Microbial Communities and Interactions of Nitrogen Oxides With Methanogenesis in Diverse Peatlands of the Amazon Basin

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Tropical peatlands are hotspots of methane (\text{CH}_4) production but present high variation and emission uncertainties in the Amazon region. This is because the controlling factors of methane production in tropical peats are not yet well documented. Although inhibitory effects of nitrogen oxides (\text{NO}_x) on methanogenic activity are known from pure culture studies, the role of \text{NO}_x in the methane cycling of peatlands remains unexplored. Here, we investigated the \text{CH}_4 content, soil geochemistry and microbial communities along 1-m-soil profiles and assessed the effects of soil \text{NO}_x and nitrous oxide (\text{N}_2\text{O}) on methanogenic abundance and activity in three peatlands of the Pastaza-Marañón foreland basin. The peatlands were distinct in pH, DOC, nitrate pore water concentrations, C/N ratios of shallow soils, redox potential, and \textsuperscript{13}C enrichment in dissolved inorganic carbon and \text{CH}_4 pools, which are primarily contingent on \text{H}_2-dependent methanogenesis. Molecular 16S rRNA and \textit{mcrA} gene data revealed diverse and novel methanogens varying across sites. Importantly, we also observed a strong stratification in relative abundances of microbial groups involved in \text{NO}_x cycling, along with a concordant stratification of methanogens. The higher relative abundance of ammonia-oxidizing archaea (Thaumarchaeota) in acidic oligotrophic peat than ammonia-oxidizing bacteria (\textit{Nitrospira}) is noteworthy as putative sources of \text{NO}_x. Experiments testing the interaction of \text{NO}_x species and methanogenesis found that the latter showed differential sensitivity to nitrite (up to 85% reduction) and \text{N}_2\text{O} (complete inhibition), which would act as an unaccounted \text{CH}_4 control in these ecosystems. Overall, we present evidence of diverse peatlands likely differently affected by inhibitory effects of nitrogen species on methanogens as another contributor to variable \text{CH}_4 fluxes.

**Keywords:** Amazon peatlands, nitrogen oxides, peat geochemistry, methanogens, microbial communities and interactions
INTRODUCTION

Estimates of methane (CH₄) emissions in the tropics have high uncertainties due to limited spatial and temporal in-situ monitoring and a poor mechanistic understanding of soil CH₄ sources and sinks (Kirschke et al., 2013). Multiple studies have evaluated the mechanisms of CH₄ flux in soils (Verchot et al., 2000; Blodau, 2002; Whalen, 2005; Elberling et al., 2011). However, spatial and temporal CH₄ flux variability in most terrestrial ecosystems is high, particularly in the Amazon basin (Bloom et al., 2010; Davidson et al., 2012), and with low modeling predictability (Parker et al., 2018). Peatlands have been overlooked as strong CH₄ sources within tropical latitudes (Sakabe et al., 2018; Tang et al., 2018a; Wong et al., 2018, 2020; Finn et al., 2020), including recently documented sites in the Peruvian Amazon (Teh et al., 2017; Winton et al., 2017; Finn et al., 2020). Large swaths of areas holding tropical peatlands have been reported in the Amazon basin (Gumbrecht et al., 2017). The Pastaza-Marañón foreland basin (PMFB) contains soil carbon stocks estimated to be at least 3.1 × 10¹² kg, or ∼32% of that of South America (Draper et al., 2014), which could be slightly underestimating true extents according to more recent predictions (Gumbrecht et al., 2017). Therefore, peatlands of the PMFB pose a major potential source for atmospheric CH₄ in the region. Given the role of CH₄ as a powerful greenhouse gas and the predicted shift in environmental conditions of the Amazon basin in the wake of global climate change (Davidson et al., 2012), it is critical to address uncertainties in CH₄ flux in these environments.

In waterlogged peat soils, oxygen (O₂) depletion due to the imbalance between slow O₂ diffusion in water and rapid O₂ consumption by heterotrophic respiration (Elberling et al., 2011) remains a main regulator of methanogenesis (Conrad, 2020). However, the influence from other high-redox species, such as nitrogen oxides (NOₓ), are rarely assessed in some microbial soil habitats. NOₓ species are a set of N-O compounds, including nitrate (NO₃⁻), nitrite (NO₂⁻), and nitric oxide (NO). The distribution of NOₓ species and methanogenesis is commonly heterogeneous, however, it has been frequently found that in peatlands vertical soil stratification under anoxic conditions dictates microbial composition (Jackson et al., 2008; Noll et al., 2005; Watanabe et al., 2010; Puglisi et al., 2014; Bai et al., 2017) as well as methanogenic activity (Cadillo-Quiroz et al., 2006), and also leads to the formation of NOₓ gradients (Pett-Ridge et al., 2006; Senga et al., 2015; Stone et al., 2015; Wang et al., 2016). Importantly, the microbial metabolism for NOₓ production and consumption has been found to change with depth in sub-tropical forest soil (Tang et al., 2018b). Within diverse microbial communities, fermentative enzymes and NOₓ reductases have been shown to correlate with chemical gradients (Chen et al., 2017). In tropical soils, heterotrophic denitrification and dissimilatory nitrate reduction to ammonium (DNRA)—a competitive reductive reaction to denitrification based on the common substrate NO₃⁻—have been identified as the main pathways of NOₓ cycling. Of these two pathways, denitrification (i.e., the reduction of NOₓ to N₂O and N₂) is the dominant nitrogen loss pathway in tropical biomes, accounting for 24–53% of total ecosystem nitrogen loss (Hall and Matson, 1999; Houlton et al., 2006). Depending on the geochemical conditions (pH, DOM, metals) and microbial activity, NOₓ are differently distributed in soils, which can affect the microbial community. For instance, NO₃⁻ concentrations are typically higher in shallow soil layers where microbial nitrification is most active, but it can also accumulate in deeper layers, where its consumption may be inhibited by humic substances with phenol moieties (Senga et al., 2010). Meanwhile, NOₓ is unstable in acidic peat pore water, because it decomposes under acidic conditions (pH < 5.5) and rapidly reacts with organic soil moieties or metals (Cleemput and Samater, 1996). The relative degree to which soil microbiota are exposed to NOₓ is controlled by the inherent soil properties in addition to the activity of microbial NOₓ-oxidoreductases or reductases. Their variable availability and interactions make NOₓ generally difficult to identify as stimulators or inhibitors of microbial processes. Effects of NOₓ have to be evaluated within the geochemical context of their soils or complementarily tested under controlled conditions.

Methanogens have been shown to be inhibited by free NOₓ in pure culture experiments (Klüber and Conrad, 1998b) and paddy soil incubations (Klüber and Conrad, 1998a). In addition, NOₓ can also serve as oxidants in the anaerobic oxidation of CH₄ (AOX). In contrast to marine taxa that rely on sulfate, members of both the domains Archaea and Bacteria possess the potential to use NO₃⁻ (Haroon et al., 2013) and NO₂⁻ (Ettwig et al., 2010) to oxidize CH₄. The metabolism of AOM-mediating bacteria (known as NC-10, and affiliated Candidatus Methyloirabilis oxyfera) has been demonstrated, and members of this clade were detected in sub-tropical peat soil (Hu et al., 2014). Thus, the concentration and species make-up of NOₓ have the potential to influence the production and flux of CH₄ in anaerobic soils, such as those in tropical peatlands.

In this study, we focused on three peatlands in the western Peruvian Amazon, locally known as San Jorge (SJO), Buena Vista (BVA), and Quistococha (QUI), with divergent formation history (Lähteenoja et al., 2009) and representing the geochemical diversity of anoxic, tropical peat soils found in the PMFB (Supplementary Figures 1, 2). Our goal was to evaluate the potential impact of nitrogen oxides on methane formation in geochemically diverse peatlands of the Amazon basin. We evaluated carbon and nitrogen pools, and the overall microbial community composition along soil profiles before we characterized the CH₄ cycling based on isotopic signatures, and correlated methanogen abundances with soil NOₓ distribution. We complemented field efforts with site specific anoxic soil slurries and enrichment cultures to test effects of NOₓ compounds on methanogenic activity. Our work provides evidence for an important role of diverse geochemical backgrounds and putative N and C cycling interactions affecting methanogenesis in Amazon peatlands.
MATERIALS AND METHODS

Study Sites and Field Sampling

The studied peatlands are located in the Pastaza-Marañón foreland basin (PMFB), Loreto Region, in the Western Peruvian Amazon. San Jorge (SJO; 4°03’41.6″ S, 73°11’48.1″ W) is a dome-type acidic (pH ~3.5) oligotrophic peatland characterized previously (Lähteenoja et al., 2009), Quistococha (QUI), located adjacent to a lagoon (3°50’5.6″ S, 73°19’24.0″ W) with a relatively high density of Mauritia flexuosa palms, is classified by vegetation as a palm swamp site with mildly acidic (pH ~4.3) and poor to intermediate nutrients, and Buena Vista (BVA; 4°14’23.3″ S, 73°12’08.2″ W) is a minerotrophic near circumneutral (pH ~6.5) nutrient rich site undergoing large riverine flood pulses reaching 5–6 meter high lasting several months (4–6) and vegetated by primary rich forest. Field work was conducted in Summer 2014 (QUI, SJO) and 2015 (BVA) after onset of the rain season when the water tables were at or close to the surface (QUI, BVA) or at ~10 cm below soil surface (SJO). Samples for isotopic analyses were collected in 2017.

To extract soil pore water from a vertical profile of 1 m depth, PTFE tubing ending in a porous teflon macro-rhizon (4.5 mm OD, 0.15 mm pore size, Sunvalley Solutions) was pushed into the peat soil. Peat pore water was pulled with a syringe and filtered (0.8/0.2 µm pore size, Acrodisc) into acid-washed HDPE plastic bottles (Nalgene Nunc Int.). Samples for ion chromatography and spectrometric analysis received the biocide Thymol (Thermo Fisher Scientific) to a 100 mg/L final concentration (Cape et al., 2001). To sample soil gas for the quantification of CH$_4$ and CO$_2$, we used a modification to a soil gas equilibration-by-diffusion through gas-permeable teflon membrane method (Hu et al., 2014). Ten PTFE tubes of 10–100 cm length with a 10 cm-long teflon stub were inserted into the soil over an area of ~1 m$^2$, closed at the top, and left at place for 24 h. After the equilibration period, a gas-tight syringe (Monoject) was used to draw sample gas and to inject it into pre-evacuated glass vials through a butyl rubber stopper. All liquid and gas samples were stored under cold or frozen conditions during transport and upon analysis. To sample soil for DNA extraction, a Russian soil corer was used to take 1 m cores. Three samples of 500 mg soil were aseptically weighted into screw cap tubes at every 10 cm, for two soil cores at each site (60 samples per site). Soil samples were kept frozen throughout the field work and transport. A 1:5, soil:water slurry was prepared and measured with a pH meter 10A (Ecosense, YSI), using the same soil depth intervals as for molecular work.

Geochemical Analyses and Determination of the Carbon Isotopic Composition

Pore water cations were analyzed by ion chromatography using a Dionex ICS2000 instrument at a 1 mL/min eluent flow rate. A CG12A pre-column, followed by a CS12A analytical column was used with an eluent of 35 mM methanesulfonic acid. DOC was determined by a TOC-V Total Organic Carbon Analyzer (Shimadzu Scientific Instruments). Inorganic nitrogen species were quantified spectrophotometrically using an AQ2 Discrete Analyzer (Seal Analytical) following the EPA-103-A Rev.10 method for ammonium (LoD 0.004 mg-N/L, range 0.02–2.0 mg-N/L) and EPA-127-A method for NO$_3^-$/NO$_2^-$ (LoD 0.003 mg-N/L, range 0.012–2 mg-N/L). TC and TN in soil pore water was determined with a TOC-V/CSN connected to a TNM-1 unit (Shimadzu Scientific Instruments) following a method based on Standard Methods 5310B and ASTM D8083. Methane δ$^{13}$C was measured with a Thermo Fisher Scientific Precon unit interfaced to a Thermo Fisher Scientific Delta V Plus isotope ratio mass spectrometer (Thermo Fisher Scientific) at the Stable Isotope Facility at U.C. Davis (Yarnes, 2013). DIC concentrations and δ$^{13}$C of DIC measurements were measured on the SHIVA platform in the EcoLab Laboratory (Toulouse, France). The δ$^{13}$C of DIC was analyzed using a mass spectrometer (Isoprime 100, Elementar) coupled with an equilibration system (MultiFlow-Geo, Elementar). Samples were acidified using phosphoric acid and flushed with helium. Standards included Na$_2$CO$_3$ and NaHCO$_3$ as well as internal water standards. All standards were analyzed every 8 samples to check for instrument stability. All samples were analyzed in replicates.

The fractionation factor $\alpha$ was derived with the formula

$$\alpha = \frac{\delta_{CO_2}^{+10^3}}{\delta_{CH_4}^{+10^3}}$$

Trace metal (Mo) soil content was determined by inductively coupled plasma mass-spectrometry (ICP-MS) after acid digestion. Briefly, an acid mix (HF/HNO$_3$+HCl) was added to soil samples in acid-cleaned Teflon vials. Soil organic matter was oxidized overnight. After another HCl addition, the sample was microwaved and the top liquid was decanted and evaporated on a hotplate. Repeated acid addition and evaporation concentrated the soil metals. A diluted sample was then measured on an iCAP-Q (Thermo Fisher Scientific) with Mo calibrated for 0.018–126 ppb (3% error range).

$E_h$ calculations. The partial pressure of CO$_2$ and CH$_4$ ($P_{CO_2}$, $P_{CH_4}$) in soil gas samples was used to derive the equilibrium redox potential ($E_h$) between the redox couple. For each depth interval, $E_h$ was pH-corrected. All calculations were based on the equation CO$_2 + 4 H_2 = CH_4 + 2 H_2O$ for methanogenesis.

$$E_{pH}^H = E_{pH,0}^H - (0.059 \frac{v_H}{n_F}) \log p_{HO}$$

The standard potential at pH = 0 ($E_{pH,0}^H$; Bratsch, 2009), the stoichiometric coefficient $v_H = 8$, and the number of electrons transferred per 1 mole of CO$_2$ and CH$_4$ ($n = 8$) were used to derive the standard potential at distinct soil depths ($E_{pH}^H$). From there, we calculated $E_h$ using the Nernst formula. The temperature ($T$) was set to 298 K and we used 8.314 J mol$^{-1}$ K$^{-1}$ for the ideal gas constant ($R$) and 96,485 C mol$^{-1}$ for the Faraday constant ($F$) according to Stumm and Morgan (2012). We used partial pressure as proxy for the activity of the redox species.

$$E_h = E_{pH}^H - 2.303 \left( \frac{R}{n_F} \right) \log \left( \frac{P_{CH_4}}{P_{CO_2}} \right)$$
DNA Extraction, Amplification, and Amplicon Sequencing of 16S rRNA and mcrA Genes

Soil DNA was extracted from stored frozen samples using a NucleoSpin Soil DNA extraction kit (Macherey-Nagel GmbH). PCR was performed with the archaeal-bacterial primers 515F/909R (Tamaki et al., 2011) and mlas/mcrA-rev (Steinberg and Regan, 2009) to target methanogenic Euryarchaeota. For 16S rRNA gene amplification reactions, we used 0.3 μM of forward and reverse primers, 0.2 mg/L bovine serum albumin, and 1× GoTaq Green Master Mix (Promega). The thermal cycling conditions were the following: Initial denaturation at 95°C for 5 min, 25 cycles of denaturation at 94°C for 30 s, annealing at 52°C for 1 min, and extension at 72°C for 1 min, as well as a subsequent final elongation step at 72°C for 10 min. The reaction chemistry for mcrA amplification reactions was used as described elsewhere (Steinberg and Regan, 2009). Thermal cycling included denaturation at 95°C for 3 min, 5 cycles of denaturation at 95°C for 30 s, annealing at 48°C for 45 s, and extension at 72°C for 30 s, accomplished by another 30 cycles of 95°C for 30 s, 55°C for 45 s, 72°C for 30 s, and one final elongation at 72°C for 10 min. Samples were multiplexed (Herbold et al., 2015), normalized (SequlPrep kit, Invitrogen), and sent for sequencing to the DNAsu core facility, with 2×300-bp paired-end Illumina MiSeq (Tempe, AZ).

For archaeal 16S rRNA and mcrA gene quantification, the reaction mix consisted of 4 mM MgCl₂, 1.5× CXR reference dye, 0.3 μM of forward and reverse primer, 0.2 mg/L bovine serum albumin (to reduce inhibition interference) and 1× GoTaq qPCR master mix (Promega). Replicate reactions were run on a QuantStudio 3 real-time PCR system (Applied Biosystems). The primers ARC787f/ARC1059r (Yu et al., 2005) and mlas/mcrA-rev (Steinberg and Regan, 2008) were used. Optimal qPCR cycling conditions in reactions with ARC787f/ARC1059r were found to be denaturation at 94°C for 10 min, 45 cycles of 94°C for 10 s and 60°C for 30 s. Cycling stages for mlas/mcrA-rev comprised primary denaturation at 95°C for 2 min, followed by 40 cycles of 95°C for 30 s, 55°C for 45 s, 72°C for 30 s, and 83°C for 8 s. Target products amplified by qPCR were confirmed by gel electrophoresis. Standard curves were created with the efficiencies $E = 1.01–1.03$ for mcrA and $E = 0.82–0.88$ for archaeal 16S, and good linearity ($r^2 > 0.99$) in the measurement range of the samples.

16S rRNA and mcrA Phylogenetic Analyses

Sequences were merged and demultiplexed with an in-house script developed in R. All subsequent analyses were conducted on the Qiime 2 platform1 (Caporaso et al., 2010). After dereplication and de-novo chimera-filtering, open-reference clustering was applied at a 97% identity level and using the SILVA database (release 128). This reduced the mean and median of sequences per sample from 87,379 and 70,016 to 54,155 and 44,371, respectively. Singleton sequences were removed with the feature-table filter-features command. An alpha-rarefaction curve was created for every sample as a visual proxy for species richness (Supplementary Figure 3). Taxonomic classification was done using VSEARCH consensus classifier with SILVAs 99 majority taxonomy (release 128). Percentage identity was set to 0.94, min-consensus was set to 0.6, and maximum hits accepted were 10. A phylogenetic tree was constructed de-novo with the built-in module FastTree 2 (Price et al., 2009). Ammonia-oxidizing microbes were classified based on their affiliation to Thaumarchaeota and Nitrosomonadaceae/Nitrospiraceae 16S rRNA gene sequences (Supplementary Table 1).

We evaluated methanogen groups based on the alpha subunit gene encoding the MCR enzyme (mcrA), which catalyzes the final step in methanogenesis (Ermler et al., 1997). Sequences of the mcrA gene were dereplicated using the -fastx_uniques command in USEARCH (Edgar, 2010). Unoise3 was applied to denoise and to filter chimeras. Singleton sequences were retained (-minsize 1) to include very low abundance OTUs. The sequences were translated and frameshift-corrected by Framebot (Wang et al., 2013) with a low loss (<10%) of sequences. Subsequent clustering was administered using an 85% identity level (Yang et al., 2014). The -cluster_fast algorithm (variant of UCLUST) was fed with centroid sequences, which were derived from a custom-made database. The database was built with sequences from the FunGene repository2 in January 2018, and complemented with additional reads from tropical mangroves (Taketani et al., 2010) and a peat swamp forest (Kanokratana et al., 2010). The output of the -cluster_fast command was used to make an OTU table, to classify centroid sequences, and to derive core diversity metrics. At this stage, OTUs with 5 or less sequences over all soil layers were removed. To classify the ~150 amino acids long centroid sequences, we tested two different search algorithms on their performance with a subset of our sample data. The standalone version of BlastP (2.7.1+) and HMMer 3.0 did not show substantial differences in sensitivity and specificity as noted previously (Orellana et al., 2014). Hence, BlastP was run on the non-aligned centroid queries and typically attained e-values of $10^{-80}$ or lower (Takeuchi et al., 2011; Penton et al., 2015).

We refined the evaluation of sequence similarity cutoffs for methanogen taxonomic levels (Steinberg and Regan, 2008) by (i) including 102 methanogen species that were all represented by mcrA sequences from isolates in swissprot database, (ii) extending the taxonomy to 14 methanogen families, (iii) using the Jone-Taylor-Thornton (JTT) algorithm, and (iv) introducing archaea-adjusted gamma-distribution based on an elongation factor specifically determined for archaea (Gaucher et al., 2001). Distance matrices and phylogenetic trees were derived in MEGA Version 7.0.26. The phylogenetic tree was constructed by aligning sequences while ambiguous positions were removed for each sequence pair. Distances were calculated using the JTT method. Phylogenetic trees of 16S and mcrA sequences were overlaid and found to be congruent (Supplementary Figure 4). The overall

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1https://qiime2.org, releases 2017.12 and 2018.8.

2http://fungene.cme.msu.edu
workflow including the molecular analysis pipeline is visualized in Supplementary Figure 5.

**Microcosm Incubations and Activity Measurements**

In a glove box with a reducing, O₂-free atmosphere (0.5% H₂ in N₂), roots and coarse particles (>5 mm) were removed from peat soil, and soil diluted 1:10 in anoxic, sterile 18.2 MΩ × cm water. The slurry was well homogenized, dispensed to culture vials, and sealed with butyl rubber stoppers. Next, an anoxic stock solution of NO₂⁻ was injected (final concentration 200 µM) to a subset before all vials’ headspace was flushed with pure N₂. In-situ NO₂⁻ would be supplied steadily from aerobic reactions. But in anoxic microcosms there is no such steady supply and the presence of NO₂⁻ had to be mimicked, which we did by one single NO₂⁻ pulse for simplicity. Soil slurries were agitated briefly to disperse the NO₂⁻ and incubated under dark and static conditions at room temperature for a total of 3 weeks. Headspace (200 µL) was sampled in even time intervals with a gas-tight, N₂-purged syringe (VICI Precision Sampling) and injected into a gas chromatograph (GC, SRI Instruments) equipped with a flame-ionization detector (FID). Two continuous HayeSep-D columns were kept at 90°C (oven temperature) with N₂ (UHP grade 99.999%, Praxair Inc.) as carrier gas and H₂ for FID combustion. Methane concentration measurements were calibrated with customized standard mixtures (Scott Specialty Gases, accuracy ±5%) over a range of 5–5,000 ppmv. Gas phase concentrations were corrected using Henry’s law and the dimensionless concentration constants \( K_H^{CH_4}(CH_4) = 0.0342 \) to account for gas dispersed into the aqueous phase at 25°C. Methane production rates were calculated based on the linear region of the concentration curve for manipulated and control incubations.

**Soil Enrichments**

Because N₂O may be derived from NO₂⁻ via denitrification (see Discussion), we chose the two sites that showed the most contrasting sensitivity to NO₂⁻ additions (SJO and BVA). From these soils, we derived methanogenic enrichment cultures as previously described (Cadillo-Quiroz et al., 2008) in order to reduce microbial complexity and exposed them to N₂O by headspace injections. For our tests, culture vials were pressurized with an H₂/CO₂ (80/20) mixture (up to 12 PSI) and N₂O was injected to ~500 ppm final headspace concentration. Methane and N₂O changes were monitored by GC injections as described before. For N₂O detection, an electron-capture detector (ECD) was used in line with the FID. Once N₂O levels dropped below detectability, the cultures were spiked again with 500 ppm N₂O. Turbidity (OD₆₀₀ measurements) and hence biomass growth was generally very low for enrichments and microbial growth was therefore confirmed by microscopy. Enrichments were incubated at 30°C.

**Statistical Analyses**

All basic statistical tests were performed with JMP Pro software (Version 13.1.0, SAS Institute Inc.). Raw sequence counts were normalized by Johnson transformation. Principal components (Supplementary Figure 6) were explored using the multivariate analysis toolbox on JMP. PERMDISP and PERMANOVA were conducted on the Qiime 2 platform using the diversity beta-group-significance command including pairwise testing on 9999 permutations. Alpha and beta diversity indices were calculated using the diversity core-metrics-phylogenetic command configured with sampling depths according to individual sample sets. **McrA phylogeny was visualized with Krona (Ondov et al., 2011). Spearman’s rank multivariate analysis (Supplementary Table 2) was conducted on mcrA sequence reads using JMP. Plotting and regression analysis was done with the MATLAB R2018a software (Version 9.4.0.813654, Mathworks Inc.).**

**RESULTS**

**PMFB Peatlands Show Differing Soil CH₄ Profiles, DOC Loads, and Inorganic Nitrogen Levels**

Equilibrated soil gas analyses (see section “Materials and Methods”) showed that CH₄ maxima per site were up to 25 g/L in SJ, 47 g/L in QUI and 69 g/L in BVA, predominantly in deep peat (Figure 1). All sites contained roughly 10 times higher N₂O levels than the mean ambient atmosphere (~320 ppb, Stocker et al., 2013). We observed that particularly soil columns of the less acidic site (BVA) showed increasing N₂O concentrations with soil depth (Figure 1). Tests for pore water NO₂⁻ concentrations in SJO and QUI did not show statistically significant differences from the method’s limit of detection (LoD, 0.01 mg/L), and only BVA samples yielded NO₂⁻ levels above the LoD (p < 0.05). Nitrate reached highest concentrations in shallow soil layers, with maxima at 0.4 mg/L (BVA), 0.05 mg/L (QUI), and 0.02 mg/L (SJO), but N₂O was generally the dominant nitrogen oxide. Ammonium (NH₄⁺) showed a vertical pattern opposite to NO₂⁻ and NO₃⁻ (Figure 1). Concentrations increased by roughly a factor of 4 from top to bottom layers in SJO, and followed a similar, but less pronounced, trend at the other two locations. Since molybdenum (Mo) is an essential trace metal in N₂ fixation (Mo cofactors are the active site in nitrogenases), we determined Mo abundances in top soils (0–30 cm depth) to exclude the possibility of Mo limitation. Acid-digested peat extracts yielded Mo concentrations of 0.53 ± 0.01 ppm (QUI), 1.25 ± 0.2 ppm (SJO), and 1.32 ± 0.1 ppm (BVA, all n = 3). Dissolved organic carbon (DOC) profiles derived from soil pore water differed significantly across sites (ANOVA, p < 0.05, Figure 1). Highest DOC concentrations were found in BVA peat (up to 29.1 mg/L). Unlike DOC profiles, C/N ratios resulted in significant variation along soil depth. A trend of increasing C/N values with increasing depth was observed in BVA where the ratio at the 60 cm-layer was ~3.6 times that of surface layers (Figure 1). In contrast, the C/N profile at SJO decreased with increasing depth with values from uppermost and lowermost layer being ~2.4-fold difference.
Overall Microbial Community Composition Along Peat Soil Profiles

The geochemical diversity of the three peatlands was reflected in their contrasting microbial community composition. A total of 4,980,584 sequencing reads were clustered to 16S rRNA operational taxonomic units (OTUs) with 3% identity radius for taxa assignment on the genus level or above. SJO with 6,636 OTUs had the lowest richness while BVA yielded about three times as many OTUs (18,649). We focused henceforth on microbial groups putatively relevant to the cycling and vertical distribution of soil NO\textsubscript{x} (Supplementary Table 1) or that were highly abundant (Figure 2). We assumed ecological coherence within the family taxonomic rank as proposed elsewhere (Philippot et al., 2010) but emphasize that likely not all taxa of a Family share NO\textsubscript{x}-cycling traits. Bathyarchaeota was a very abundant phylum frequently making up 20% of total OTU counts, predominantly in deeper regions of the peat soils. In contrast, Euryarchaeota were less abundant and did not show a depth preference. The surface soil layer in BVA cores was among the richest in Euryarchaeota with ~3% of total OTUs. In QUI and SJO, maxima in Euryarchaeota OTUs (1.5 and 1%, respectively) were found in moderate depths (Figure 2). Xanthobacteraceae relative abundances peaked at mid profile depth in QUI (5.4 and 3.5% of total OTUs in each core). In SJO peat soil, the group reached even higher values of 17.9% and remained at ~1% of total OTUs in a depth of 75 cm before ceasing in the bottom layers. BVA showed maximum relative abundances for Xanthobacteriaceae (4.7 and 5.4%) exclusively in shallow layers. As potential nitrifiers (NO\textsubscript{x} producing), Thaumarchaeota showed high relative abundances in QUI and SJO (up to 23.7 and 16.8%), and low relative abundances in BVA. Nitrospiraceae were most abundant in BVA in soil layers (3.3–3.7%) slightly below the top layer (15–25 cm in both duplicate cores). Potentially denitrifying (NO\textsubscript{3}− consuming) Bacillaceae and Paenibacillaceae seem to be a unique occurrence in the palm swamp of QUI with relative abundances of up to 8.4 and 2.7%, respectively.

Methane Isotopic Signatures and Soil Redox Potential

The isotopic composition (δ\textsuperscript{13}C) of dissolved CH\textsubscript{4} and inorganic carbon (DIC) shows active methanogenesis in all peatlands and points to signatures of distinct pathways. Values of δ\textsuperscript{13}C in CH\textsubscript{4} and DIC from BVA and QUI were indistinguishable from each other but distinct from SJO, which had a more depleted δ\textsuperscript{13}C signature (Figure 3). In SJO, δ\textsuperscript{13}C values in CH\textsubscript{4} also showed variation between duplicate cores with a core showing a sudden depletion at 20 cm depth by 6‰ (Figure 3).

The average redox potential (E\textsubscript{h}) based on the CH\textsubscript{4}/CO\textsubscript{2} pair decreased with more positive values in SJO, followed in order by QUI and BVA (Figure 3), consistent with observed levels of CH\textsubscript{4} emissions and abundance trends of the mcrA/16S rRNA fraction (see below). Along soil profiles, E\textsubscript{h} in SJO decreased from ~0.03 to ~0.035 V, increased in QUI from ~0.098 V to ~0.067 V, while BVA showed negligible change at ~0.21 V.

Methanogen Diversity and Distribution

The methanogen communities in BVA and SJO diverged the most and BVA exhibited the highest methanogen diversity of all studied peatlands as evaluated through the mcrA gene (Figure 4).
PERMANOVA tests on \textit{mcrA} amplicons from all cores indicated significant differences among peatlands. Site replicates were not distinguishable \((F = 1.11, \ p = 0.2494)\), but values across sites showed increasing dissimilarity with BVA closer but distinct to QUI \((F = 7.5 \pm 1.6, \ p < 0.0005)\), and further from and more distinct to SJO \((F = 11.4 \pm 0.7, \ p < 0.0001)\). \textit{McrA} amplicon analysis identified 264, 509, and 1,104 OTUs from SJO, QUI, and BVA, respectively. Alpha diversity analysis confirmed BVA as the site with the most diverse methanogen community (Shannon index, SI = 4.3 ± 0.4) followed by QUI (SI = 3.6 ± 0.4), and SJO (SI = 2.7 ± 0.5) as the least diverse. This order reflects the more favorable physiological conditions for methanogenesis under low \(E_h\) (Figure 3).

\textbf{Figure 4} illustrates Methanomicrobiales as most dominant across sites (40–72\%) particularly in SJO, while Methanobacteriales reached its highest fraction (25\%) in BVA but lowest (7\%) in SJO. Furthermore, Methanomicrobiales sequence alignment on the amino acid level showed their clustering with environmental clones outside the established phylogeny (Supplementary Figure 7), with OTUs less than 67\% similar to any known family and likely novel groups in the order. Additionally, OTU fractions (Figure 4) also evidenced the putative acetoclastic contribution by \textit{Methanosetaecae} and \textit{Methanosarcinaceae} particularly in BVA (~21\%), a lesser degree in QUI (14\%) and SJO (8\%). In BVA, the site with highest pH and minerotrophic conditions, \textit{Methanosaetaceae} (including \textit{Methanothrix}) represented nearly a fifth (18\%) of the community. Meanwhile, in QUI \textit{Methanosarcinaceae} (including \textit{Methanosarcina}) made up a bigger fraction (8\%) than \textit{Methanosetaecae} (6\%), suggesting differential acetoclastic contributions likely related to acetate availability and the significantly higher acetate affinity of \textit{Methanothrix}.  

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure2.png}
\caption{16S rRNA gene relative abundance heatmap of archaeal (left) and bacterial (right) taxa with known nitrogen metabolic potential. Archaeal 16S rRNA gene relative abundances were summarized on the phylum level with Bathyarchaeota, Euryarchaeota, and Thaumarchaeota consistently making up >90\% of all archaeal taxa. Only bacterial taxa at the family level that were either most dominant (at least 2\% of total OTUs), had the potential of a syntrophic relationship to methanogens, or were affiliated to known metabolic reactions involving NO\(_x\), are depicted. Confirmed metabolic capacities to produce NO\(_x\), consume NO\(_x\), or for a syntrophic lifestyle are marked with closed circles. Hypothesized metabolic capacities (unsupported by pure cultures) are marked with open circles. The number of unassigned sequences (no match in the SILVA database) increased notably in deeper layers. Relative abundances for individual cores and bacterial metabolic capacities at the family level are provided in Supplementary Table 1.}
\end{figure}
Nitrogen Oxides and Methanogenesis Interactions

FIGURE 3 | Redox gradient and carbon isotope composition of CH$_4$ and dissolved inorganic carbon along soil profiles of three tropical peatlands (BVA, QUI, SJO). Redox potential (Eh) is based on the ratio of the CH$_4$-CO$_2$ redox couple and soil pH-corrected. VPDB, Vienna Pee Dee Belemnite. Each data point corresponds to one replicate soil core.

Composition of Methanogenic Community Along Vertical Profiles Correlates With NO$_x$ Values

Our statistical analyses exhibited different correlation trends between NO$_x$ and the relative abundance of distinct methanogenic groups along soil profiles. Together with the principal components analysis (PCA, Figure 5), Spearman correlation pointed to an overall positive correlation of NO$_3^-$ with methanogen relative abundances, while NO$_2^-$ did not show significant relationships. Specifically, PERMANOVA tests indicated a significant shift of the overall mcrA-sequenced methanogenic community between the depth interval that was relatively enriched in NO$_x$ (0–50 cm) and the one relatively depleted in NO$_x$ (50–100 cm) for both cores in BVA ($F = 5.39$, $p = 0.0170$), QUI ($F = 4.13$, $p = 0.0190$), and SJO ($F = 3.71$, $p = 0.0493$). The differences in variation were unaffected by dispersion ($p > 0.05$). Spearman’s rank further indicated sequence counts of Methanosetaeaceae and Methanosarcinaceae correlated positively with NO$_3^-$ ($\rho = 0.76$ and 0.72 correspondingly, $p < 0.005$) in SJO, while Methanoregulaceae and NO$_3^-$ correlated negatively ($\rho = -0.79$, $p < 0.005$) in BVA (Supplementary Table 2 and Supplementary Figure 8). Other geochemical parameters, such as pH ($|\rho| < 0.49$), phosphate ($|\rho| < 0.56$), sulfate ($|\rho| < 0.50$), and ammonium ($|\rho| < 0.47$), did not reveal more significant relationships with any of the selected methanogen taxa (Supplementary Table 2). Principal component analysis showed well separated data points clustered into the three peat soils (Figure 5). OTU relative abundances of Bathyarchaeota, Methanomicrobia, and Methanobacteria were similarly associated to principal component 1 (PC 1), while CH$_4$ concentration was related to PC 2.

Given the negative correlation of NO$_2^-$ and a methanogenic group in BVA and the overall variation of methanogenic communities in NO$_x$ gradients across all sites, we conducted a further in-depth evaluation beyond field measurements testing methanogen sensitivity to NO$_x$ species.

CH$_4$ Production Potentials in SJO, QUI, BVA Peat Soils Show Different Responses to NO$_2^-$ Amendment and N$_2$O Halts Methanogenesis

Our tests on the effects of NO$_2^-$ on methanogenesis rates in amended (200 µM NO$_2^-$) anoxic soil slurries or effects of N$_2$O on methanogenic enrichment cultures revealed responses consistent with our field observations showing inhibitory effects and variable responses. We found that NO$_2^-$ exerted differential, mostly negative effects on the CH$_4$ production potential across the contrasting tropical peatlands (Figure 6A). Samples from
**FIGURE 4** | McrA/archaeal 16S rRNA gene copy ratios along soil depth (left) and McrA phylogenetic distribution of cumulative reads at all levels (right) in each peatland. Gene copy ratios are based on qPCR analysis and data from both soil cores are shown (dark and light gray lines). The gene ratios were used to better illustrate methanogen proportions relative to the total number of archaea, which explicitly revealed differences between peatlands. Absolute gene quantities ranged from $1.3 \times 10^4$ (SJO) to $1.8 \times 10^9$ (BVA) McrA gene copies per g of dry soil and $8.7 \times 10^5$ (SJO) to $7.3 \times 10^{11}$ (QUI) archaeal 16S rRNA gene copies per g of dry soil. Relative abundance percentages refer to the total number of OTUs recovered at each site. Higher level taxa were collapsed if these were comprised of 100% the lower-level taxa. Mr., Methanoregulaceae; Mst., Methanosaetaceae; Mr., Methanosarcinaceae; Mc., Methanocellaceae; Mb., Methanobacteriaceae; Mmc., Methanomassiliicoccaceae.

**FIGURE 5** | PCA ordination plots of microbial and environmental data from three soil profiles of contrasting Amazon peatlands. Samples were derived from QUI (red triangles), SJO (green circles) and BVA (blue squares) with abbreviations as in Figure 1. Methane concentration ([CH$_4$]), 16S rRNA gene OTU relative abundances, and McrA gene copy number (A), and Bathyarchaeota alpha (Shannon) and beta (Unifrac) diversity indices, NO$_x$ concentrations ([NO$_2$], [NO$_3$]) and pH (B) were evaluated against microbial distribution. We used Bathyarchaeota abundance and diversity indices as a not yet physiologically resolved close archaeal group important to evaluate but not as assumed methanogens.
deeper soils showed the highest residual activity reduction (down to ~10% or below detection) across all sites. However, shallow, presumably most active, soil samples showed the most variation in CH₄ output. The oligotrophic site (SJO) with the lowest potential methanogenic rates was not affected or instead stimulated marginally (90% or >100% residual activity, respectively). The intermediate site (QUI) had a mild or moderate reduction (retaining ~60–80% residual activity), and the minerotrophic and most CH₄-producing site (BVA) had the harshest reduction (~15–30% residual activity). The latter is consistent with our expectations based on the relatively steeper field NO₂⁻ gradients observed in BVA.

Using recently developed methanogenic enrichments from BVA and SJO, we found N₂O inhibits methanogenesis, with a strong threshold-based behavior (Figure 6B). Under H₂/CO₂ overpressure (controls, no organic substrates added) and without added N₂O, SJO, and BVA enrichments showed linear CH₄ production by 0.6 and 4.7 nmol day⁻¹, respectively. Enrichments receiving N₂O injections showed N₂O reduction concomitant to CH₄ production. Methane accumulation did not progress in a linear fashion as seen in controls and halted when cultures were spiked with 28 µM N₂O (aqueous concentration). Methane formation appeared to resume when N₂O fell below a threshold of ~10 µM in BVA (after 7 days) and to < 0.004 µM in SJO (after 17 days). The recovery of methanogenesis was less pronounced in SJO whose enrichment had low methanogenic biomass likely leading to lagging periods longer than the 7 days between N₂O injections used in our assays (Figure 6B). N₂O consumption observed in the SJO enrichment (pH 3.5) is noteworthy because it occurred despite the known ceasing of N₂O reductase activity at pH < 5 (Liu et al., 2010, 2014). These results show that N₂O—a stable nitrogen oxide species under peat soil conditions (contrary to NO₂⁻)—that can only be removed enzymatically, inhibits methanogenesis if present above a distinct threshold. We determined thresholds of 0.004–10 µM pointing at a wide range in individual N₂O sensitivity among methanogens.

**DISCUSSION**

**Contrasting Overall CH₄ Patterns Across SJO, QUI, and BVA Peatland**

Our study revealed distinct vertical patterns in the distribution of several carbon (including methane) and nitrogen pools among contrasting Amazon peatlands. In the acidic nutrient-poor SJO peats, low NO₂⁻ concentrations throughout the soil profile raised the C/N ratio, indicating relative nitrogen scarcity (Hall and Matson, 2003). SJO peatland also yielded the most oxidized CH₄/CO₂ redox balance and showed signs of methane oxidation in the isotopic abundances. The “hook” shape of the ¹³C/CH₄ profile (Figure 3) likely indicates aerobic methane oxidation, which preferentially converts ¹²CH₄ to CO₂, resulting in an enrichment of ¹³CH₄ in the residual dissolved CH₄ pool. A similar concentration pattern as in oligotrophic SJO has been also described in an oligotrophic tropical peatland in Panama (Holmes et al., 2015). The depletion of CH₄ pools could explain why moderate mcrA gene frequencies would not lead to higher CH₄ soil gas concentrations in SJO peats. In the mildly acidic QUI peatland, the observed C/N ratios are consistent with previous measurements underlining the moderate to poor
trophic conditions relative to the other peat soils (Lawson et al., 2014). The steep decrease of CH$_4$ concentrations in surface levels but absence of a strong signature of CH$_4$ oxidation in the isotopic data suggest that CH$_4$ is lost from soils perhaps mainly through diffusion from water saturated soil to the atmosphere or through palms and trees as reported for this site (Van Haren and Cadillo-Quiroz, 2016). Aerobic oxidation of methane likely operates in QUI as methanotrophic sequences were detected in this and previous study (Finn et al., 2020), however, frequent near or above soil surface water table in this site likely limit this activity to dryer seasons or elevated soils. The minerotrophic, near-circumneutral pH, peat soils of BVA were characterized by relatively high NO$_3^-$ concentrations in shallow soil layers, leading to lower C/N ratios and a higher nitrogen nutrient richness that in the other sites. This trophic condition could be the basis of the greater diversity and relative abundance of methanogens observed in BVA soils, which also presented the most favorable redox conditions for methanogens. As a result, relatively high CH$_4$ concentrations up to more shallow soil layers lead to increased emission potential. Aerobic methane oxidation is likely similarly limited by water table and diffusion processes as in QUI, but more research is needed on that component. The isotopic CH$_4$ and DIC proportions yield fractionation factors ($\alpha$) of 1.075 ± 0.0015 in SJO, 1.071 ± 0.0009 in QUI, and 1.067 ± 0.0015 in BVA (SD, $n = 10$–12), and are within a previously reported range (Holmes et al., 2015). According to the current consensus, $\alpha < 1.055$ and $\alpha > 1.065$ are characteristic for environments dominated by acetate-using (acetoclastic) or H$_2$-using (hydrogenotrophic) methanogenesis, respectively (Whiticar, 1999). Thus, the source of CH$_4$ is preferentially H$_2$/CO$_2$ in the studied peatlands, with a more noticeable contribution of acetoclastic methanogenesis in BVA.

**Source Pathways and Microbial Origin of NO$_x$ in Peat Soils**

The dynamics of the nitrogen cycle in tropical peatlands has not been detailed but our measurements allow us to postulate some likely pathways leading to NO$_x$ production by different microbial groups. Ammonium (NH$_4^+$) sources could include release from organic matter through ammonification, N$_2$ fixation, or DNRA. The latter would not serve as a net source of dissolved inorganic nitrogen, because NH$_4^+$ is produced from NO$_3^-$ Plant debris with low C/N ratio tends to trigger net N mineralization (Manzoni et al., 2010) which would result in increasing NH$_4^+$ concentrations as observed in SJO deep peat. Also, microbial fixation of N$_2$ mediated by the Mo-based nitrogenase enzyme is another likely source of NH$_4^+$. The unusual temperature dependence of the enzyme grants a high N$_2$-fixing capacity to tropical peats (Houlton et al., 2008), which can lead to accumulatio of inorganic N. In fact, the measured Mo content among sites is above the limiting range for N$_2$ fixation by free-living heterotrophic bacteria in tropical forest soils (Barron et al., 2008).

In contrast to NH$_4^+$, NO$_x$ species were more abundant in shallow than deeper soils, irrespective of the site-specific geochemistry, which is consistent with previous reports (Hu et al., 2014; Palmer and Horn, 2015; Tang et al., 2018b). We found that the relative fraction of Thaumarchaeota and Nitrospiraceae were highly abundant (Figure 2) and likely play an important role in regulating pulses of NO$_x$ through peat soils. Thaumarcheaota are mostly ammonia-oxidizing archaea (AOA), while Nitrosonomadaceae (genus Nitrosonomas) and Nitrospiraceae (genus Nitrospira) are members of the ammonia-oxidizing bacteria (AOB). AOA and AOB generally prefer inorganic nitrogen sources, even though AOA have been shown to metabolize organic nitrogen, too (Weber et al., 2015). Both AOA and AOB aerobically convert NH$_4^+$ into NO$_2^-$. Several studies have pointed out that AOA are more dominant in acidic soils than their bacterial counterparts (Zhang et al., 2012), which is likely due to indirect pH effects on the preferred substrates (Höfferle et al., 2010; Yao et al., 2011; Daebeler et al., 2014; Weber et al., 2015). The partitioning of both groups across the studied peatlands reflects this pattern with the dominance of AOB over AOA in BVA, and the opposite pattern in mildly acidic QUI and highly acidic SJO. We propose nitrification to be mostly carried out by archaea residing in top (0–30 cm) soil layers of the oligotrophic and acidic sites and AOB-mediated nitrification to prevail in the minerotrophic, closer to neutral pH site. This preferential niche selection would have further implications on the NO$_3^-$ to NO$_2^-$ ratio. Some species of the Nitrospiraceae (AOB) are capable of oxidizing NO$_2^-$ further to NO$_3^-$. Therefore, the capacity for complete nitrification (Daims et al., 2015; van Kessel et al., 2015) could explain the higher levels of NO$_3^-$ detected in the NO$_x$ profile from BVA (Figure 1).

**NO$_x$ Affect Peat Methanogenesis Directly or Indirectly, via Reaction to N$_2$O**

Based on the correlation of distinct methanogenic taxa with NO$_x$ distribution and sensitivity of methane formation to NO$_x$, we infer an inhibition of microbial methanogenesis and propose 3 plausible explanations. First, the introduction of NO$_2^-$ can change redox potential and thus decrease CH$_4$ production. NO$_x$ have naturally high redox potentials. Methanogenesis operates preferentially at negative potentials and soil communities have been shown to be sensitive to changes in redox conditions (Fetzer and Conrad, 1993; Jugsujinda et al., 2008; Hirano et al., 2013). Moreover, the magnitude of observed inhibition is consistent with the redox gradients measured in-situ. Methanogens of BVA soil would experience the greatest difference in redox because the CH$_4$/CO$_2$ balance in BVA is most reduced (Figure 3), and hence, exposure to oxidized radicals would result in harsh disruption of the redox balance. Second, denitrifying microorganisms can deplete H$_2$ or acetate, thus, competing with methanogens for the common substrates. The predominance of the hydrogenotrophic or acetoclastic methanogenic pathway together with the substrate preference of the indigenous denitrifying community (NO$_x$ consumers, Figure 2) could lead to the apparent pattern (Klüber and Conrad, 1998a; Conrad, 1999). Third, NO$_2^-$ negatively affects methanogens on a physiological level. Here, derivatives of transformation processes from NO$_2^-$ might be stronger agents than NO$_2^-$ itself. Under natural soil conditions, NO$_2^-$ can biotically or abiotically be transformed into NO and N$_2$O.
Elevated NO concentrations affect a variety of cellular processes, often leading to cytotoxicity (Saraiva et al., 2004). Similarly, N₂O at ~0.1 mM levels has been found to be an inhibitor of methanogenesis in environmental samples (Balderston and Payne, 1976). N₂O has been shown to bind and inactivate vitamin B₁₂ (Drummond and Matthews, 1994), a key metabolite for methanogens because its cobalt(I) center serves as a methyl acceptor during methanol to methane transformation (Matthews, 2001). The resistance of some methanogenic groups to N₂O exposure by vitamin B₁₂ synthesis or the presence of complementing phyla that can make vitamin B₁₂ (e.g., Thaumarchaeota, Doxey et al., 2014) may result in the differential suppression of CH₄ production across sites.

Conversely, the measured CH₄ pools may also be indirectly affected by NOX. For instance, NO₃⁻-based anaerobic methane oxidation (AOM) could affect the results without direct effects on methanogenesis since our experiment only accounted for net CH₄ accumulation. However, considering the slow CH₄ oxidation rates observed in other freshwater experiments (Zhu et al., 2012; Segarra et al., 2015; Valenzuela et al., 2017), it is unlikely that CH₄ levels can be significantly affected by AOM during our incubations.

Steady-state N₂O could have retarding effects on CH₄ accumulation at SJO peatland but is insufficient to affect methanogenesis at BVA. The equilibrated N₂O soil gas concentrations we measured in-situ (Figure 1) correspond to 0.05–0.1 μM N₂O dissolved in soil pore water. The determined thresholds of ~10 μM in BVA and approximate <0.004 μM in SJO are above and below the N₂O soil gas concentrations. This may contribute to the general emission pattern of higher CH₄ emissions from BVA peat and low CH₄ emissions from SJO peat (Finn et al., 2020). We should note that the enrichments used for the incubations represented only a fraction of the microbial community present and active in-situ. Nevertheless, the NO₃⁻ or N₂O effects on CH₄ accumulation in soil incubations or culture enrichments suggest that complex links between the nitrogen and carbon cycle occur in Amazon peatlands. These may likely comprise interactions of nitrifying microbes and methanogens, which would contribute to the high spatial variation in CH₄ production observed in peatlands.

CONCLUSION

Taken together, the PMFB harbors peatlands with diverse edaphic chemistries, which is especially reflected in pH, DOC, NO₃⁻ pore water concentrations and C/N ratios of shallow soils. Based on the physicochemical conditions in the waterlogged peats (redox, trace metal availability, substrates) the tropical soils provide feasible conditions for microbial methanogenesis, predominantly via the hydrogenotrophic pathway. The overall microbial communities show strong patterns of vertical stratification in the top 1 m. Thaumarchaeota and Nitrospira have similar ecological niches as producers of NO₃⁻ species. Thaumarchaeota are more abundant in the low-pH sites SJO and QUI, whereas Nitrospira appear more dominant in the more neutral-pH soil BVA. Our data reveal high potential of novel and diverse methanogens affiliated with the Methanomicrobiales order. We present evidence that soil NO₃⁻ species impact methanogen relative abundances and activities and our laboratory incubations show significant effects of NO₂⁻ and N₂O on CH₄ production.

Our findings may have broad implications on how pulses of nitrogen oxides, which are controlled by nitrifying/denitrifying groups and abiotic factors, could contribute to the spatial and seasonal variation observed in CH₄ emissions from peatlands of the Amazon. For instance, drought-induced drainage of peatlands may fuel nitrification and the production of nitrogen oxides, which, in turn, lower CH₄ production. Lowering of the water table and the oxic-anoxic interface would shift the NOₓ profile down toward soil layers with higher methane content. Subsequently, nitrogen oxides become a more dominant electron acceptor than CO₂ resulting in increased CO₂ production and decreased CH₄ production. With a higher diffusion range than O₂, nitrogen oxides reach more methanogenic cells and may inhibit them before they actually encounter O₂. Because of the variable effects of NO₂⁻ and N₂O, and the divergent vertical distribution of these compounds, further work is needed to assess effects of NOₓ species on individual soils. Regardless, our study highlights an underestimated link between the aerobic branch of the nitrogen cycle and the anaerobic branch of the carbon cycle yielding a generally negative feedback on methane formation in tropical peat soils. Because Amazonian peatlands represent large stocks of organic carbon, the magnitude of this feedback may be critical to regulate how much carbon in the form of CH₄ would be exported into the atmosphere.

DATA AVAILABILITY STATEMENT

All geochemical data can be found in the paper and Supplementary Material. The sequence data have been deposited in the GenBank, EMBL and DDBJ databases as SRA Bioproject PRJEB36841.

AUTHOR CONTRIBUTIONS

SB and HC-Q designed the study and wrote the manuscript. SB, JvH, JU, AMH, and HC-Q conducted the field work. SB and AFS performed the molecular work at the laboratory. SB and DRF analyzed the sequence data. SB and ZZ conducted incubations and geochemical analytical work. SB, AMH, and HC-Q analyzed the geochemical data. All authors contributed to the final draft of the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2021.659079/full#supplementary-material
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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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