., 2020; Haroon et al., 2013; Shen et al., 2016; Welte et al., 2016).

Despite the important role of the Amazonian floodplain forests for the global CH_4 emissions (Gedney et al., 2019; Sawakuchi et al., 2014), the identities of their CH_4 cycling microbes remain largely unknown. Although the methanogenic communities have been explored in the last decade (Conrad et al., 2010, Conrad et al., 2013; Pazinato et al., 2010; Ji et al., 2016; Hernández et al., 2019), the diversity of groups related to CH_4 consumption is far less understood. To date, there was no effort to characterise the methanotrophic groups in situ. In addition, the environmental variability present in floodplain ecosystems is likely to result in major differences in the CH_4 cycling communities not only in temporal, but also in spatial scales. For instance, the riverine origin is a very important factor, as some waters, such as the Amazon River, may carry large amounts of inorganic suspensoids, while others, such as the Tapajós River, are comparatively poor in dissolved solids (Junk et al., 2011). In addition to the effects on the proportions of CH_4 producers and consumers, these environmental variabilities could affect dominant metabolic routes in each process. Such information is essential to build a framework to understand the current state and predict future scenarios under environmental alterations, such as those related to climate change.

Here, we provide the first in-depth characterisation of microbial communities responsible for CH_4 cycling in Amazonian floodplain sediments and their dynamics in situ. We selected three floodplain areas with contrasting characteristics to explore part of the vast environmental variability found in this ecosystem and assessed their microbial diversity under both flooded and unflooded conditions. We considered how the abiotic factors inherent in each area may affect microbial community composition, focusing on the CH_4 cycling taxa. To further inspect the methanotrophic communities, we investigated the presence of their groups and hypothesised that taxa with distinct metabolic routes could present a different distribution across the floodplains. In addition, to examine the same dynamics in an area without flooding influences, an upland forest site was also studied. To tackle these questions, we used high-resolution sequencing of the 16S rRNA genes to assess archaeal and bacterial diversities, along with the quantification of key genes in the CH_4 production (*mcr*A) and aerobic oxidation (*pmo*A). The results highlight the importance of the local environmental factors to drive both chemistry and microbiology of the floodplain forests, and also the potential role of different methanotrophic metabolisms in these areas.

2 | MATERIALS AND METHODS

2.1 | Sediment and soil sampling

The studied sites are located in the region of Santarém and Belterra, in the central-western area of the state of Pará, Brazil. The regional climate is classified as Am (Köppen), tropical humid, with a mean annual temperature of 26 ± 2°C, and annual precipitation above 2500 mm (Alvares et al., 2013). There are two well defined seasons,

dry (DS; July–November) and wet (WS; December–June), with more than 70% of the rain concentrated in the latter.

Triplicate sediment samples were collected from three floodplain areas in May (WS) and October (DS) 2016, when the rivers reached maximum and minimum levels, respectively. These areas differed regarding their vegetation (Moura et al., 2008) and the adjacent river: FP1 (Floodplain 1 at Igarapé Jamaraquá, 2°49'04.6" S 55°02'04.6" W), in the Tapajós river; FP2 (Floodplain 2 at Igarapé Maicá, 2°28'11.2" S 54°38'49.9" W), in the Amazonas river; and FP3 (Floodplain 3 at Igarapé Açu, 2°22'44.8" S 54°44'21.1" W), in the intersection of both rivers. In addition, we collected soil samples from an upland primary forest area, PFO (2°51'19.6" S 54°57'30.1" W), located in the Tapajós National Forest (Figure S1).

Sediment and soil samples were collected using a corer (5 cm diameter × 10 cm depth) and transported on ice to the laboratory. All samples were homogenised thoroughly and stored (−20°C for DNA analyses and 4°C for chemical analyses) and processed within two weeks. During the wet season sampling, the water column in the floodplains ranged from 0.5 to 3 m. Dissolved oxygen (DO) and pH in the sediment-water interface were assessed using a YSI Professional Plus Instrument (Pro Plus). No water column was observed during the dry season sampling; however, site FP1 was water-logged.

2.2 | Chemical analyses

Sediment and soil samples were processed in the Laboratory of Chemical Analysis of the Department of Soil Science of the Luiz de Queiroz College of Agriculture (ESALQ/USP, Brazil), following procedures described by Camargo et al., (2009). The following parameters were determined: pH in $CaCl_2$; total nitrogen (N) by Kjeldahl method; phosphorus (P), potassium (K), calcium (Ca), and magnesium (Mg) by ion exchange resin extraction; sulphur (S) by calcium phosphate 0.01 mol L^{-1} extraction and turbidimetry determination; aluminium (Al) extraction by potassium chloride extraction 1 mol L^{-1}; organic matter (OM) by the dichromate/titrimetric method; boron (B) by extraction with hot water; and the micronutrients copper (Cu), iron (Fe), manganese (Mn) and zinc (Zn) with a chelating agent, according to Lindsay and Norvell (1978).

2.3 | DNA extraction

The extraction of DNA from 0.25 g of sediment and soil samples was carried out in duplicate reactions using the PowerLyzer PowerSoil DNA Isolation Kit (MoBIO Laboratories Inc.), with an optimised protocol for tropical soils described by Venturini et al., (2020). Briefly, the adaptations included an extension in the vortex time to 15 min, followed by a 3 min centrifugation at 10,000 × g and incubation with C2 and C3 solutions at −20°C. DNA quantity and quality were assessed in 1% agarose gel and using a Nanodrop 2000c spectrophotometer (Thermo Fisher Scientific Inc.) set for determining absorbance at the following wavelengths: 230, 260, 280, and 320 nm. Purified DNA samples were stored at −20°C until processed.

2.4 | Archaeal and bacterial 16S rRNA gene sequencing

The diversity of archaeal and bacterial communities was assessed by high-throughput sequencing of the V4 region of 16S rRNA gene, using the following primer sets, respectively: 519f/915r (Klindworth et al., 2013; Stahl and Amann, 1991) and 515f/806r (Caporaso et al., 2011). Paired-end sequencing, with 2 × 250 bp reads, was performed in Illumina Hiseq 2500 platform at Novogene Bioinformatics Technology, using standard procedures.

2.5 | Quantitative PCR

Quantitative PCR (qPCR) was used to assess the abundance of archaeal and bacterial 16S rRNA genes, as well as functional gene markers indicative of the presence of methanogenic (methyl coenzyme-M reductase – *mcr*A) and methanotrophic (particulate methane monooxygenase – *pmo*A) microorganisms. Cycle conditions and primers used are described in Table S1. The 10 μl reactions contained 5 μl of SYBR Green ROX qPCR (Thermo Fisher Scientific Inc), 0.2 μl of bovine serum albumin (Thermo Fisher Scientific Inc., 20 mg ml^{-1}), 1 μl of each primer (5 pmol), 1 μl of DNA template (10 ng) and 1.8 μl of ultrapure water. Reactions were performed in triplicate using a StepOne Plus instrument (Applied Biosystems). Gene abundance was estimated using a standard curve constructed with 10^0 to 10^{10} copies of the targeted gene fragments amplified from the strains presented in Table S1.

2.6 | Bioinformatics and statistical analyses

All bioinformatics and statistical analyses were performed on R studio 3.5.1 (Rstudio Team, 2018). Raw sequences were analysed by inferring the amplicon sequence variants (ASVs) using the Dada2 1.9.3 package (Callahan et al., 2016). We obtained approximately 1.3 M sequences of Archaea and 4.1 M sequences of Bacteria. Reads with phred score >30 were truncated at the positions 220 and 180 for Archaea and 200 and 190 for Bacteria. Sequences were error-corrected, dereplicated, merged, and chimera-filtered. After quality control, 700,396 archaeal sequences with an average length of 383 bp and 3,054,201 bacterial sequences with an average length of 253 bp were obtained. Taxonomy was assigned using the SILVA database (release 138, 27.03.2020). The ASV counts classified in the same taxonomic groups were summarised and normalised to within-sample relative abundance (TSS - total sum scaling method, Lin & Peddada, 2020).

Statistical analyses and graphical visualisation were carried out using vegan 2.5–1 (Oksanen et al., 2018), ARTool 0.10.5 (Kay

& Wobbrock, 2018), lsmeans 2.30–0 (Lenth, 2018), dunn.test 1.3.5 (Dinno, 2017) and ggplot2 3.1.0 (Wickham & Chang, 2016) packages. Microbial taxa with reported methanogenic or methanotrophic capabilities were manually filtered and grouped at the genus level (except for the family Methylomirabilaceae, which was grouped at this level) using the databases PhyMet2 (http://phymet2.biotech.uni.wroc.pl/) and Methanotroph Commons (http://www.methanotroph.org/wiki/taxonomy/), respectively. Shapiro-Wilk normality test and Levene's homogeneity test were performed in order to define the most appropriate statistical test to be used to detect significant differences among treatments. Nonmetric multidimensional scaling (NMDS) and permutational multivariate analysis of variance (PERMANOVA) were used to assess the similarities among samples regarding chemical properties (Gower distance) and community composition (Bray-Curtis distance). Envfit analysis was also carried out, aiming to identify environmental variables significantly correlated with the microbial community structure. Kruskal-Wallis with post hoc Dunn's test was used to determine statistical differences among the chemical properties of the studied areas. Two-way ANOVA of aligned rank transformed data was used to investigate the effect of location and sampling time on the relative abundance of CH_4 cycling-related taxa and functional gene quantities in the floodplains, the later followed by pairwise comparisons using Tukey's adjustment.

3 | RESULTS

3.1 | Variability in chemical properties

Lower values ($p < .05$) of N, P, K, Ca, Mg, Al, Cu, Mn, and Zn were observed for FP1 compared to FP2 and FP3. Forest (PFO) soils presented higher OM and N contents in relation to the floodplains (Table S2). Multivariate analysis indicated that the sites were highly different ($R^2 = .81$, $p = .001$) regarding their chemical profiles. There was also a significant effect of sampling time, albeit explaining only a small fraction of the variability ($R^2 = .05$, $p = .004$) (Figure 1; Table 1).

3.2 | Archaeal and bacterial communities

PERMANOVA (Table 1), NMDS ordination, and Envfit analysis (Figure 2a, Table S3) indicated significant differences in the composition of the archaeal communities among the three floodplain sites. We did not observe a significant effect of sampling time on the archaeal communities. Higher pH values correlated significantly with the PF1 archaeal community, while P, K, Ca, Mg, Al, Cu, Mn, and Zn were correlated with some PF3 communities. In addition, increasing B and OM contents were positively correlated with PFO communities. Bathyarchaeia, Nitrososphaeria, and Methanobacteria were the dominant classes in all floodplain sites (reaching up to 59, 65, and 52%, respectively; Figure S2a). In addition, Nanoarchaeia also presented a high relative abundance in FP1 (ranging from 1.1 to 28%). By contrast, forest soils formed a separated cluster (Figure 2a), indicating major differences in archaeal community composition in relation to the floodplains - this observation was confirmed by PERMANOVA, which had the R^2 increased from .38 to .76 when the forest site was included in the analysis. The forest soils were largely dominated by the class Nitrososphaeria, which represented >92% of the archaeal communities (Figure S2a).

The composition of the bacterial communities was found to be significantly different across the studied sites (Figure 2b, Table 1, Table S3). The sampling area explained 46% of the variability in floodplain bacterial composition and 62% when the forest samples were included in the analyses. Sampling time was found to

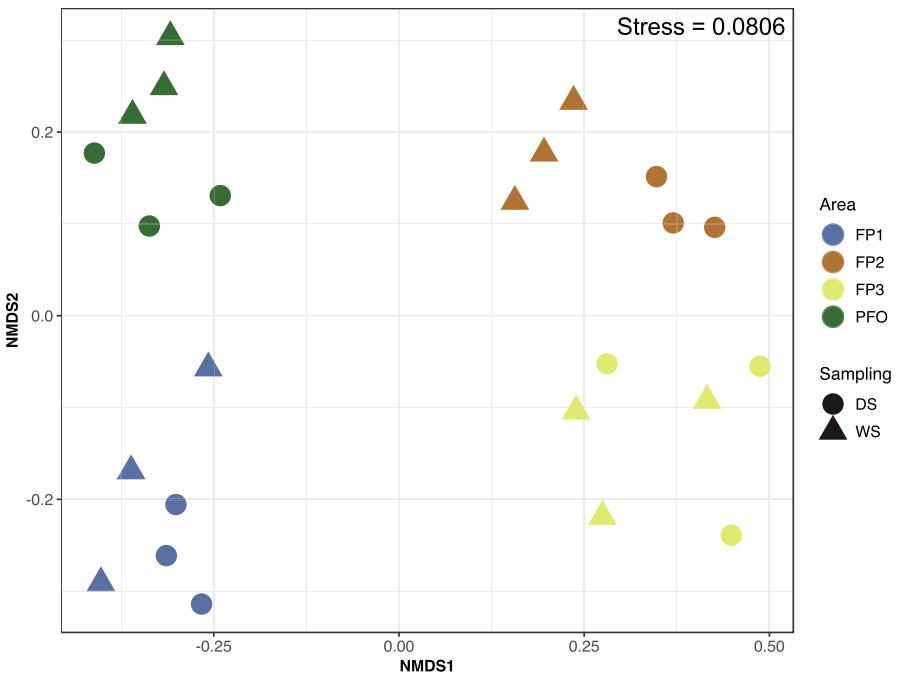

FIGURE 1 Clustering of the chemical properties of the floodplain sediments (FP1, FP2, and FP3) and upland forest soils (PFO) during wet (WS) and dry (DS) sampling times. Plot is based on the nonmetric multidimensional scaling (NMDS) using the Gower distance index [Colour figure can be viewed at wileyonlinelibrary.com]

TABLE 1 Permutational Multivariate Analysis of Variance of the chemical properties and the microbial communities' taxonomic profile in the floodplain sediments and upland forest soils

	Area			Sampling Time			Area x Sampling		
Data	R^2	*F*	*p*-value	R^2	*F*	*p*-value	R^2	*F*	*p*-value
Including upland forest data									
Chemical properties	.806	41.108	**.001**	.047	7.123	**.004**	.042	2.148	.065
16S Archaeal Sequencing	.761	21.211	**.001**	.008	0.682	.541	.039	1.098	.357
16S Bacterial Sequencing	.628	14.146	**.001**	.048	3.265	**.008**	.087	1.969	**.028**
Only floodplain data									
Chemical propertiers	.754	32.786	**.001**	.078	6.780	**.006**	.030	1.310	**.028**
16S Archaeal Sequencing	.384	4.682	**.002**	.028	0.670	.614	.096	1.169	.319
16S Bacterial Sequencing	.469	8.535	**.001**	.113	4.090	**.003**	.088	1.595	**.082**

Abbreviations: FP1, Floodplain 1; FP2, Floodplain 2; FP3, Floodplain 3; PFO, Upland Forest. Bold values indicate statistical significance at p-value <0.05. Distance index: Gower (chemical properties) and Bray-Curtis (amplicon sequencing at genus level).

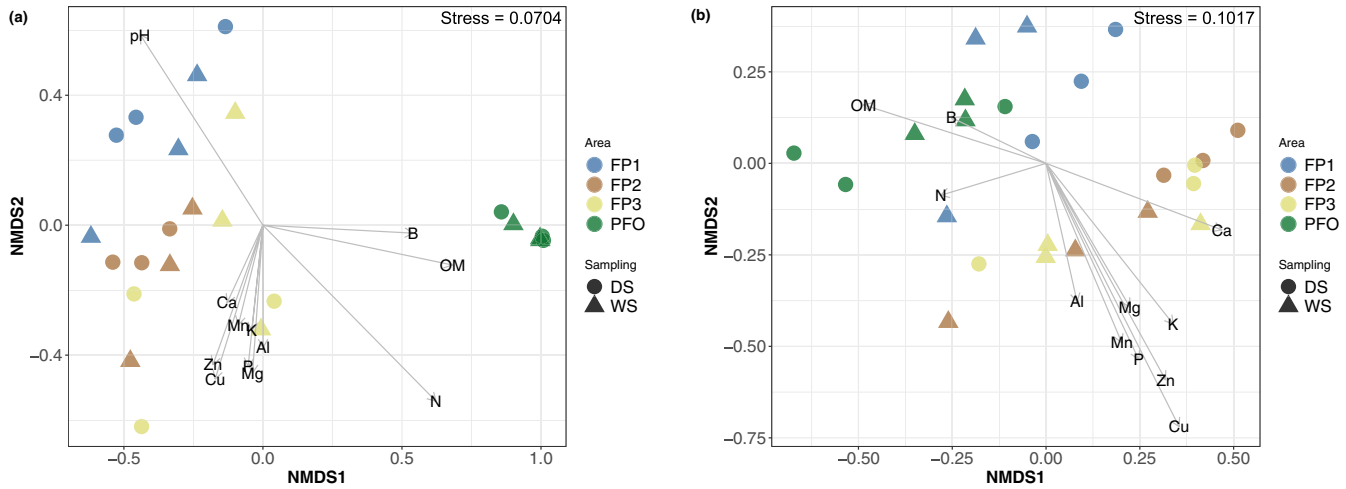

FIGURE 2 Clustering of the (a) archaeal and (b) bacterial communities at genus level in the floodplain sediments (FP1, FP2, and FP3) and upland forest soils (PFO) during wet (WS) and dry (DS) sampling times, and correlation with environmental factors. Only environmental factors that share significant correlation ($p < .05$) with community structure are shown with vectors: Al, aluminium; B, boron; Ca, calcium; Cu, copper; K, potassium; Mg, magnesium; Mn, manganese; N, nitrogen; OM, organic matter; P, phosphorus; pH, hydrogen potential; Zn, zinc. Plot is based on the nonmetric multidimensional scaling (NMDS) using the Bray-Curtis distance index [Colour figure can be viewed at wileyonlinelibrary.com]

have a weak but significant effect on the bacterial composition (R^2: .05 and .11, when the analyses were carried out with and without the forest site, respectively). While the bacterial communities from FP1 showed positive correlation only with pH, FP2 and FP3 were associated with increasing concentrations of Al, Mn, Zn, Mg, K, Cu, and P. Similar to the observed for Archaea, the PFO bacterial communities were correlated with OM and B. Despite oscillations in relative abundance, the following bacterial classes were found to be the most predominant across all sites: Acidobacteriae, Actinobacteria, Alphaproteobacteria, Gammaproteobacteria, and Thermophilia (Figure S2b).

The abundance of archaea and bacteria in the samples was assessed by qPCR. Standard curves presented R^2 values above .99 and amplification efficiencies between 75% and 99%. The average abundance (gene copies ng DNA^{-1}) ranged from 3×10^3 to 4×10^4 for archaeal 16S rRNA and 3×10^5 to 1×10^6 for bacterial 16S rRNA (Figure S3, Tables S4 and S5). Sampling time was found to be the only factor significantly affecting the bacterial abundance, while archaea responded only to site x season interaction (Table 2).

3.3 | Methanogenic and methanotrophic communities

We searched the 16S rRNA gene sequence data for taxa with a reported role in CH_4 production or consumption. The overall relative abundance of methanogenic archaea in the floodplain sediments varied significantly across sites (Table 2), ranging from 1.4% to 53% of the archaeal communities. By contrast, methanogens comprised less than 1.3% of forest soil archaea (Figure 3). *Methanobacterium*

TABLE 2 Two-way ANOVA of the aligned rank transformed qPCR and sequencing data from the floodplains

	Area			Sampling Time			Area x Sampling Time		
Data	***df***	***F***	***p*-value**	***df***	***F***	***p*-value**	***df***	***F***	***p*-value**
Gene quantification (copies ngDNA^{-1})									
16S rRNA Archaea	2, 12	3.745	.055	1, 12	4.708	.051	2, 12	4.401	**.037**
16S rRNA Bacteria	2, 12	3.843	.051	1, 12	13.286	**.003**	2, 12	0.406	.675
*mcr*A	2, 12	1.388	.287	1, 12	0.001	.971	2, 12	0.180	.837
*pmo*A	2, 12	0.013	.987	1, 12	8.768	**.012**	2, 12	0.056	.945
Ratio *mcr*A:*pmo*A	2, 12	2.439	.129	1, 12	3.314	.094	2, 12	0.502	.618
Archaeal methane-cycling taxa (Relative abundance)									
Total	2, 12	12.590	**.001**	2, 12	0.676	.437	2, 12	2.957	.090
Candidatus *Methanoperedens* - AOM	2, 12	7.841	**.006**	2, 12	11.982	**.005**	2, 12	6.397	**.013**
Methanobacterium	2, 12	12.195	**.001**	2, 12	0.439	.520	2, 12	2.122	.159
Methanosarcina	2, 12	2.728	.106	2, 12	10.203	**.008**	2, 12	18.017	**.001**
Methanomassiliicoccus	2, 12	2.708	.107	2, 12	2.545	.137	2, 12	1.164	.345
Bacterial methane-cycling taxa (Relative abundance)									
Total	2, 12	1.123	.357	2, 12	0.989	.340	2, 12	0.371	.698
Methanotrophs Type I	2, 12	2.678	.109	2, 12	9.188	**.010**	2, 12	4.674	**.032**
Methanotrophs Type II	2, 12	0.018	.982	2, 12	0.014	.909	2, 12	0.235	.794
Methylomirabilaceae - AOM	2, 12	0.599	.565	2, 12	4.764	**.049**	2, 12	3.943	**.048**
Candidatus *Methylospira*	2, 12	4.617	**.033**	2, 12	11.132	**.006**	2, 12	6.674	**.011**
Methylobacter	2, 12	1.205	.334	2, 12	5.329	**.040**	2, 12	4.065	**.045**
Methylobacterium	2, 12	4.594	**.033**	2, 12	4.600	.053	2, 12	6.522	**.012**
Methylocystis	2, 12	0.177	.840	2, 12	0.038	.848	2, 12	0.240	.790
Methylomonas	2, 12	3.068	.084	2, 12	2.122	.168	2, 12	0.900	.432

Abbreviations: *df*, degrees of freedom, *F*, F-values. Bold values indicate statistical significance at *p*-value <.05.

was the most dominant methanogenic genus in all samples. The proportions of ASVs assigned to this genus changed significantly across sites, with higher values observed for FP2 and FP3, but it was not influenced by sampling time. *Methanomassiliicoccus* was detected in lower proportions, mainly in FP1, and was not affected by any of the tested factors, while *Methanosarcina* relative abundance only changed significantly between sampling times (Table 2).

Methanotrophic taxa represented only a small fraction of the bacterial and archaeal communities. The relative abundance of bacterial methanotrophs ranged from 0.7% to 3.3% in the floodplain sediments, while it was below 0.6% in the forest soils. *Methylocystis* was the most dominant methanotrophic genus in all sites. The family Methylomirabilaceae was also a prevalent taxon across the studied sites, mainly in FP2. Furthermore, *Methylomonas* and *Methylobacter* were found to be enriched in FP1 (Figure 3). We detected taxa that comprised four types of aerobic and anaerobic methanotrophic metabolisms: Type I, Candidatus *Methylospira*, *Methylobacter*, and *Methylomonas*; Type II, *Methylobacterium* and *Methylocystis*; AOM - Bacteria, Methylomirabilaceae; and AOM - Archaea, Candidatus *Methanoperedens* (Figure 4). Taxa related to type I methanotrophy and AOM - Bacteria were significantly affected by sampling time and by site x sampling interaction, while the AOM - Archaea taxon was affected by both factors and their interaction. By contrast, Type II was not affected by any factor (Table 2).

We assessed the abundance of the gene markers for methanogenesis (*mcr*A) and aerobic methanotrophy (*pmo*A). In floodplain sediments, regardless of the season, there was a significantly higher abundance of methanogens and methanotrophs in comparison to forest soil (Figure 5, Tables S4 and S5). The *mcr*A gene average abundance ranged from 2×10^3 to 5×10^3 copies ng DNA^{-1} in the floodplain sediments, while in the forest soils, it was below the detection limit. Gene counts for *pmo*A in floodplain sediments ranged from 1×10^2 to 4×10^2 copies ng DNA^{-1}, while forest soils presented less than 5×10^1 copies ng DNA^{-1}. Considering only the floodplains, the abundance of the *mcr*A gene was not significantly affected by area or sampling time, while *pmo*A varied with sampling time (Table 2). When the floodplain sites were inspected separately regarding *pmo*A gene abundance, we observed a reduction during the wet season for all sites, but it was deemed significant only in FP1 and FPO (Table S5). Furthermore, the *mcr*A:*pmo*A ratios were not significantly affected by any factor (Table 2, Figure 5c).

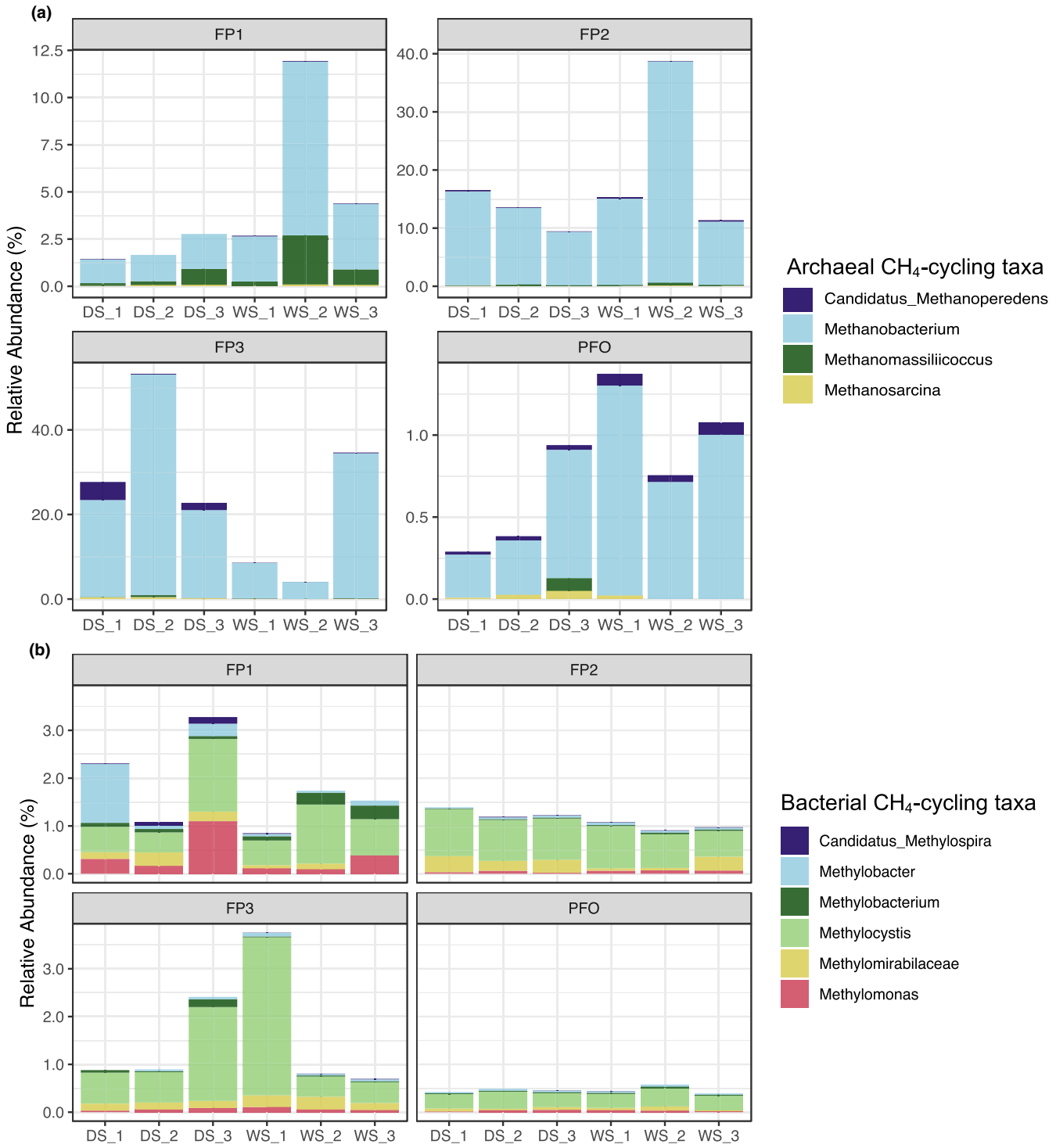

FIGURE 3 Relative abundance of the (a) archaeal and (b) bacterial CH_4-cycling taxa in the floodplain sediments (FP1, FP2, and FP3) and upland forest soils (PFO) during wet (WS) and dry (DS) sampling times [Colour figure can be viewed at wileyonlinelibrary.com]

4 | DISCUSSION

The Amazon River, termed "white-water", transports large amounts of nutrient-rich sediments, which are carried to the floodplain forests during the wet season. By contrast, the Tapajós river termed "clear-water", delivers low amounts of sediments and dissolved solids (Junk et al., 2011). The transfer of materials by the Amazon River to sediments at sites FP2 and FP3 was indicated by their higher contents of trace metals, such as Cu, Mn, Fe, and Zn when compared to FP1, which is exposed exclusively to the Tapajós River. In fact, in relation to the chemistry, FP1 clustered separately from the other floodplains, suggesting that due to the low nutrient content of the Tapajos, forest-related factors may have a stronger influence on the chemical composition of these sediments.

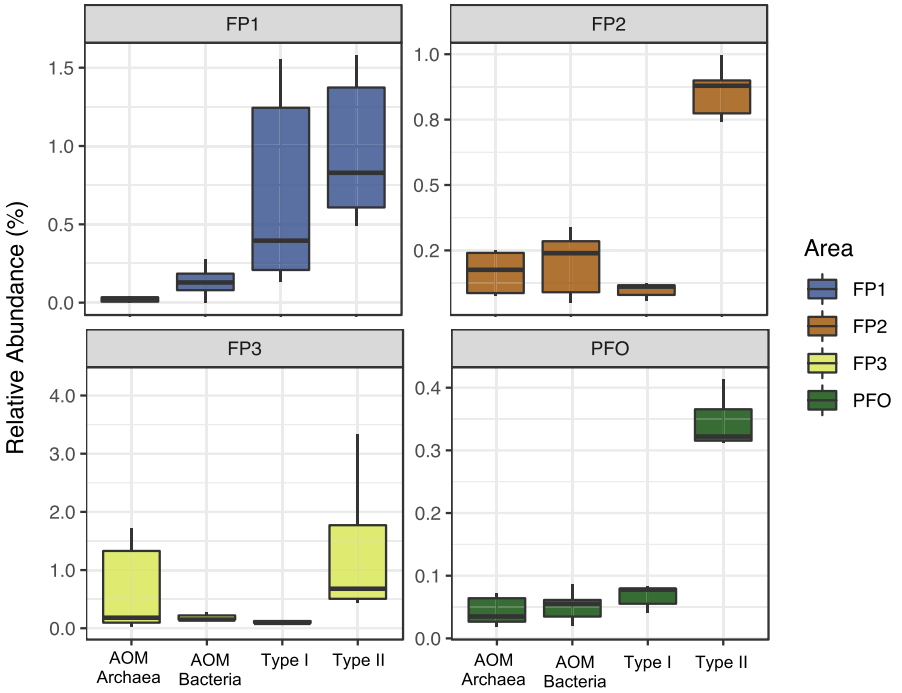

FIGURE 4 Relative abundance of the different methanotrophic metabolism types in the floodplain sediments (FP1, FP2, and FP3) and upland forest soils (PFO). AOM Archaea: Candidatus *Methanoperedens*. AOM Bacteria: *Methylomirabilaceae*. Type I: Candidatus *Methylospira*, *Methylobacter*, and *Methylomonas*. Type II: *Methylobacterium* and *Methylocystis*. Taxa with same metabolism type were grouped and their proportions were presented per site [Colour figure can be viewed at wileyonlinelibrary.com]

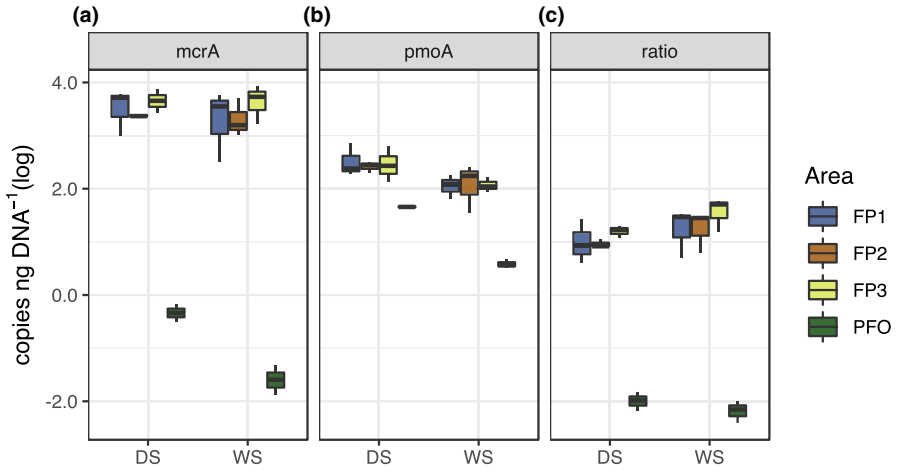

FIGURE 5 Functional gene copy number. Number of copies per ng of DNA (copies ng DNA-1) of (a) *mcr*A, (b) *pmo*A genes, and (c) *mcr*A:*pmo*A ratio in the floodplain sediments (FP1, FP2, and FP3) and upland forest soils (PFO) during wet (WS) and dry (DS) sampling times. Means and standard deviations from the raw data are available in Table S3 [Colour figure can be viewed at wileyonlinelibrary.com]

In the Amazon region, several studies already showed the importance of soil chemical properties on the soil microbial community composition (Rodrigues et al., 2013) and function (Lammel et al., 2015; Meyer et al., 2020; Paula et al., 2014). Here we show that archaeal and bacterial communities in sediments from the three floodplains analysed are significantly distinct from upland forest soil communities regarding their composition. In addition, we observed major differences in those communities among floodplain areas with distinct environmental characteristics. This information is valuable to disentangle which environmental factors drive the variations in microbe-driven ecosystem processes. It is interesting that the archaeal and bacterial communities from the FP1 did not cluster together with upland forest soil communities, differently from what was observed for the chemical properties. This might indicate that periodic flooding, and the changes caused by it, could be a major determinant of the composition of the microbial communities in the floodplain forests, and also that environmental factors not assessed in this study are likely to be affecting these communities.

Our data indicated that the composition of the microbial communities is related to the sampling area, while only the bacterial community was little (but significantly) influenced by the sampling time. In addition, time also affected the total bacterial abundance. Microbes are known for their high degree of metabolic flexibility and physiological adaptations that may allow them to endure under changing environmental conditions (Meyer et al., 2004; Ye et al., 2018). The periodic flooding events may favour microbial taxa adapted to these environmental oscillations, and the endurance of the archaeal communities under such contrasting conditions is noteworthy.

Upland forest soils were largely dominated by the class Nitrososphaeria, while in the floodplains, in addition to this class, Bathyarchaeia and Methanobacteria were also among the dominant groups. Recent metagenomic studies have suggested that the class Bathyarchaeia may have a potential function in processes related to anaerobic degradation of organic matter, including acetogenesis (He et al., 2016; Maus et al., 2018) and methanogenesis (Evans et al., 2015). However, the demonstration of these physiological

capabilities is still missing. The prevalence of Bathyarchaeia in the floodplain sediments could be an indication of their role in this environment.

We also observed a higher relative abundance of the class Nanoarchaeia (with 100% of the ASVs assigned to the order Woesearchaeales) in FP1, in relation to the other floodplain areas. Although scant information is available for this class, studies have suggested that some groups may establish syntrophic interactions with methanogens and provide metabolic complementation (Liu et al., 2018, 2021). This might indicate a potential role of this group in the CH_4 cycle of FP1, which has oligotrophic characteristics if compared to the other floodplains. Together, Bathyarchaeia, Methanobacteria and Nanoarchaeia comprised more than half of the total archaeal community in the floodplains, highlighting the importance of the CH_4 metabolism in the biogeochemistry of these ecosystems.

Among the methanogens, *Methanobacterium* was the dominant genus in all areas. This group is widely distributed in anaerobic environments across the globe and is known to produce CH_4 mainly through the hydrogenotrophic pathway (Evans et al., 2019). *Methanosarcina*, which accounted only for a small fraction of the methanogens, is a metabolically versatile group and is able to utilise H_2/CO_2, acetate, methylamines, and methanol as substrates for methanogenesis (Evans et al., 2019; Fournier, 2009; Welander & Metcalf, 2008). *Methanomassiliicoccus*, which was part of the dominant groups only in FP1, is known to have the metabolic capability to perform methylotrophic and hydrogen-dependent methanogenesis (Nkamga & Drancourt, 2016). In our study, we observed a prevalence of microbes with the potential to perform the hydrogenotrophic pathway. Yet, investigations on the RNA level and the isotopic signal would be required to determine the dominant metabolic route in the Amazonian floodplains. The dominance of hydrogenotrophic methanogens could have implications for CH_4 production under climate change influence. It has been proposed that temperature rise can favour this metabolism over the acetoclastic one in freshwater ecosystems (Zhu et al., 2020), which could result in an amplifying effect.

We did not find significant variations in both relative and absolute abundance of methanogens between both sampling times, i.e., during wet and dry seasons. Despite the lack of knowledge in the mechanisms underpinning this process, the resistance of methanogens to drainage was also reported by Ma and Lu (2011). This resistance may be related to the protection against oxidative stress, as already reported for methanogens (Angle et al., 2017; Lyu et al., 2018). According to Hernández et al., (2019), the abundance of methanogens could be an indication of the flooding history of the Amazon floodplain. In a microcosm experiment, the authors observed the survival of the methanogenic populations to short but not to long periods of desiccation. Here, we report at the DNA level the persistence of these communities in situ under conditions with and without flooding.

Regarding the aerobic CH_4-oxidising microbes, *Methylocystis and Methylobacterium*, classified as the Type II methanotrophs, were found to comprise large fractions of the communities and did not seem to be affected by sampling time. These organisms are known to endure under fluctuations in the environment, such as variable O_2 and CH_4 availability. Their stability is owed to several strategies, including dormancy (Eller et al., 2005; Ho et al., 2013; Krause et al., 2012) and ability to use different carbon substrates (Dedysh et al., 2005, 2016). Such versatility could confer an advantage in the floodplain environment, which may present not only temporal but also spatial variation in CH_4 availability (Moura et al., 2008). By contrast, the relative abundance of Candidatus *Methylospira*, *Methylobacter*, and *Methylomonas*, classified as Type I, were significantly different between sampling times (Table 2). Often, members of this group are very responsive to high substrate availability (Ho et al., 2013). They also have their abundance reduced quickly under O_2 limitation or other adverse conditions (Knief, 2015), which could be comparable to the contrasting wet and dry seasons in the Amazon basin.

In the floodplain sediments, we also detected both archaeal and bacterial taxa with the reported capability to carry out anaerobic CH_4 oxidation. The archaeal genus Candidatus *Methanoperedens* has the capability to carry out anaerobic methanotrophy using the nitrate-dependent reverse methanogenesis (Yan & Ferry, 2018) or in consortia with sulphate-reducing bacteria (Su et al., 2019). In addition, it has been demonstrated that members of the archaeal anaerobic methanotrophic group, including the Candidatus *Methanoperedens*, can couple CH_4 oxidation to the reduction of metals such as Fe(III) and Mn(IV) (Gabriel et al., 2020; Leu et al., 2020). In fact, we detected this genus mainly in FP2 and FP3, which were also the floodplains with the highest quantities of those metals. Another group, the bacterial family Methylomirabilaceae, which is among the dominant methanotrophs detected in this study, comprises taxa with the capability to oxidise CH_4 under anaerobic conditions using nitrite as an electron acceptor. This process generates oxygen internally, which is subsequently used to oxidise CH_4 as a carbon source (Ettwig et al., 2010; Padilla et al., 2016). This unique metabolism allows Methylomirabilaceae to thrive in CH_4-rich and oxygen-depleted environments (Shen et al., 2016).

The rate of CH_4 emission in wetlands is a result of the balance between its production and consumption (Malyan et al., 2016). Although many studies showed that Amazonian floodplains are an important source of CH_4 (Barbosa et al., 2020; Potter et al., 2014; Ringeval et al., 2014), Koschorreck (2000) demonstrated that the CH_4 emission rates may decrease to zero when the sediments become exposed to air. In fact, we observed a small but significant increase in counts of *pmo*A copies from aerobes under nonflooded conditions across all floodplain areas (Figure 5; Table 2). The drainage of the floodplains allows for the oxygenation of the sediment, which could promote an increase in abundance and/or activity of the aerobic methanotrophs. However, *mcr*A:*pmo*A ratios were not significantly different under flooded and nonflooded conditions. Therefore, at the DNA level, the ratio between methanogens and aerobic methanotrophs does not seem to reflect the differences in CH_4 emissions during contrasting flooding conditions reported previously (Koschorreck, 2000). As presented in Figure 4, taxa with the potential for different methanotrophic metabolisms play different

contributions to the pool of methanotrophs across the studied sites. The changes in pools of methanotrophs occupying distinct niches, including different electron acceptors and endurance to environmental variability (reviewed by Stein, 2020), could be related to the contrasting physical and chemical properties of the studied sites. Deciphering the links between CH_4 emissions and microbial pools in the Amazonian floodplains is, therefore, a complex task and requires further investigations monitoring these microbial groups. This information is also relevant in the context of climate change, which could lead to shifts in the proportions of different methanotrophic groups. For instance, increase in hypoxia due to temperature rise (Diaz & Rosenberg, 2008) could lead to a higher contribution of AOM in relation to the aerobic process. The action of these organisms could play a key role to offset predicted increases in methanogenesis with higher temperatures (Marotta et al., 2014; Yvon-Durocher et al., 2014). However, the effect of the temperature per se on the anaerobic methanotrophs from tropical floodplains has not been explored. The contributions of the different groups could also suffer from the influence of the rainfall regime, which is predicted to be affected by climate change (Evans & Wallenstein, 2013; Trenberth et al., 2014). AOM may represent an important CH_4 sink under flooding and contribute to reduce part of the emissions from this environment. In fact, using isotopic signal, Sawakuchi et al., (2016) estimated that biological CH_4 oxidation in Amazonian rivers may prevent large amounts of CH_4 to be released to the atmosphere, which could be the equivalent to 7% of the oxidation estimated for upland soils.

We described the composition of CH_4 cycling microbial communities in three different types of floodplains located in the Eastern Amazon. We observed that methanogens are present in high abundance and they seem to resist the dramatic environmental changes that occur between the contrasting sampling times. Methanotrophs that use different pathways to oxidise CH_4 were detected and presented distinct distribution patterns, indicating that a wide metabolic diversity may be harboured in this highly variable environment. This environmental variability, which is remarkably affected by the river origin, drives not only the floodplain sediment chemistry but also the composition of the microbial communities. The results improve our knowledge on the CH_4 cycling microbes in the Amazonian floodplains and provide insights into their vastly unexplored methanotrophic diversity. Such information is essential for deciphering the complexity of the CH_4 cycling in the Amazonian floodplains, as microbial groups occupying distinct niches may respond differently to ongoing and future environmental changes, including those related to climate change.

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AUTHOR CONTRIBUTIONS

Júlia B. Gontijo, Siu M. Tsai, Brendan J. M. Bohannan, Klaus Nüsslein, and Jorge L. Mazza Rodrigues designed the research; Júlia B. Gontijo, Andressa M. Venturini, José Mauro S. Moura and Clovis D. Borges collected the samples; Júlia B. Gontijo and Andressa M. Venturini performed the benchwork; Júlia B. Gontijo, Andressa M. Venturini and Caio A. Yoshiura analysed the data; Júlia B. Gontijo and Fabiana S. Paula wrote the manuscript; all authors contributed to the final manuscript version.

DATA AVAILABILITY STATEMENT

16S rRNA sequence data have been deposited on NCBI's Sequence Read Archive (SRA) under the accession number PRJNA629547.

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REFERENCES

Alvares, C. A., Stape, J. L., Sentelhas, P. C., Gonçalves, J. L. M., & Sparovek, G. (2013). Köppen's climate classification map for Brazil. *Meteorologische Zeitschrift*, *22*(6), 711–728. https://doi.org/10.1127/0941-2948/2013/0507

Angle, J. C., Morin, T. H., Solden, L. M., Narrowe, A. B., Smith, G. J., Borton, M. A., Rey-Sanchez, C., Daly, R. A., Mirfenderesgi, G., Hoyt, D. W., Riley, W. J., Miller, C. S., Bohrer, G., & Wrighton, K. C. (2017). Methanogenesis in oxygenated soils is a substantial fraction of wetland methane emissions. Nature. *Communications*, *8*(1), https://doi.org/10.1038/s41467-017-01753-4

Barbosa, P. M., Melack, J. M., Amaral, J. H. F., MacIntyre, S., Kasper, D., Cortés, A., Farjalla, V. F., & Forsberg, B. R. (2020). Dissolved methane concentrations and fluxes to the atmosphere from a tropical floodplain lake. *Biogeochemistry*, *148*(2), 129–151. https://doi.org/10.1007/s10533-020-00650-1

Barros, D. J., de Souza Bento, M., de Souza Ferreira, M., Braga do Carmo, J., Hartung Toppa, R., Neu, V., & Aparecido Navarrete, A. (2020). Methane, microbes and models in Amazonian Floodplains: State of the art and perspectives. In L. Hufnagel (Eds.), *Changing ecosystems and their services* (1st ed., Vol. *1*, pp. 270–287). IntechOpen. https://doi.org/10.5772/intechopen.90247

Bridgham, S. D., Cadillo-Quiroz, H., Keller, J. K., & Zhuang, Q. (2013). Methane emissions from wetlands: Biogeochemical, microbial, and modeling perspectives from local to global scales. *Global Change Biology*, *19*(5), 1325–1346. https://doi.org/10.1111/gcb.12131

Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J. A., & Holmes, S. P. (2016). DADA2: High-resolution sample inference

from Illumina amplicon data. *Nature Methods*, *13*(7), 581–583. https://doi.org/10.1038/nmeth.3869

Camargo, O. A., Moniz, A. C., Jorge, J. A., & Valadares, J. M. A. (2009). Métodos de análise química, mineralógica e física de solos do Instituto Agronômico de Campinas. *Boletim Técnico*, *106*, 77.

Caporaso, J. G., Lauber, C. L., Walters, W. A., Berg-Lyons, D., Lozupone, C. A., Turnbaugh, P. J., Fierer, N., & Knight, R. (2011). Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. *Proceedings of the National Academy of Sciences of the United States of America*, *108*(1), 4516–4522. https://doi.org/10.1073/pnas.1000080107

Conrad, R. (2009). The global methane cycle: Recent advances in understanding the microbial processes involved. *Environmental Microbiology Reports*, *1*, 285–292. https://doi.org/10.1111/j.1758-2229.2009.0

Conrad, R., Ji, Y., Noll, M., Klose, M., Claus, P., & Enrich-Prast, A. (2013). Response of the methanogenic microbial communities in Amazonian oxbow lake sediments to desiccation stress. *Environmental Microbiology*, *16*(6), 1682–1694. https://doi.org/10.1111/1462-2920.12267

Conrad, R., Klose, M., Claus, P., & Enrich-Prast, A. (2010). Methanogenic pathway, 13C isotope fractionation, and archaeal community composition in the sediment of two clear-water lakes of Amazonia. *Limnology and Oceanography*, *55*(2), 689–702. https://doi.org/10.4319/lo.2009.55.2.0689

Cui, M., Ma, A., Qi, H., Zhuang, X., & Zhuang, G. (2015). Anaerobic oxidation of methane: An "active" microbial process. *MicrobiologyOpen*, *4*, 1–11. https://doi.org/10.1002/mbo3.232

Dedysh, S. N., Haupt, E. S., & Dunfield, P. F. (2016). Emended description of the family Beijerinckiaceae and transfer of the genera Chelatococcus and Camelimonas to the family Chelatococcaceae fam. nov. *International Journal of Systematic and Evolutionary Microbiology*, *66*(8), 3177–3182. https://doi.org/10.1099/ijsem.0.001167

Dedysh, S. N., & Knief, C. (2018). Diversity and phylogeny of described aerobic methanotrophs. *Methane Biocatalysis: Paving the Way to Sustainability*, *17*, 42. https://doi.org/10.1007/978-3-319-74

Dedysh, S. N., Knief, C., & Dunfield, P. F. (2005). Methylocella species are facultatively methanotrophic. *Journal of Bacteriology*, *187*(13), 4665–4670. https://doi.org/10.1128/JB.187.13.4665-4670.2005

Diaz, R. J., & Rosenberg, R. (2008). Spreading dead zones and consequences for marine ecosystems. *Science*, *321*(5891), 926–929. https://doi.org/10.1126/science.1156401

Dinno, A. (2017). dunn.test: Dunn's Test of Multiple Comparisons Using Rank Sums. R package version 1.3.5.

Eller, G., Krüger, M., & Frenzel, P. (2005). Comparing field and microcosm experiments: A case study on methano- and methylo-trophic bacteria in paddy soil. *FEMS Microbiology Ecology*, *51*(2), 279–291. https://doi.org/10.1016/j.femsec.2004.09.007

Ettwig, K. F., Butler, M. K., Le Paslier, D., Pelletier, E., Mangenot, S., Kuypers, M. M. M., Schreiber, F., Dutilh, B. E., Zedelius, J., de Beer, D., Gloerich, J., Wessels, H. J. C. T., van Alen, T., Luesken, F., Wu, M. L., van de Pas-Schoonen, K. T., Op den Camp, H. J. M., Janssen-Megens, E. M., Francoijs, K.-J., … Strous, M. (2010). Nitrite-driven anaerobic methane oxidation by oxygenic bacteria. *Nature*, *464*(7288), 543–548. https://doi.org/10.1038/nature08883

Evans, P. N., Boyd, J. A., Leu, A. O., Woodcroft, B. J., Parks, D. H., Hugenholtz, P., & Tyson, G. W. (2019). An evolving view of methane metabolism in the Archaea. *Nature Reviews Microbiology*, *17*, 219–232. https://doi.org/10.1038/s41579-018-0136-7

Evans, P. N., Parks, D. H., Chadwick, G. L., Robbins, S. J., Orphan, V. J., Golding, S. D., & Tyson, G. W. (2015). Methane metabolism in the archaeal phylum Bathyarchaeota revealed by genome-centric metagenomics. *Science*, *350*(6259), 434–438. https://doi.org/10.1126/science.aac7745

Evans, S. E., & Wallenstein, M. D. (2013). Climate change alters ecological strategies of soil bacteria. *Ecology Letters*, *17*(2), 155–164. https://doi.org/10.1111/ele.12206

Fournier, G. (2009). Horizontal gene transfer and the evolution of methanogenic pathways. *Methods in Molecular Biology (Clifton. N.J.)*, *532*, 163–179. https://doi.org/10.1007/978-1-60327-853-9_9

Gabriel, G. V. M., Oliveira, L. C., Barros, D. J., Bento, M. S., Neu, V., Toppa, R. H., Carmo, J. B., & Navarrete, A. A. (2020). Methane emission suppression in flooded soil from Amazonia. *Chemosphere*, *250*, 126263. https://doi.org/10.1016/j.chemosphere.2020.126263

Gedney, N., Huntingford, C., Comyn-Platt, E., & Wiltshire, A. (2019). Significant feedbacks of wetland methane release on climate change and the causes of their uncertainty. *Environmental Research Letters*, *14*(8), 84027. https://doi.org/10.1088/1748-9326/ab2726

Haque, M. F. U., Xu, H. J., Colin Murrell, J., & Crombie, A. (2020). Facultative methanotrophs – diversity, genetics, molecular ecology and biotechnological potential: A mini-review. *Microbiology*, *166*(10), 894–908. https://doi.org/10.1099/mic.0.000977

Haroon, M. F., Hu, S., Shi, Y., Imelfort, M., Keller, J., Hugenholtz, P., Yuan, Z., & Tyson, G. W. (2013). Anaerobic oxidation of methane coupled to nitrate reduction in a novel archaeal lineage. *Nature*, *500*(7464), 567–570. https://doi.org/10.1038/nature12375

He, Y., Li, M., Perumal, V., Feng, X., Fang, J., Xie, J., Sievert, S. M., & Wang, F. (2016). Genomic and enzymatic evidence for acetogenesis among multiple lineages of the archaeal phylum Bathyarchaeota widespread in marine sediments. *Nature Microbiology*, *1*(6). https://doi.org/10.1038/nmicrobiol.2016.35

Hernández, M., Klose, M., Claus, P., Bastviken, D., Marotta, H., Figueiredo, V., Enrich-Prast, A., & Conrad, R. (2019). Structure, function and resilience to desiccation of methanogenic microbial communities in temporarily inundated soils of the Amazon rainforest (Cunia Reserve, Rondonia). *Environmental Microbiology*, *21*(5), 1702–1717. https://doi.org/10.1111/1462-2920.14535

Hess, L. L., Melack, J. M., Affonso, A. G., Barbosa, C., Gastil-Buhl, M., & Novo, E. M. L. M. (2015). Wetlands of the Lowland Amazon Basin: Extent, Vegetative Cover, and Dual-season Inundated Area as Mapped with JERS-1 Synthetic Aperture Radar. *Wetlands*, *35*(4), 745–756. https://doi.org/10.1007/s13157-015-0666-y

Ho, A., Kerckhof, F. M., Luke, C., Reim, A., Krause, S., Boon, N., & Bodelier, P. L. E. (2013). Conceptualizing functional traits and ecological characteristics of methane-oxidizing bacteria as life strategies. *Environmental Microbiology Reports*, *5*(3), 335–345. https://doi.org/10.1111/j.1758-2229.2012.00370.x

Ji, Y., Angel, R., Klose, M., Claus, P., Marotta, H., Pinho, L., Enrich-Prast, A., & Conrad, R. (2016). Structure and function of methanogenic microbial communities in sediments of Amazonian lakes with different water types. *Environmental Microbiology*, *18*(12), 5082–5100. https://doi.org/10.1111/1462-2920.13491.

Junk, W. J. (1997). The central Amazon floodplain: ecology of a pulsing system. In The central Amazon floodplain: ecology of a pulsing system.

Junk, W. J., Piedade, M. T. F., Schöngart, J., Cohn-Haft, M., Adeney, J. M., & Wittmann, F. (2011). A classification of major naturally-occurring Amazonian lowland wetlands. *Wetlands*, *31*(4), 623–640. https://doi.org/10.1007/s13157-011-0190-7

Kay, M., & Wobbrock, J. O. (2018). Aligned Rank Transform for Nonparametric Factorial ANOVAs. R package version 0.10.5.

Klindworth, A., Pruesse, E., Schweer, T., Peplies, J., Quast, C., Horn, M., & Glöckner, F. O. (2013). Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. *Nucleic Acids Research*, *41*(1), https://doi.org/10.1093/nar/gks808

Knief, C. (2015). Diversity and habitat preferences of cultivated and uncultivated aerobic methanotrophic bacteria evaluated based on pmoA as molecular marker. *Frontiers in Microbiology*, *6*, 1346. https://doi.org/10.3389/fmicb.2015.01346

Koschorreck, M. (2000). Methane turnover in exposed sediments of an Amazon floodplain lake. *Biogeochemistry*, *50*, 195–206. https://doi.org/10.1023/A:1006326018597

Krause, S., Lüke, C., & Frenzel, P. (2012). Methane source strength and energy flow shape methanotrophic communities in oxygen-methane counter-gradients. *Environmental Microbiology Reports*, *4*(2), 203–208. https://doi.org/10.1111/j.1758-2229.2011.00322.x

Lammel, D. R., Feigl, B. J., Cerri, C. C., & Nüsslein, K. (2015). Specific microbial gene abundances and soil parameters contribute to C, N, and greenhouse gas process rates after land use change in Southern Amazonian Soils. *Frontiers in Microbiology*, *6*(OCT), https://doi.org/10.3389/fmicb.2015.01057

Lenth, R. (2018). lsmeans: Least-Squares Means. R package version 2.30-0

Leu, A. O., Cai, C., McIlroy, S. J., Southam, G., Orphan, V. J., Yuan, Z., Hu, S., & Tyson, G. W. (2020). Anaerobic methane oxidation coupled to manganese reduction by members of the Methanoperedenaceae. *ISME Journal*, *14*(4), 1030–1041. https://doi.org/10.1038/s41396-020-0590-x

Lin, H., & Peddada, S. D. (2020). Analysis of microbial compositions: a review of normalization and differential abundance analysis. *Npj Biofilms and Microbiomes*, *6*(1), https://doi.org/10.1038/s41522-020-00160-w

Lindsay, W. L., & Norvell, W. A. (1978). Development of a DTPA Soil Test for Zinc, Iron, Manganese, and Copper. *Soil Science Society of America Journal*, https://doi.org/10.2136/sssaj1978.03615995004200030009x

Liu, X., Li, M., Castelle, C. J., Probst, A. J., Zhou, Z., Pan, J., Liu, Y., Banfield, J. F., & Gu, J.-D. (2018). Insights into the ecology, evolution, and metabolism of the widespread Woesearchaeotal lineages. *Microbiome*, *6*(1), https://doi.org/10.1186/s40168-018-0488-2

Liu, X., Wang, Y., & Gu, J. D. (2021). Ecological distribution and potential roles of Woesearchaeota in anaerobic biogeochemical cycling unveiled by genomic analysis. *Computational and Structural Biotechnology Journal*, *19*, 794–800. https://doi.org/10.1016/j.csbj.2021.01.013

Luton, P. E., Wayne, J. M., Sharp, R. J., & Riley, P. W. (2002). The mcrA gene as an alternative to 16S rRNA in the phylogenetic analysis of methanogen populations in landfill. *Microbiology*, *148*(11), 3521–3530. https://doi.org/10.1099/00221287-148-11-3521

Lyu, Z., Shao, N., Akinyemi, T., & Whitman, W. B. (2018). Methanogenesis. *Current Biology*, *28*, R727–R732. https://doi.org/10.1016/j.cub.2018.05.021

Ma, K., & Lu, Y. (2011). Regulation of microbial methane production and oxidation by intermittent drainage in rice field soil. *FEMS Microbiology Ecology*, *75*(3), 446–456. https://doi.org/10.1111/j.1574-6941.2010.01018.x

Malyan, S. K., Bhatia, A., Kumar, A., Gupta, D. K., Singh, R., Kumar, S. S., Tomer, R., Kumar, O. M., & Jain, N. (2016). Methane production, oxidation and mitigation: A mechanistic understanding and comprehensive evaluation of influencing factors. *Science of the Total Environment*, *572*, 874–896. https://doi.org/10.1016/j.scitotenv.2016.07.182

Marotta, H., Pinho, L., Gudasz, C., Bastviken, D., Tranvik, L. J., & Enrich-Prast, A. (2014). Greenhouse gas production in low-latitude lake sediments responds strongly to warming. *Nature Climate Change*, *4*(6), 467–470. https://doi.org/10.1038/nclimate2222

Maus, I., Rumming, M., Bergmann, I., Heeg, K., Pohl, M., Nettmann, E., Jaenicke, S., Blom, J., Pühler, A., Schlüter, A., Sczyrba, A., & Klocke, M. (2018). Characterization of Bathyarchaeota genomes assembled from metagenomes of biofilms residing in mesophilic and thermophilic biogas reactors. *Biotechnology for Biofuels*, *11*(1), https://doi.org/10.1186/s13068-018-1162-4

Meyer, A. F., Lipson, D. A., Martín, A. P., Schadt, C. W., & Schmidt, S. K. (2004). Molecular and Metabolic Characterization of Cold-Tolerant Alpine Soil Pseudomonas Sensu Stricto. *Applied and Environmental Microbiology*, *70*(1), 483–489. https://doi.org/10.1128/AEM.70.1.483-489.2004

Meyer, K. M., Morris, A. H., Webster, K., Klein, A. M., Kroeger, M. E., Meredith, L. K., Brændholt, A., Nakamura, F., Venturini, A., Fonseca de Souza, L., Shek, K. L., Danielson, R., van Haren, J., Barbosa de Camargo, P., Tsai, S. M., Dini-Andreote, F., de Mauro, J. M. S., Barlow, J., Berenguer, E., … Bohannan, B. J. M. (2020). Belowground changes to community structure alter methane-cycling dynamics in Amazonia. *Environment International*, *145*, https://doi.org/10.1016/j.envint.2020.106131

Moreira-Turcq, P., Seyler, P., Guyot, J. L., & Etcheber, H. (2003). Exportation of organic carbon from the Amazon River and its main tributaries. *Hydrological Processes*, *17*(7), 1329–1344. https://doi.org/10.1002/hyp.1287

Moura, J. M. S., Martens, C. S., Moreira, M. Z., Lima, R. L., Sampaio, I. C. G., Mendlovitz, H. P., & Menton, M. C. (2008). Spatial and seasonal variations in the stable carbon isotopic composition of methane in stream sediments of eastern Amazonia. *Tellus*, *Series B: Chemical and Physical Meteorology*, *60*(1), 21–31. https://doi.org/10.1111/j.1600-0889.2007.00322.x

Nkamga, V. D., & Drancourt, M. (2016). Methanomassiliicoccales. In Bergey's Manual of Systematics of Archaea and Bacteria (pp. 1–2). doi: 10.1002/9781118960608.obm00127

Oksanen, J., Guillaume Blanchet, F., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., & Stevens, M. H. H. (2018). vegan: Community Ecology Package.

Padilla, C. C., Bristow, L. A., Sarode, N., Garcia-Robledo, E., Gómez Ramírez, E., Benson, C. R., Bourbonnais, A., Altabet, M. A., Girguis, P. R., Thamdrup, B. O., & Stewart, F. J. (2016). NC10 bacteria in marine oxygen minimum zones. *ISME Journal*, *10*(8), 2067–2071. https://doi.org/10.1038/ismej.2015.262

Pangala, S. R., Enrich-Prast, A., Basso, L. S., Peixoto, R. B., Bastviken, D., Hornibrook, E. R. C., Gatti, L. V., Marotta, H., Calazans, L. S. B., Sakuragui, C. M., Bastos, W. R., Malm, O., Gloor, E., Miller, J. B., & Gauci, V. (2017). Large emissions from floodplain trees close the Amazon methane budget. *Nature*, *552*(7684), 230–234. https://doi.org/10.1038/nature24639

Paula, F. S., Rodrigues, J. L. M., Zhou, J., Wu, L., Mueller, R. C., Mirza, B. S., Bohannan, B. J. M., Nüsslein, K., Deng, Y. E., Tiedje, J. M., & Pellizari, V. H. (2014). Land use change alters functional gene diversity, composition and abundance in Amazon forest soil microbial communities. *Molecular Ecology*, *23*(12), 2988–2999. https://doi.org/10.1111/mec.12786

Pazinato, J. M., Paulo, E. N., Mendes, L. W., Vazoller, R. F., & Tsai, S. M. (2010). Molecular characterization of the archaeal community in an Amazonian wetland soil and culture-dependent isolation of methanogenic archaea. *Diversity*, *2*(7), 1026–1047. https://doi.org/10.3390/d2071026

Potter, C., Melack, J. M., & Engle, D. (2014). Modeling methane emissions from amazon floodplain ecosystems. *Wetlands*, *34*(3), 501–511. https://doi.org/10.1007/s13157-014-0516-3

Ringeval, B., Houweling, S., Van Bodegom, P. M., Spahni, R., Van Beek, R., Joos, F., & Röckmann, T. (2014). Methane emissions from floodplains in the Amazon Basin: Challenges in developing a process-based model for global applications. *Biogeosciences*, *11*(6), 1519–1558. https://doi.org/10.5194/bg-11-1519-2014

Rodrigues, J. L. M., Pellizari, V. H., Mueller, R., Baek, K., Jesus, E. D. C., Paula, F. S., Mirza, B., Hamaoui, G. S., Tsai, S. M., Feigl, B., Tiedje, J. M., Bohannan, B. J. M., & Nusslein, K. (2013). Conversion of the Amazon rainforest to agriculture results in biotic homogenization of soil bacterial communities. *Proceedings of the National Academy of Sciences of the United States of America*, *110*(3), 988–993. https://doi.org/10.1073/pnas.1220608110

RStudio Team (2018). RStudio: Integrated development for R. Retrieved from http://www.rstudio.com/.

Sawakuchi, H. O., Bastviken, D., Sawakuchi, A. O., Krusche, A. V., Ballester, M. V. R., & Richey, J. E. (2014). Methane emissions from Amazonian Rivers and their contribution to the global methane budget. *Global Change Biology*, *20*(9), 2829–2840. https://doi.org/10.1111/gcb.12646

Sawakuchi, H. O., Bastviken, D., Sawakuchi, A. O., Ward, N. D., Borges, C. D., Tsai, S. M., Richey, J. E., Ballester, M. V. R., & Krusche, A. V. (2016). Oxidative mitigation of aquatic methane emissions in large Amazonian rivers. *Global Change Biology*, *22*(3), 1075–1085. https://doi.org/10.1111/gcb.13169

Shen, L. D., Wu, H. S., Gao, Z. Q., Liu, X., & Li, J. (2016). Comparison of community structures of Candidatus Methylomirabilis oxyfera-like bacteria of NC10 phylum in different freshwater habitats. *Scientific Reports*, *6*, https://doi.org/10.1038/srep25647

Stahl, D. A., & Amann, R. (1991). Development and application of nucleic acid probes in bacterial systematics. In E. Stackebrandt, & M. Goodfellow (Eds.), *Nucleic Acid Techniques in Bacterial Systematics* (pp. 205–248). John Wiley & Sons Ltd.

Su, G., Zopfi, J., Yao, H., Steinle, L., Niemann, H., & Lehmann, M. F. (2019). Manganese/iron-supported sulfate-dependent anaerobic oxidation of methane by archaea in lake sediments. *Limnology and Oceanography*, *65*(4), 863–875. https://doi.org/10.1002/lno.11354

Trenberth, K. E., Dai, A., van der Schrier, G., Jones, P. D., Barichivich, J., Briffa, K. R., & Sheffield, J. (2014). Global warming and changes in drought. *Nature Climate Change*, *4*(1), 17–22. https://doi.org/10.1038/nclimate2067

Venturini, A. M., Nakamura, F. M., Gontijo, J. B., da França, A. G., Yoshiura, C. A., Mandro, J. A., & Tsai, S. M. (2020). Robust DNA protocols for tropical soils. *Heliyon*, *6*(5), https://doi.org/10.1016/j.heliyon.2020.e03830

Welander, P. V., & Metcalf, W. W. (2008). Mutagenesis of the C1 oxidation pathway in Methanosarcina barkeri: New insights into the Mtr/Mer bypass pathway. *Journal of Bacteriology*, *190*(6), 1928–1936. https://doi.org/10.1128/JB.01424-07

Welte, C. U., Rasigraf, O., Vaksmaa, A., Versantvoort, W., Arshad, A., Op den Camp, H. J. M., Jetten, M. S. M., Lüke, C., & Reimann, J. (2016). Nitrate- and nitrite-dependent anaerobic oxidation of methane. *Environmental Microbiology Reports*, *8*(6), 941–955. https://doi.org/10.1111/1758-2229.12487

Wickham, H., & Chang, W. (2016). ggplot2': Create elegant data visualisations using the grammar of graphics. R package version 3.1.0.

Wilson, C., Gloor, M., Gatti, L. V., Miller, J. B., Monks, S. A., McNorton, J., Bloom, A. A., Basso, L. S., & Chipperfield, M. P. (2016). Contribution of regional sources to atmospheric methane over the Amazon Basin in 2010 and 2011. *Global Biogeochemical Cycles*, *30*(3), 400–420. https://doi.org/10.1002/2015GB005300

Yan, Z., & Ferry, J. G. (2018). Electron bifurcation and confurcation in methanogenesis and reverse methanogenesis. *Frontiers in Microbiology*, *9*, https://doi.org/10.3389/fmicb.2018.01322

Ye, F., Ma, M.-H., Op den Camp, H. J. M., Chatzinotas, A., Li, L., Lv, M.-Q., Wu, S.-J., & Wang, Y. U. (2018). Different recovery processes of soil ammonia oxidizers from flooding disturbance. *Microbial Ecology*, *76*(4), 1041–1052. https://doi.org/10.1007/s00248-018-1183-3

Yvon-Durocher, G., Allen, A. P., Bastviken, D., Conrad, R., Gudasz, C., St-Pierre, A., Thanh-Duc, N., & del Giorgio, P. A. (2014). Methane fluxes show consistent temperature dependence across microbial to ecosystem scales. *Nature*, *507*(7493), 488–491. https://doi.org/10.1038/nature13164

Zhu, Y., Purdy, K. J., Eyice, Ö., Shen, L., Harpenslager, S. F., Yvon-Durocher, G., Dumbrell, A. J., & Trimmer, M. (2020). Disproportionate increase in freshwater methane emissions induced by experimental warming. *Nature Climate Change*, *10*, 685–690. https://doi.org/10.1038/s41558-020-0824-y

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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