Pedobiologia - Journal of Soil Ecology 113 (2025) 151093

Contents lists available at ScienceDirect

Pedobiologia - Journal of Soil Ecology

journal homepage: www.elsevier.com/locate/pedobi

Variability in depth-mediated shifts in methanogen and methanotroph communities across tropical Andean mountain peatlands

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ARTICLE INFO

Keywords:
methanogens
methanotrophs
andean mountain peatlands
grazing

ABSTRACT

The balance between methane producing and methane consuming microorganisms is partially responsible for the magnitude and direction of soil-atmosphere methane fluxes. Therefore, understanding the effects of vegetation and land use on relative abundance, community structure and vertical distribution of methane cycling microorganisms should help us interpret patterns of net emissions of methane. Mountain peatlands in the tropics are abundant and are important in global carbon cycling, but little is known about the communities of methane-cycling microorganisms in these ecosystems. We sampled peat from eight Andean peatlands in Peru, Ecuador and Colombia and described the dominant vegetation, grazing intensity and other site characteristics at each site. We characterised the microbial community of each peat sample at different depths and identified the methane cycling microorganisms. We observed a higher proportion of methanogens and methanogen:methanotroph in the shallow peat of grazed sites than ungrazed sites. At ungrazed sites we found relatively high methanotroph abundance relative to methanogens, even at the deepest sampling depths, suggesting plant aerenchymal oxygen transport structures communities at depth. We found changes in the methane cycling microbial communities among sites and with depth, e.g., in the superficial peat the hydrogenotrophic methanogens were more abundant but at depth there was more metabolic diversity. Our study provides new insights into the community structure of methane cycling microorganism of the tropical mountain peatlands, and raises interesting questions regarding the drivers of methanotroph abundance deeper in peat.

1. Introduction

Methane (CH_4) is the second most important greenhouse gas, after carbon dioxide, contributing to global warming. Thus, understanding factors affecting its global atmospheric concentration is of paramount importance (Zhang et al., 2017). Peatlands are a major source of CH_4 to the atmosphere (Frolking et al., 2011; Ma et al., 2022; Saunois et al., 2020), but they do vary greatly in CH_4 efflux. The rate partially depends on the balance between two specific groups of microorganisms specialized in CH_4 production (methanogens) and CH_4 oxidation (methanotrophs) (Lai, 2009). Two key environmental factors driving the relative balance of methanogens and methanotrophs in peatlands include 1) the redox potential and substrate quality and quantity, which are affected by peat depth, plant functional types (PFTs), and environmental conditions (such as water table and temperature), and 2) disturbances such as land use change for agriculture (Angel and Conrad, 2009; Blodau, 2002; Conrad, 2009).

At increasing depths, peat is often more decomposed, peat quality decreases, and the pore spaces are smaller (Patiño et al., 2021). These factors, interacting with water table levels, lead to a vertical gradient in oxygen diffusion that affects the redox potential and microbial communities. In deeper layers where oxygen and other highly oxidized

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https://doi.org/10.1016/j.pedobi.2025.151093
Received 9 June 2025; Received in revised form 12 October 2025; Accepted 13 October 2025
Available online 14 October 2025

electron acceptors become limited and biochemical redox gradients are small, a specialized group of methanogenic Archaea create CH_4 as a terminal decomposition product (Conrad, 2009; Tate, 2015). In contrast, closer to the surface more oxygen or other electron acceptors are available, which creates a more favourable niche for CH_4 oxidising bacteria, at least when methanogens are active in deeper horizons or anoxic microsites, and consequently CH_4 is available (Conrad, 2009; Hanson and Hanson, 1996; Tate, 2015). In response to this vertical gradient, changes in the community composition of both methanogens and methanotrophs have been described (He et al., 2015; Peltoniemi et al., 2016).

Sphagnum, graminoids, and cushion plants (in the Juncaceae, Asteraceae, and Plantaginaceae) are the dominant functional types of Andean mountain peatlands (Benavides et al., 2023; Boucher et al., 2016; Rodríguez-Echeverría et al., 2021; Whinam and Hope, 2005). Graminoids and cushion plants produce dense and deep aerenchymatous root biomass which affect both substrate supply and quality (electron donors) through rhizodeposition, and oxidative potential (electron acceptors) via radial oxygen loss. This leads to shifts in microbial communities, promoting CH_4 oxidation even in deep layers where CH_4 production is typically dominant (Fritz et al., 2011; Saarnio et al., 2000; Strack et al., 2016). In addition, cushion plants are characterised by a low stature, that protects from environmental stress by increasing temperature and reducing evapotranspiration (Cavieres et al., 2007) allowing them to thrive at higher elevations under cold conditions. The presence of aerenchymatous vascular plants has been widely associated with higher CH_4 emissions due to the ability of these plants to transport CH_4 produced in deeper layers to the atmosphere (Turetsky et al., 2014). However, in Andean mountain and cool temperate low elevation austral peatlands the presence of aerenchymatous cushion plants has been associated with low CH_4 emissions, likely as consequence of a better soil aeration due to the aerenchyma and differences in radial oxygen loss from roots into the rhizosphere (Armstrong et al., 1992; Fritz et al., 2011) that could affect the balance between methanogens and methanotrophs, favouring the latter (Dullo et al., 2017; Fritz et al., 2011; Sanchez et al., 2017). However, studies of microbial communities in Andean peatlands are lacking.

Another impact on CH_4 efflux is grazing, which is a main cause of degradation in Andean peatlands (Machaca et al., 2018). Grazing increases soil bulk density and root density (Elschot et al., 2013; Marin-Diaz et al., 2021) and affects plant community composition and cover (Machaca et al., 2018). Moreover, the addition of manure, urine, and faeces-associated methanogens all can have a direct impact on CH_4 cycling and microbial communities (Lammel et al., 2015; Sanchez et al., 2017; Veber et al., 2018). In Andean peatlands, based on preliminary studies, a working framework of CH_4 dynamics may be that emissions are low in undisturbed conditions, and the introduction of cattle can lead to increased CH_4 emissions (Sanchez et al., 2017). This would likely be reflected in transformation of methanogen and methanotroph community structure in surface horizons.

Microbial communities in mountain peatlands of the tropical Andes have not been characterised, since most peatland research has been carried out in boreal or low elevation tropical peatlands. However, recent research has revealed that mountain peatlands are abundant in the tropics, increasing their relevance for global carbon cycling (Benavides et al., 2023; Chimner et al., 2019; Hribljan et al., 2024), pointing to the importance of characterizing microbial communities in these ecosystems. The oxidising effects of PFTs and cooler climatic conditions of Andean peatlands (Fritz et al., 2011; Jauhiainen et al., 2016; Planas-Clarke et al., 2020; Sanchez et al., 2017), and intensive disturbance in cushion-dominated pastures should help to structure vertical patterns of methanogen and methanotroph communities.

In this study we sampled soil from eight peatlands, which had a gradient in cattle grazing, in Colombia, Ecuador, and Peru, from three depths (10–20, 30–40 and 60–70 cm). We hypothesized, (H1) that with increasing depth the ratio of methanogens to methanotrophs would increase. We also expected that (H2) the presence of cattle would increase the proportion of methanogens and methanogen:methanotoph ratios (Mgen:Mtroph) in near-surface peat.

2. Material and methods

2.1. Study region and sites

This study includes sites in Ecuador, Colombia, and Peru, covering a latitudinal gradient from − 9° to 5°. We sampled peatlands from eight different sites covering an elevation gradient from 3152 m to 4773 m and mean annual temperature and annual precipitation from 1.5 °C to 8.7 °C and 674 mm to 1568 mm, respectively (Table 1. Fig. 1). Due to their proximity and similarities in climate, land use, and soil properties, the Cayambe Coca site in Ecuador, consisted of a cluster of two cores taken in two adjacent peatlands separated by < 300 m. We classified grazing intensity as either no to light grazing (N-L grazing) or med to high grazing (M-H grazing)) for each site based on disturbances (i.e. animal tracks and faeces) observed on site (Table 1).

2.2. Field sampling

Samples were taken opportunistically as part of a study of long-term carbon cycling in mountain peatlands of the tropical Andes (Hribljan et al., 2024). At each site, one to four cores were collected using a 100 cm long × 6.35 cm diameter stainless steel metal gouge auger (AMS, Idaho, USA). From most cores, the 10–20 cm, 30–40 cm and 60–70 cm depths were subsampled for microbial community characterization and soil chemical and physical properties. Sub samples for microbial community analysis were taken using sterile conditions and the rest of the core was reserved for the physic and chemical analyses. In a limited number of cores, for logistical reasons only the shallowest target depth increments were collected, and for some sites deeper segments were included in addition to the target three depth increments (Table 1). After the core was extracted, the water table position in the open hole was allowed to stabilize for approximately one hour and then recorded as depth from the peat surface. All samples were put immediately on ice packs, frozen within 8 h, and transported frozen to the US Forest Service Northern Research Station Forestry Science Laboratory in Houghton, Michigan, USA for further processing.

2.3. Environmental and peat properties

Standardised climate data (mean annual temperature and precipitation) for all sites were obtained from WorldClim (www.worldclim.org), a high resolution (30 arc s or c. 1 km at the equator) database generated from a high number of climate observations and topographical data (Fick and Hijmans, 2017). Soil C and N were determined from 75 mg of dried ground peat on a combustion based elemental analyser (Watmough et al., 2022), 57 elements were analysed from ashed peat after acid digestion on an inductively coupled plasma mass spectrometer (Birnbaum et al., 2023). Only Cu, Fe, Ni, and S were used in further analyses due to their role in aerobic oxidative enzymes (Cu), methanogenesis limiting factor (Fe, Ni) or methanogenesis inhibitor (Cu, Ni). Soil pH was measured in a 2:1 ratio of distilled water to peat (Table1).

2.4. Methanogen and methanotroph communities

Microbial communities were characterised with DNA amplicon sequencing. Approximately 10 ml of peat from a sample was placed in a 50 ml tube, followed by twenty 3.2 mm chrome-steel beads, and pulverised with a modified mini-beadbeater-96 (BioSpec Products, Bartlesville, OK, USA) bead beater for 2 min. DNA was extracted from 0.5 g subsample of pulverised peat using a PowerSoil® DNA Isolation Kit (MoBio Laboratories Inc., Carlsbad, California, USA, now Qiagen) following the manufacturer's instructions, with the addition of a 30-

Table 1
Site description table. Antisana (Ant) and Cayambe Coca (CC) sites are located in Ecuador, Chingaza National Natural park (Chi) and Páramo de Guerrero (P De G) are located in Colombia, and Cahuish (Cah), Pastoruri (Pas) and Quilcayhuanca (Qui) are located in Peru. MAT: Mean Annual Temperature, MAP: Mean Annual Precipitation. Med-H: Medium to High grazing, Not-L: No to Light grazing, Vegetation: dominant vegetation observed on the peatland.

Core	Latitude	Longitude	Elevation (m)	MAT (°C)	MAP (mm)	Grazing	Vegetation	Year sampled	Month sampled
Ant1	− 0.494657	− 78.276000	3931	4.7	1167	Med-H	Mixed cushion	2014	6
Ant2	− 0.494657	− 78.276000	3931	4.7	1167	Med-H	Mixed cushion	2014	6
Ant3	− 0.494639	− 78.276200	3930	4.7	1167	Med-H	Mixed cushion	2014	6
Ant4	− 0.494569	− 78.276200	3930	4.7	1167	Med-H	Mixed cushion	2014	6
CC 1	− 0.321631	− 78.199400	4235	4.5	1310	Not-L	Mixed cushion	2014	6
CC 2	− 0.319201	− 78.200100	4252	4.5	1310	Not-L	Mixed cushion	2014	6
Chi	4.677459	− 73.787223	3152	8.7	1568	Med-H	Other	2015	10
P De G	5.214600	− 74.006960	3636	7.9	1299	Not-L	Other	2014	2
Cah1	− 9.687670	− 77.247810	4416	3.5	674	Not-L	Other	2014	5
Cah2	− 9.687210	− 77.248040	4421	3.5	674	Not-L	Other	2014	5
Cah3	− 9.688110	− 77.247770	4418	3.5	674	Not-L	Other	2014	5
Pas1	− 9.884810	− 77.188270	4758	1.5	712	Med-H	Mixed cushion	2014	5
Pas2	− 9.885310	− 77.187930	4762	1.5	712	Med-H	Mixed cushion	2014	5
Pas3	− 9.885679	− 77.187673	4773	1.5	712	Med-H	Mixed cushion	2014	5
Qui1	− 9.466210	− 77.378940	3925	1.5	712	Med-H	Mixed cushion	2014	5
Qui2	− 9.466720	− 77.379260	3935	1.5	712	Med-H	Mixed cushion	2014	5
Qui3	− 9.464650	− 77.377610	3940	3.9	736	Med-H	Mixed cushion	2014	5

minute incubation at 65 °C in the C1 lysis buffer following the 10-min vortexing step. The extracted DNA was cleaned using a MoBio PowerClean® Pro DNA Clean-Up Kit and quantified with a Qubit Fluorometer (Invitrogen, Life Technologies, Carlsbad, CA, USA). The cleaned DNA extracts were sequenced at the U.S. Department of Energy Joint Genome Institute (JGI, Walnut Creek, California, USA; it has subsequently moved to Berkeley, California, USA) using an Illumina MiSeq (Illumina, Inc., San Diego, CA) with 2 × 300 bp chemistry. Bear et al. (2021), in a study in North American peatlands, highlighted the better characterization of the methanogenic communities targeting 16S than the mcrA gene sequences; consequently, we targeted the 16S V4 region using 515 F and 806 R primers which also contained Illumina sequencing adaptors and the reverse primer contained an 11 bp index unique to each sample (Caporaso et al., 2011).

From the raw sequence reads, Illumina adaptors and PhiX 174 were removed with BBDuk (sourceforge.net/projects/bbmap/), and 3′ and 5′ PCR primers were trimmed with Cutadapt 3 (Martin, 2011). Sequences were merged into ASVs (Amplicon Sequence Variants), filtered for quality (expected error rate = 2), chimeras removed, and reads joined, all using DADA2 (Callahan et al., 2016). Before creating the ASVs forward and reverse V4 sequence reads were truncated at 200 bp and 150 bp, respectively. Taxonomic assignment was performed with the naive Bayes feature-classifier (Bokulich et al., 2018; Pedregosa et al., 2011); and Qiime formatted SILVA 16S + 18S dataset (Silva 138 SSURef NR99 515F/806R region; (Quast et al., 2013; Robeson et al., 2021). ASVs with taxonomic assignments to mitochondria, chloroplast and eukaryote 18S sequences were filtered from the V4 dataset. All aforementioned steps, from processing with DADA2 to filtering of non-target taxa, took place within the QIIME2 environment (Bolyen et al., 2019). The datasets were then normalised by scaling with ranked subsampling (SRS) prior to all statistical analyses (Beule and Karlovsky, 2020). Tentative functional groups were assigned with faprotax_1.2.4 (Louca et al., 2016), with further refinement for methanogens and methanotrophs based on literature searches. Due to the importance of the archaeal phylum Bathyarchaeota in C cycling and their possible but unconfirmed role in CH_4 metabolism (Evans et al., 2015), we retained the Bathyarchaeota in our assessment. This phylum occurs in diverse habitats from marine to freshwater and terrestrial environments (Zhou et al., 2018) however, they have been described across other anoxic environments as metabolically diverse. For these reasons, the Bathyarchaeota have been analysed separately from the confirmed methanogens and methanotrophs.

2.5. Statistical analyses

We performed permutational multivariate analyses of variance (PERMANOVA) with Bray-Curtis distance measure to evaluate the effect of depth and location on the communities of methanogens and methanotrophs independently and after normalising the data, using the adonis2 function and 1000 permutations in the vegan package (Oksanen et al., 2020). When statistical differences were found we performed pairwise comparisons for all levels of the factor with the adonis.pair() function in the EcolUtils package (Salazar, 2021). We used Kruskal-Wallis one-way analysis of the variance to determine significance of the grazing intensity on the Mgen:Mtroph at each depth. We performed a principal component analyses (PCA) to evaluate the relationships between the Mgen:Mtroph ratio and the environmental variables (i.e., relative position of the sample to the water table, sample depth, peat pH and temperature, elevation, C, C/N ratio, Ni, Cu, S, Fe and grazing intensity), using the prcomp () function from the stats package (RCore, 2021). All variables were scaled to unit variance prior to PCA. We evaluated the relationships between methanogen and methanotroph microbial community and environmental variables through distance-based redundancy analyses (dbRDA) (Legendre and Anderson, 1999) based on Bray-Curtis dissimilarities, using the capscale function in the vegan package (Oksanen et al., 2020) and the phyloseq package (McMurdie and Holmes, 2013). We used the ordistep function in the vegan package to select the best model with permutation tests (Oksanen et al., 2020). We used logarithm transformations of the soil C/N ratio and Fe to improve residual normality. All analyses were performed using R 4.1.0 (RCore, 2021). Data associated with this study are available from figshare (Lafuente et al., 2023) and SWAMP Dataverse.

3. Results

3.1. Methanogen and methanotroph relative abundance

Methanogens were detected in all core depth samples except in the shallowest (10–20 cm) at Páramo de Guerrero and Quilcayhuanca, whereas methanotrophs were present in all samples (Figs. 2–4). The combined relative abundance of methanogens and methanotrophs was under 5 % of the total amplified prokaryote community in all samples. Both Antisana and Chingaza had a high relative abundance of methanogens in the upper depth sample (1.4 % and 1.5 % respectively). With increasing depth, the relative abundance of methanogens increased in all sites except Antisana where the surface had the highest methanogen relative abundance (Fig. 2A). Antisana and Chingaza also had the greatest relative abundance of methanotrophs at the surface (~ 2 %).

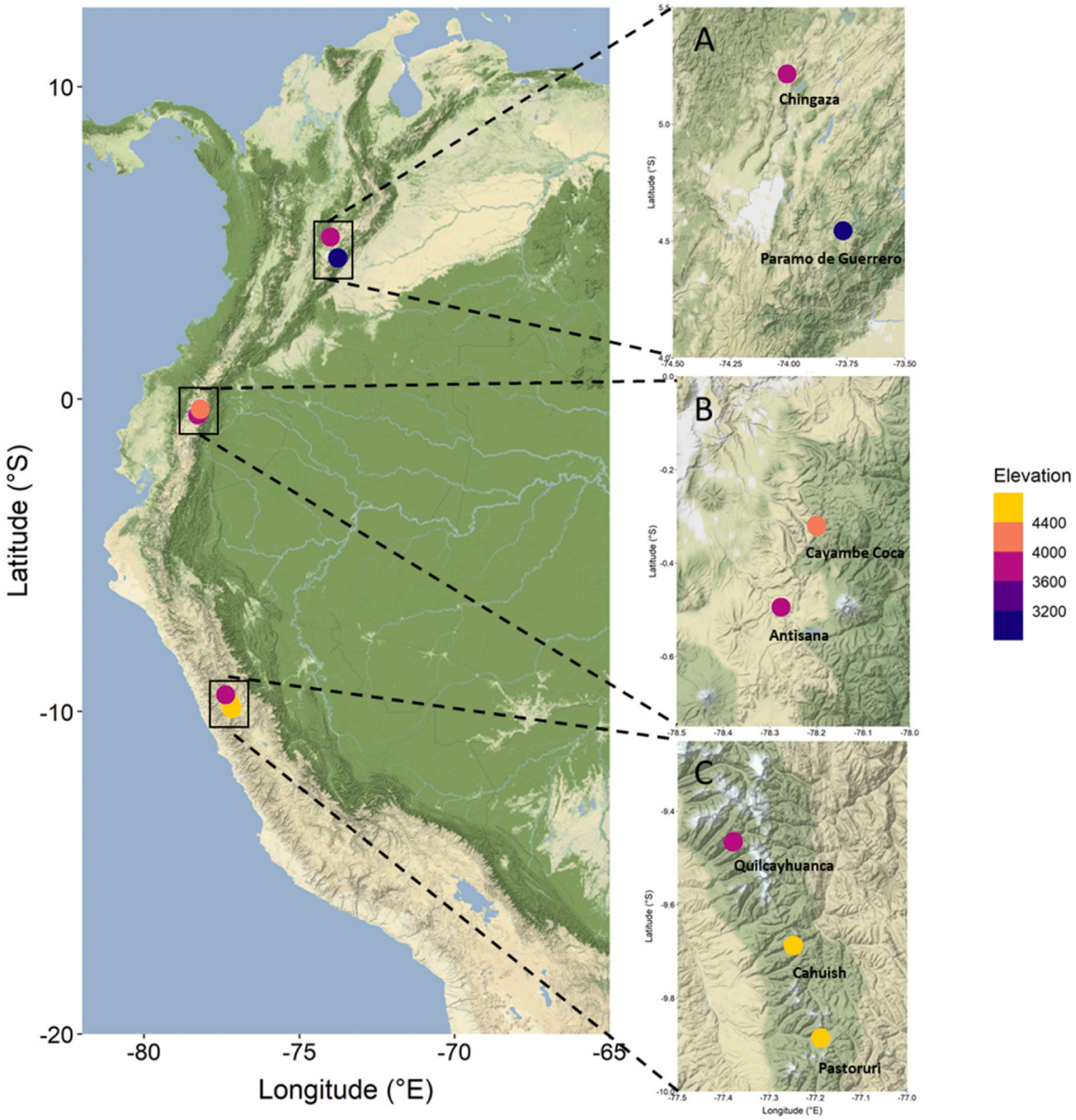

Fig. 1. Map of the study region in the tropical Andes Mountains. Insets detail locations sampled. A. Colombian sites, B. Ecuadorian sites, C. Peruvian sites.

The relative abundance of methanotrophs was higher than that of methanogens in all cores and depths except in Antisana at 30 cm and Cayambe Coca at 147 cm and 200 cm (Fig. 2B). The Mgen:Mtroph increased with depth, except in Antisana and Pastoruri where the 30 cm depth had the highest Mgen:Mtroph. This paper focused only on strict CH_4 related microorganisms thus we have not included Bathyarchaeota in our analyses because not all are likely methanogens. Interestingly, we found abundant Bathyarchaeota in all samples, and high relative abundance at depth (Fig. S1).

3.2. Methanogen and methanotroph community composition

The composition of the methanogen communities varied with depth ($F(3) = 2.43$, $p < 0.0001$) and among sites ($F(6) = 2.41$, $p < 0.0001$). Whereas the order Methanobacteriales was the dominant in the top sample (10–20 cm depth) in the site with the highest grazing intensity (Antisana), the Methanomicrobiales dominated at one of the other sites with M-H grazing (Chingaza). The former order was also dominant in the top sample at one of the sites with no evidence of grazing (Cayambe Coca). However, unlike the grazed sites, the relative abundance of methanogens in this sample was small as a proportion of total reads (below 0.003 %). The other two sites with M-H and N-L grazing intensity (Pastoruri and Cahuish, respectively) had a combination of Methanomicrobiales with Methanobacteriales or Methanomassiliicoccales in the top samples. However, the total relative abundances of methanogens in these two sites, Pastoruri and Cahuish, were small at 0.04 % and 0.004 %, respectively. At deeper layers taxa in the orders Methanocellales, Methanomicrobiales, Methanosarcinales and Methanomassiliicoccales dominated (Fig. 3).

The composition of the methanotroph communities also varied with depth ($F(3) = 2.32$, $p < 0.0001$) and among sites ($F(6) = 3.35$, $p < 0.0001$). The order Methylococcales was the dominant in the sites with M-H grazing intensity (Antisana and Pastoruri), whereas Rhizobiales was the dominant order in the sites with N-L grazing intensity (Cayambe Coca, Páramo de Guerrero and Cahuish). In addition, Methylomirabilales together with Rhizobiales were the co-dominant orders in one of the sites with M-H grazing (Chingaza; Fig. 4). The order Methylomirabilales was more abundant with depth in all sites regardless of grazing intensity.

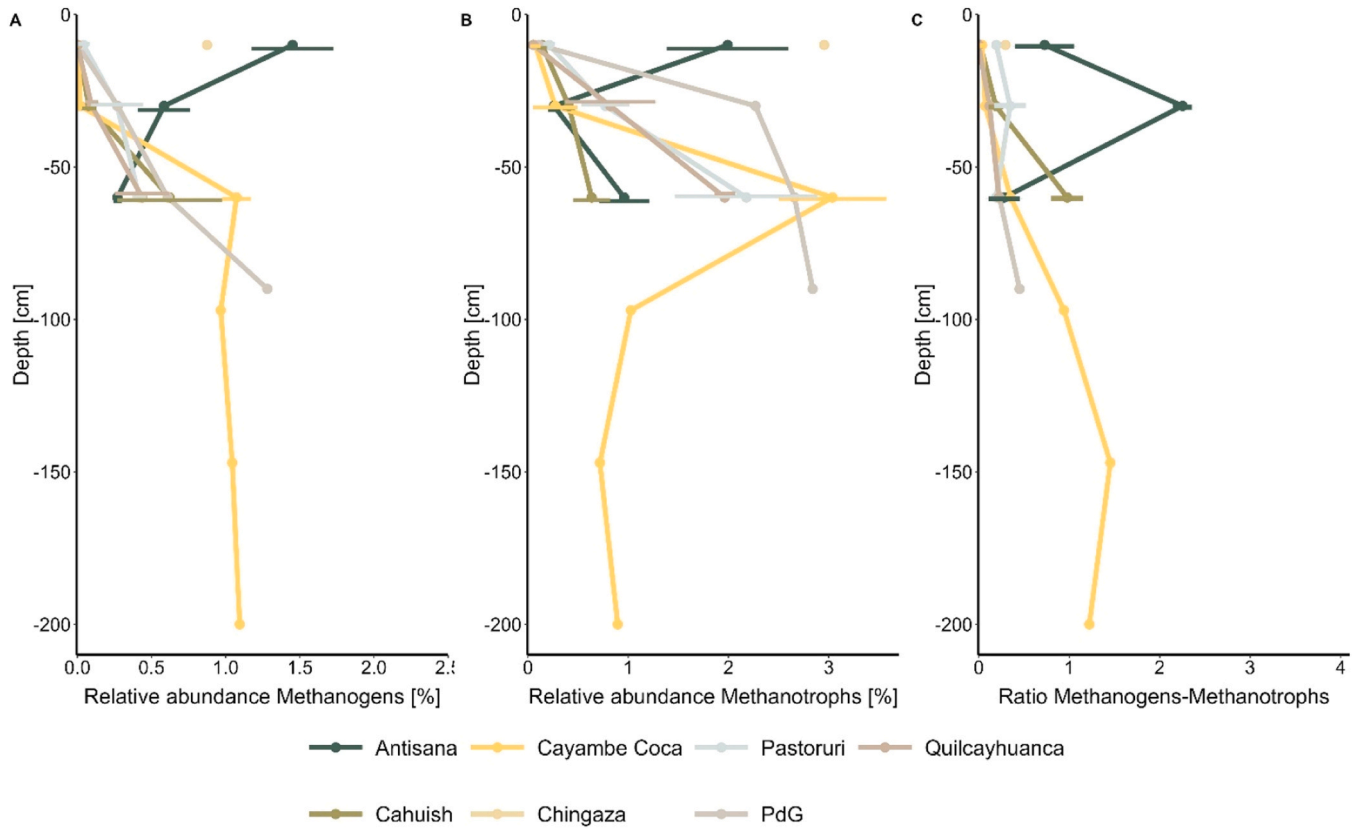

Fig. 2. Relative abundance of methanogens (A), methanotrophs (i.e. methane oxidising bacteria. B) and the methanogens:methanotroph ratio across depths in the studied sites. The relative abundances were calculated in relation to the total number of sequence reads per sample. PdG: Páramo de Guerrero. Data are means ± SE.

3.3. Predictors of the Mgen:Mtroph

Forty-one percent of variation in environmental variables was explained by the first two axes of the PCA (23 % and 18 %). Axis 1 primarily captured variation in soil physicochemical properties, while axis 2 captured variation in depth and the relative position of the water table (Fig. 5). The samples were divided in three clusters by depth. Within the cluster for the top and middle depth samples, those from the M-H grazing intensity sites (Antisana and Quilcayhuanca) clustered together on the bottom right side. However, in the deep samples, the differentiation by grazing intensity was less evident (Fig. 5). The Mgen:Mtroph of Antisana, the highest grazing intensity site, was higher in the top and middle depth samples, while two of the sites with N-L grazing intensity (Cayambe Coca and Cahuish) had lower Mgen:Mtroph in the top and middle depth samples.

3.4. Predictors of the community composition

The dbRDA model explained a total 16 % and 27 % of the methanogen and methanotroph community variability, respectively (Figs. 6A and 6B). The position of the water table relative to the peat sample was an important predictor of both methanogen and methanotroph community composition. In addition, copper (Cu) was an important predictor of the methanogen community composition, and peat temperature and pH were the main predictors of the methanotroph community composition.

4. Discussion

The CH_4 cycling microbial communities from seven Andean peatlands located in Ecuador, Colombia and Peru showed that methanotrophs were widely distributed and present in all depths and sites, whereas methanogens were rare or absent on the surface at five of the seven sites, and only abundant at two of the sites (Antisana and Chingaza). Methanogen and methanotroph abundances were likely influenced by the interactions of aerenchymatous roots abilities to both transport labile e^- and C substrates and oxygen to lower depths, combined with grazing alteration of the normal vertical patterns of substrate/electron acceptor availability (H2)(Fritz et al., 2011; Strack et al., 2016). There was not clear statistical evidence that grazing intensity had an effect on the Mgen:Mtroph (Top sample Chi square = 2.72, p = 0.26, df = 2). This may have been due to the difficulty in classifying the grazing intensity from observed evidence or that other characteristics among the sites might have overshadowed the grazing effect. Nevertheless, we observed an increase in the range of the Mgen:Mtroph with higher grazing in the near-surface samples, evident in the known highly grazed site of Antisana (Fig. S2A). Among the environmental variables studied, Cu was a strong predictor of the methanogen community composition, perhaps for its potential to inhibit certain methanogens (Bear et al., 2021), especially given the relatively high concentrations (> 400 mg/kg).

4.1. Vertical patterns in methanogen relative abundance

Methanogens are expected to be more abundant and active below the water table, under waterlogged, anoxic conditions (Freitag et al., 2010). The sites were all relatively wet (with the 10–20 cm soil depth at or beneath the water table) at the time of sample collection. However, the water table depth does fluctuate between wet and dry periods and thus we expected an increase in their relative abundance with increasing depth. For instance, Cayambe Coca, similar to the majority of sites, had very low methanogen relative abundance near the soil surface, which as expected increased with depth (Fig. 2A) (Freitag et al., 2010). In contrast, Antisana followed the opposite pattern, with a clear decline in

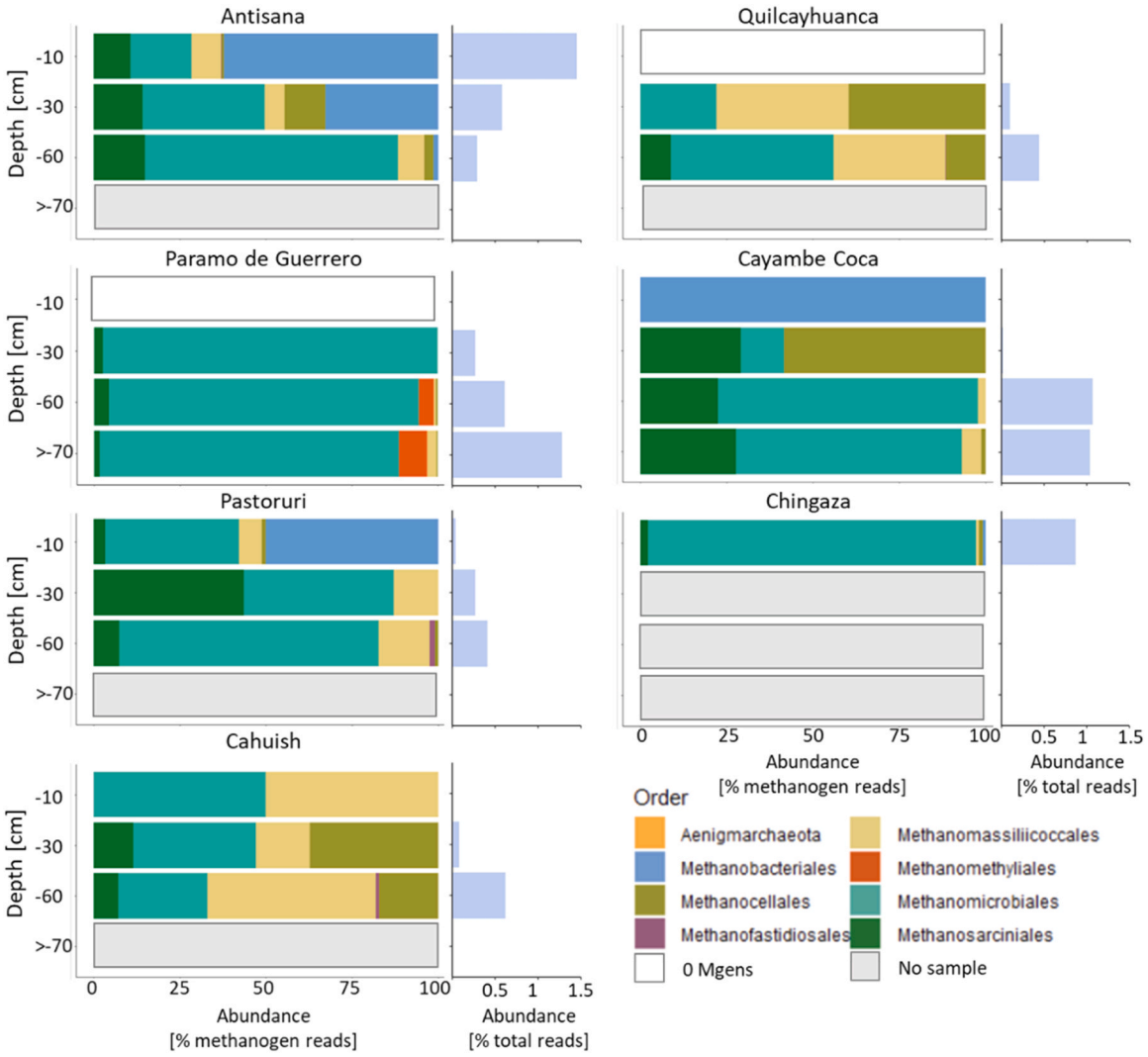

Fig. 3. Relative abundance of the most abundant orders of methanogens at each site and depth (left) out of all methanogens. The % of the total relative abundance that methanogenic community represent (< 1.5 %) in each site and depth (right) out of all the sequences in the data set. First row: from 10 to 20 cm, second row: from 30 to 40 cm, third row: from 60 to 70 cm, fourth row: over 70 cm.

methanogen relative abundance with depth. Chingaza (identified as a M-H intensity grazed peatland) similarly to Antisana showed high total abundance of methanogens in the shallow layers of peat, unfortunately we do not have deeper samples of peat and thus we do not know if Chingaza followed the same vertical pattern. This could be due to the high grazing disturbance at the site. The abundant bovine ruminants in addition to disturb the peat surface and disrupted plant production, they provide manure rich in labile substrates and methanogenic archaea, altering the methanogen community observed in the shallow layers of peat on sites with high grazing pressure like Antisana (Fritze et al., 2021; Kroeger et al., 2021; Radl et al., 2007). Further research would help us to better understand whether high grazing intensity is responsible of shifts on the vertical pattern of methanogenic communities.

4.2. *Vertical patterns of methanotroph relative abundance*

The consistent pattern of methanotroph persistence and even higher relative abundance with depth suggests a consistent availability of both CH_4 and electron acceptors that could support methanotrophy at depth (Lai et al., 2009). Given that the relative abundance of putatively aerobic CH_4 oxidising bacteria (Alphaproteobacteria, Gammaproteobacteria and Verrucomicrobia lineages (Bridgham et al., 2013; Le Mer and Roger, 2001) increased with depth well below the water table in some cases, it was likely that plant roots played important roles in microbial CH_4 cycling. As noted earlier, cushion plants and sedges have high amounts of aerenchyma tissue, which facilitates the transport of oxygen below the water table to support aerobic metabolism of roots in an otherwise anoxic environment (Bräuer et al., 2020; Lai, 2009). These roots provided oxygen for CH_4 oxidising bacteria, and also labile organic substrates at depth, which could have fed methanogens if compounds migrate into anoxic areas further from roots or into anoxic microsites in the rhizosphere. Many cushion plants have low associated CH_4 emissions, likely related to high rhizosphere oxidation by aerenchyma (Fritz et al., 2011), favouring methanotrophs in close proximity to the roots, hence providing a barrier to CH_4 diffusion into and transport by roots. This might explain the increase in methanotrophs with depth at most sites, which kept the Mgen:Mtroph relatively low even down to 200 cm depth, consistent with the presence of oxic microsites at depth (H1). Suárez et al. (2021) found that production of *Distichia muscoides* Nees & Meyen and *Plantago rigida* Kunth roots were substantial down to 40–50 cm, but they did not sample deeper, so the deep root distribution and dynamics of these species was unclear. More informally, we have observed roots of aerenchymal plants down to at least the 60–70 cm depth.

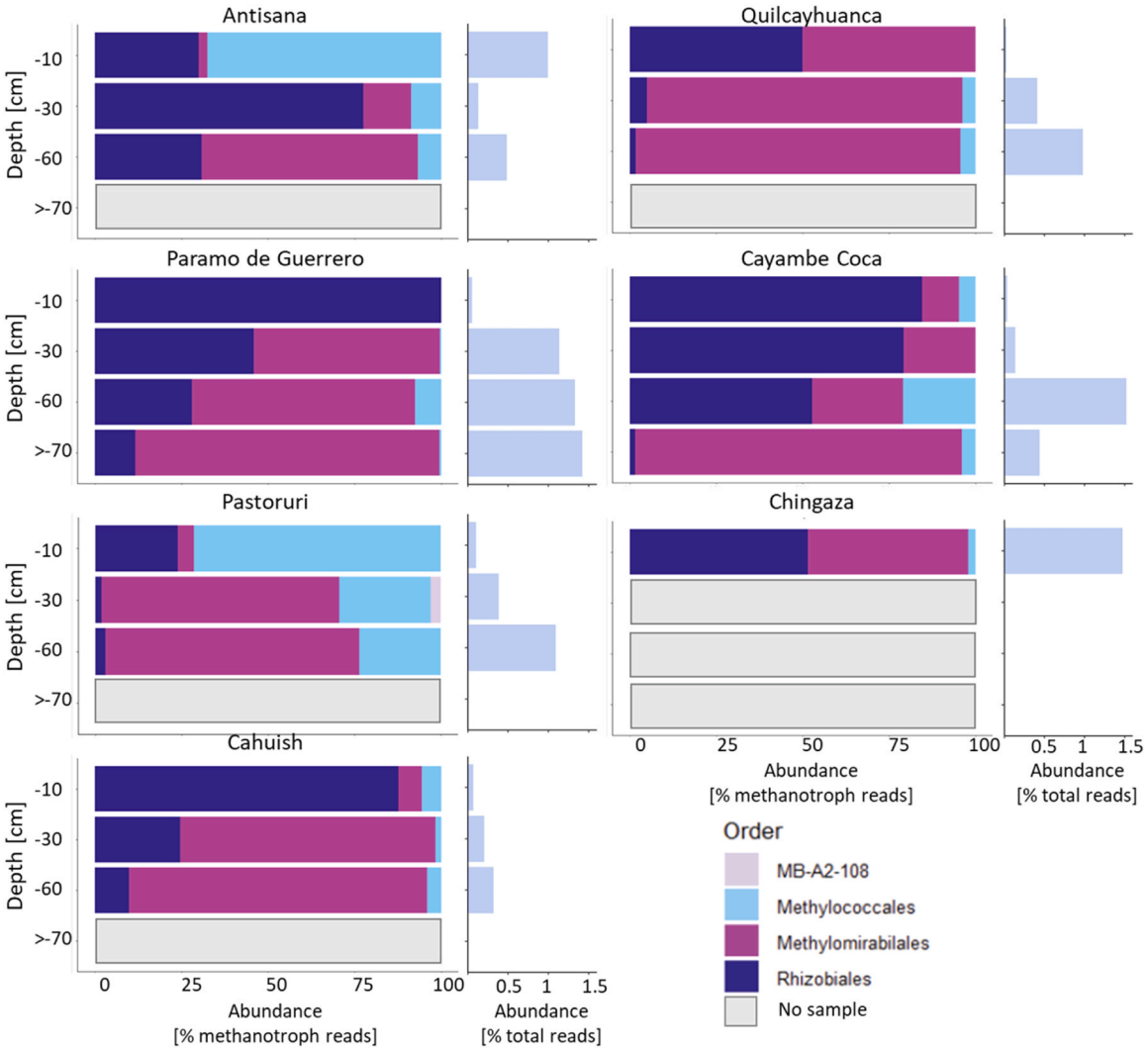

Fig. 4. Relative abundance of the most abundant orders of methanotrophs at each site and depth (left) out of all methanogens. The % of the total relative abundance that methanotrophic community represent (< 1.5 %) in each site and depth (right) out of all the sequences in the data set. First row: from 10 to 20 cm, second row: from 30 to 40 cm, third row: from 60 to 70 cm, fourth row: over 70 cm.

4.3. Vertical patterns of Mgen:Mtroph

In the absence of root inputs or disturbance, the Mgen:Mtroph was expected to be lower in the shallow peat and increase with depth (H1). Overall, we observed that Mgen:Mtroph are sometimes but not always higher at deeper layers, likely reflecting the patterns of root proliferation and substrate input described above. We would expect to find more CH_4 oxidising bacteria than methanogens near the surface where conditions were oxic, and an increase in the relative abundance of methanogens with depth relative to methanotroph, increasing the Mgen:Mtroph. Although we did observe this pattern at several sites, we did not in Antisana and Pastoruri, which were M-H grazed sites. In particular, Antisana had a high relative abundance of both methanogen and methanotrophs on the surface and was the site with the highest ratio in the shallow layers of peat (Fig. 2C). However, the intermediate depth samples had a higher ratio due to a notable reduction in the methanotroph relative abundance, perhaps reflecting disturbance to aerenchymal roots and their transport of oxygen below the water table (Sanchez et al., 2017). Disturbance and turnover of the aerenchymal plant roots at Antisana could have favoured the anaerobic metabolism of methanogens over the aerobic metabolism of methanotrophs, driving the shift in ratios of methanogens to methanotrophs and high CH_4 emissions (H2). Chingaza was one of the peatlands with M-H signs of grazing. Here we also found a very high relative abundance of methanogens in the shallow layer, but the relative abundance of methanotrophs was also very high, and consequently the ratio was small. Unfortunately, on this site we did not have samples at deeper layers. Our data showed some evidence that heavy grazing may have altered the Mgen and Mtroph relative abundance (Fig. S2), but only in very high grazing areas. Hence, Antisana (and possibly Chingaza) shows a shift in the expected vertical gradient of Mgen:Mtroph that could be explained by the presence of cattle on both sites (Kroeger et al., 2021; Radl et al., 2007). However, the lack of clear statistical evidence, may have been due to the difficulty in classifying the grazing intensity from observed evidence or that other characteristics among the sites might have overshadowed the grazing effect.

4.4. Methanogen and methanotroph communities

Both acetoclastic and hydrogenotrophic methanogenesis have been described in northern and tropical peatlands, generally with hydrogenotrophic metabolism dominant (Bräuer et al., 2020; Finn et al., 2020; Too et al., 2018). Similarly, we found that hydrogenotrophic methanogens (Methanobacteriales and Methanomicrobiales) were dominant in the surface samples suggesting that the primary source of CH_4 comes from hydrogenotrophic methanogenesis. In contrast, in deeper layers we

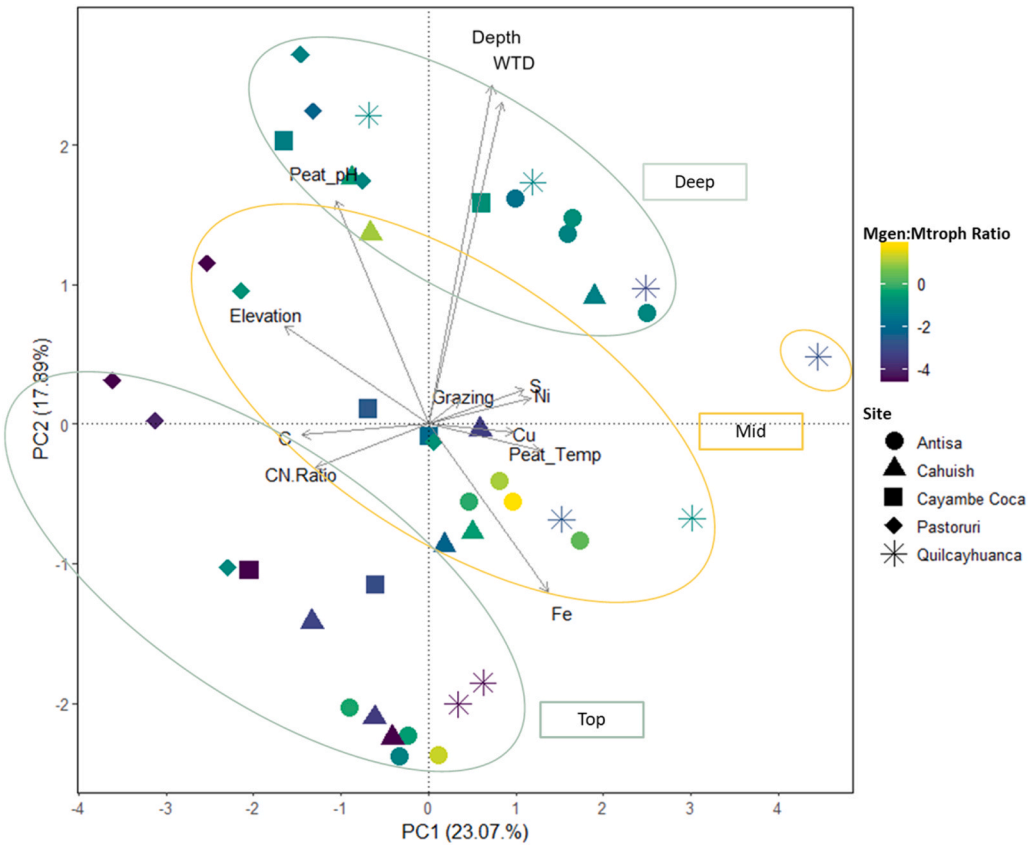

Fig. 5. Principal Components Analyses of the environmental variables. Methanogen-Methanotroph ratio has been log transformed. The ellipses have been drawn to highlight the grouping of the samples by depth. Top 10–20 cm, mid 30–40 cm, deep 60–70 cm.

found more variety of metabolisms with the increasing relative abundance of Methanocellales (hydrogenotrophic), Methanomicrobiales (hydrogenotrophic), Methanosarcinales (hydrogenotrophic, methylotrophic, reductive methylotrophic, acetoclastic) and Methanomassiliicoccales (reductive methylotrophic). Furthermore, Antisana, the site most heavily affected by grazing, was the only site with high Methanobacteriales relative abundance at the surface. Although Cayambe Coca also had high Methanobacteriales as a proportion of the methanogen community, they were at extremely low abundance relative to other members of the overall prokaryote community. Methanobacteriales are the common enteric methanogens associated with rumen fermentation in cattle (Matthews et al., 2019) as is *Methanosarcina barkeri* from the order Methanosarcinales. The genus *Methanosarcina* was found in all near-surface samples of Antisana and Chingaza and in one of the near-surface samples of Pastoruri. It is unclear whether the high relative abundance of Methanobacteriales and Methanosarcinales reflects their continued inputs from faeces, or the creation of favourable conditions by the combination of labile faecal substrate addition and physical disturbance. In a parallel finding, hot spots of CH_4 emissions have been described in boreal fen peatlands of North America and Eurasia, possibly attributable to the presence of caribou and reindeer, respectively (Fritze et al., 2021; Turetsky et al., 2014). Additionally, previous field and laboratory experiments have observed changes in the CH_4-related microbial communities and also increasing CH_4 fluxes with the addition of grazer faeces (Fritze et al., 2021; Hahn et al., 2018; Laiho et al., 2017). Methanogenesis at the soil surface is more likely to contribute to emissions, all else being equal, because of the shorter diffusive pathway to the atmosphere. Sanchez et al. (2017) measured CH_4 fluxes in two of the sites in the present study, Antisana and Cayambe Coca, concurrent with the microbial sampling for the present study. Notably, Cayambe Coca (N-L grazing disturbance) had low CH_4 fluxes (10.1 mg CH_4 m^{-2} d^{-1}) and the intensely grazed site (Antisana) had elevated CH_4 fluxes (132.3 mg CH_4 m^{-2} d^{-1}). Similarly, our finding of high emissions of CH_4 associated with a high Mgen:Mtroph has been found in other studies in which high Mgen:Mtroph (i.e. high methanogen and low methanotroph relative abundance) are found concurrent with high CH_4 emissions in different wetlands (Rey-Sanchez et al., 2019; Wen et al., 2018). This points to a potential area of research to evaluate if a rapid microbial community assay that captures this ratio could be a good predictor of ecosystem fluxes of CH_4, which could be of use for environmental monitoring purposes.

Methanotroph communities in the surface layers were dominated by the order Rhizobiales, similar to other northern and Amazonian peatlands (Finn et al., 2020; Perryman et al., 2022; Wang et al., 2022). In contrast, the top layer of Antisana and Pastoruri were dominated by Methylococcales which have been observed in more minerotrophic peatlands (Finn et al., 2020). In all samples at deeper layers Methylomirabilales gained importance, which could be related to the ability of anaerobic CH_4 oxidation coupled to nitrite oxidation that has been described in this group. This requires further investigation, as sequences of known anaerobic CH_4 oxidising prokaryotes were not found in our samples.

In conclusion, our results indicate that both methanogens and methanotrophs are abundant in tropical Andean mountain peatlands and these communities change with depth. The abundance of plants with aerenchymatous roots and their ability to transport labile substrates and oxygen to deeper layers of peat likely created the appropriate conditions for oxic microorganisms at great depths by altering the vertical patterns of substrate and electron acceptor availability. Our results highlight the need to better understand the composition and relative abundance of CH_4 related communities in order to better predict atmospheric CH_4 fluxes from Andean peatlands under the ongoing

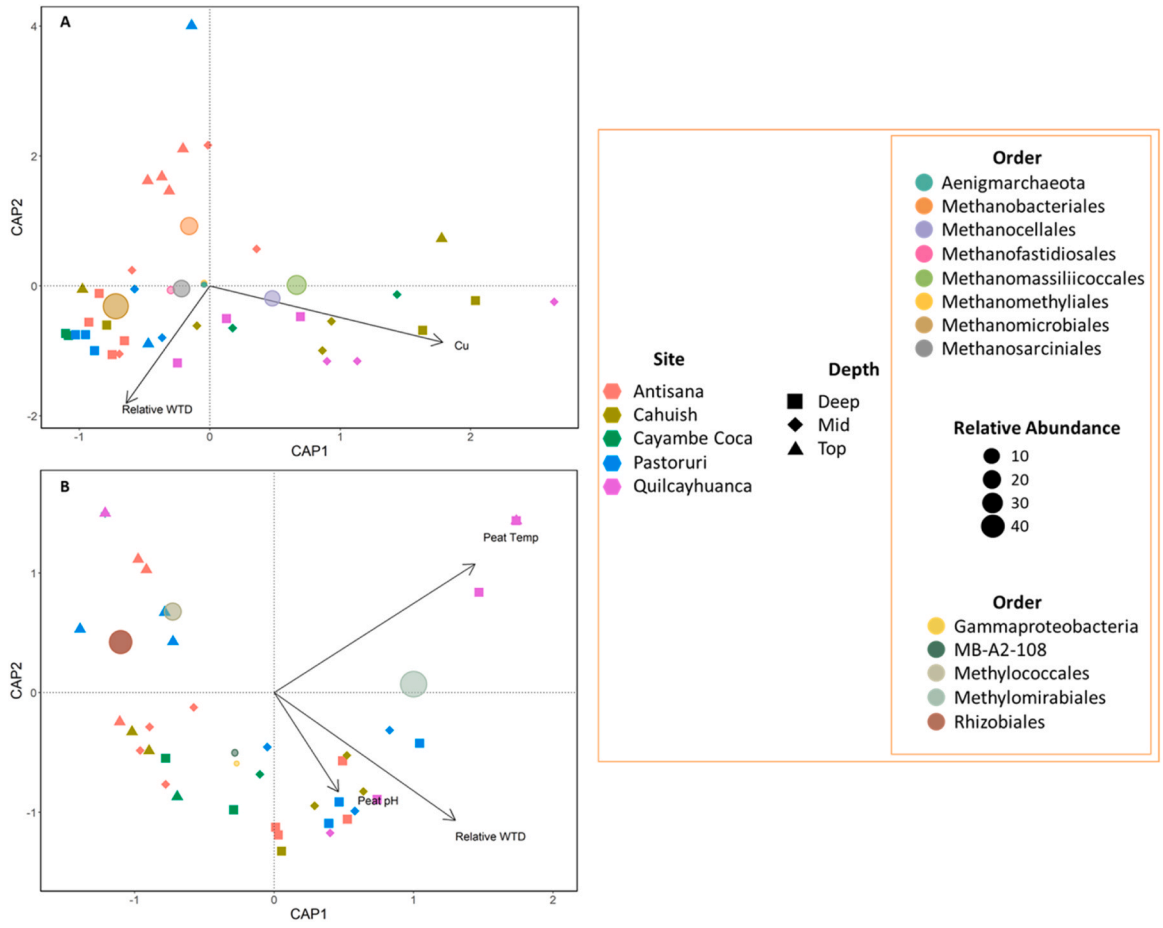

Fig. 6. Methanogen (A) and Methanotroph (B) db-RDA.

environmental changes.

CRediT authorship contribution statement

Juan C. Benavides: Writing – review & editing, Data curation. **Nathan Basiliko:** Writing – review & editing, Data curation. **John Hribljan:** Writing – review & editing, Data curation. **Rodney A. Chimner:** Writing – review & editing, Methodology. **Louis J. Lamit:** Writing – review & editing, Software, Methodology, Data curation. **Angela Lafuente:** Writing – review & editing, Writing – original draft, Formal analysis, Data curation. **Erik A. Lilleskov:** Writing – review & editing, Supervision, Methodology, Funding acquisition, Conceptualization.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Erik Lilleskov reports financial support was provided by National Science Foundation. Erik Lilleskov reports equipment, drugs, or supplies was provided by DOE Joint Genome Institute. Erik Lilleskov reports financial support was provided by USAID. Erik Lilleskov reports financial support, administrative support, equipment, drugs, or supplies, and statistical analysis were provided by USDA Forest Service Northern Research Station. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We thank A. Bales, K. Beesley, M. Behrmann, S. Harttung, S Kelso, E. Matthys, L. Merchán, AM Rozo, R. Schwartz, L. Theobold and C. Tucker for their help in the field and lab analyses. We are grateful to Shaun Watmough (School of the Environment, Trent University, Canada), Graeme Spiers (Laurentian University, Canada), and their respective lab members who contributed to the analyses of the peat chemistry through the Global Peatland Microbiome Project. This work was supported by the USDA Forest Service Northern Research Station, the US National Science Foundation (DEB-1 146 149), the USAID-funded Sustainable Wetlands Adaptation and Mitigation Program (SWAMP), and the U.S. Department of Energy Joint Genome Institute Community Science Program (Proposal ID 1445). The work conducted by the U.S. Department of Energy Joint Genome Institute, a DOE Office of Science User Facility, was supported by the Office of Science of the U.S. Department of Energy under Contract no. DE-AC02-05CH11231.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.pedobi.2025.151093.

Data availability

Data in support of these finding are available on Figshare at Lafuente et al. (2023) and gene data at JGI.

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