

Supplementary Material

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

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1 Supplementary Discussion

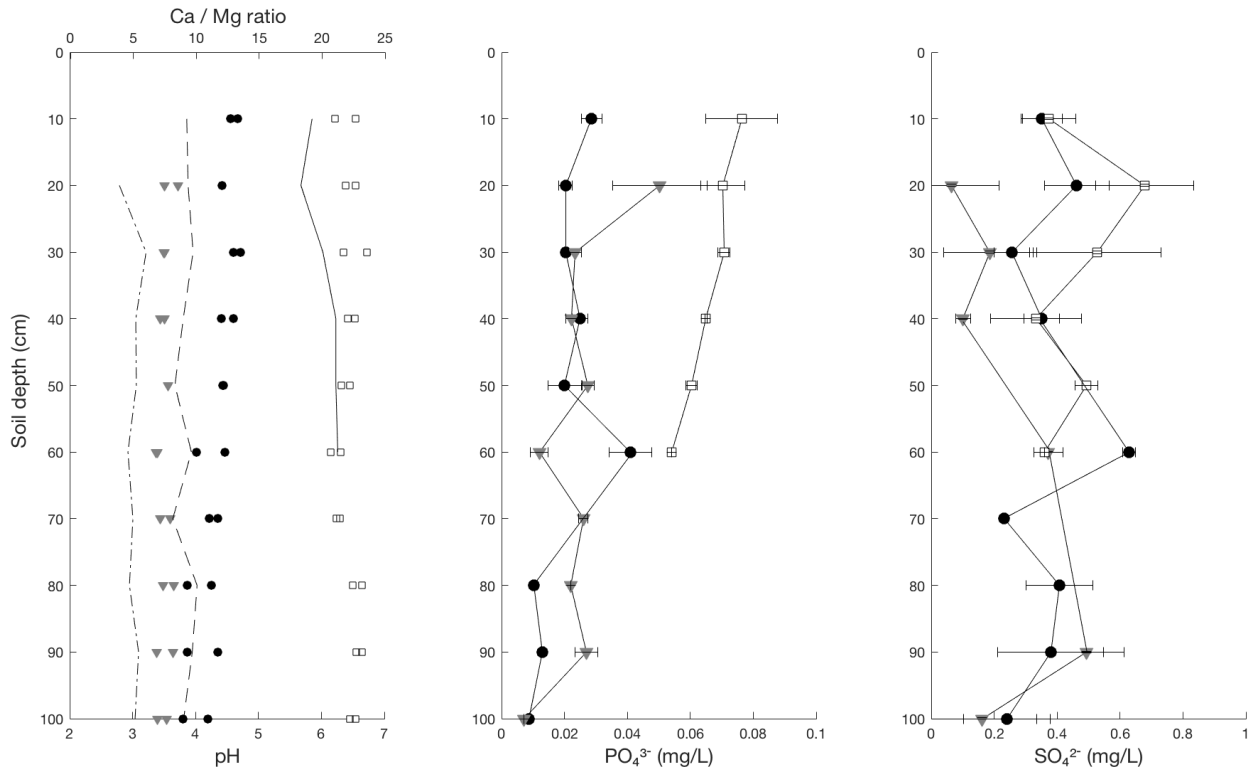
Dissolved organic carbon (DOC) profiles. DOC has emerged as an important predictor of CH₄ production in wetland soils (Liu et al., 2012; Morrissey et al., 2013). In comparison, DOC concentrations obtained in this study are at the lower end of values previously reported from Borneo peats (Gandois et al., 2014). All concentration profiles appear stable along soil depth with no indication of sinks. These would conceivably occur in more shallow regions where the fraction of refractory soil carbon is less prevalent (Artz et al., 2006). A DOC sink due to mineralization is not present based on our data. The DOC pool is comprised of diverse organic molecules characterized by a wide molecular size range, organic acids, humic substances of different aromaticities, and protein groups. These compounds may vary with depth even though the bulk concentration may be stagnant. Extracellular enzymes and humic substances may play a crucial role in diverting electron flow from methanogenesis, given the thermodynamic preference over CO₂ (Heitmann et al., 2007; Knorr and Blodau, 2009), that would ultimately suppress CH₄ production. Based on our results, we can exclude fluctuations of the total DOC pool to have major effects on carbon mineralization in distinct soil layers, but organic compounds could structurally differ along depth and impact specific microbial activities.

***Xanthobacteriaceae* and Bathyarchaeota are potentially important decomposers of organic matter in tropical peat soils.** The high abundance of *Xanthobacteriaceae* and Bathyarchaeota (Fig. S3) across sites is likely related to a putative role as degraders of peat organic matter. Particularly under O₂-limiting conditions, *Xanthobacteriaceae* have a broad suite of organic substrates, including alkanes, alkenes, (poly)aromatic compounds, thiopenes, organic acids, or xylose and xylan (Zaichikova et al., 2010). Those substrates are common and accumulate in peat soils

(Moers et al., 1990). Bathyarchaeota is an abundant group likely to have been overlooked by the lack of recognition of the phyla in older databases (as in SILVA 115 or priors; (Bai et al., 2018), or because several primers are incapable of amplification (like the *mcrA* set in this study that has 10-18 base pair mismatches; (Evans et al., 2015)). The genetic potential of Bathyarchaeota includes enzymes to hydrolyze plant-derived carbohydrates and detrital proteins, and to produce acetate for energy conservation (Lazar et al., 2016). A putative relationship Bathyarchaeota-NO_x is supported by a report indicating that NO₂⁻/NO₃⁻ transporter proteins, an enzymatic set to support DNRA, were detected in reconstructed Bathyarchaeota metagenomes (Lazar et al., 2016). PC analysis indicates such interactions, where Bathyarchaeota diversity, pH and NO_x concomitantly explain over 50% of data variation along PC 1 (Fig. 2B).

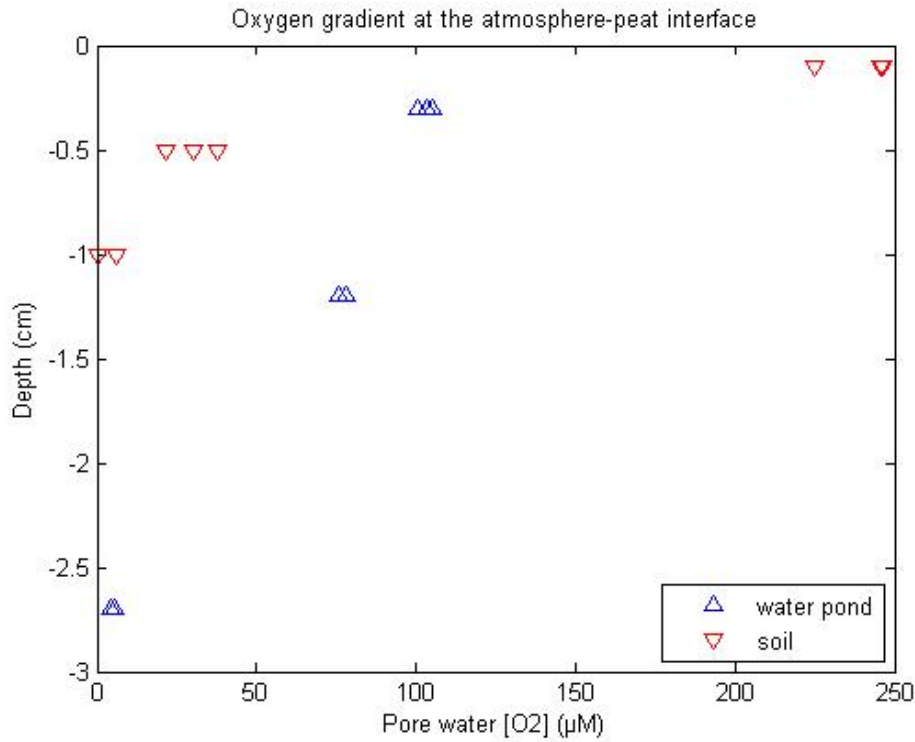
2 Supplementary Figures and Tables

2.1 Supplementary Figures



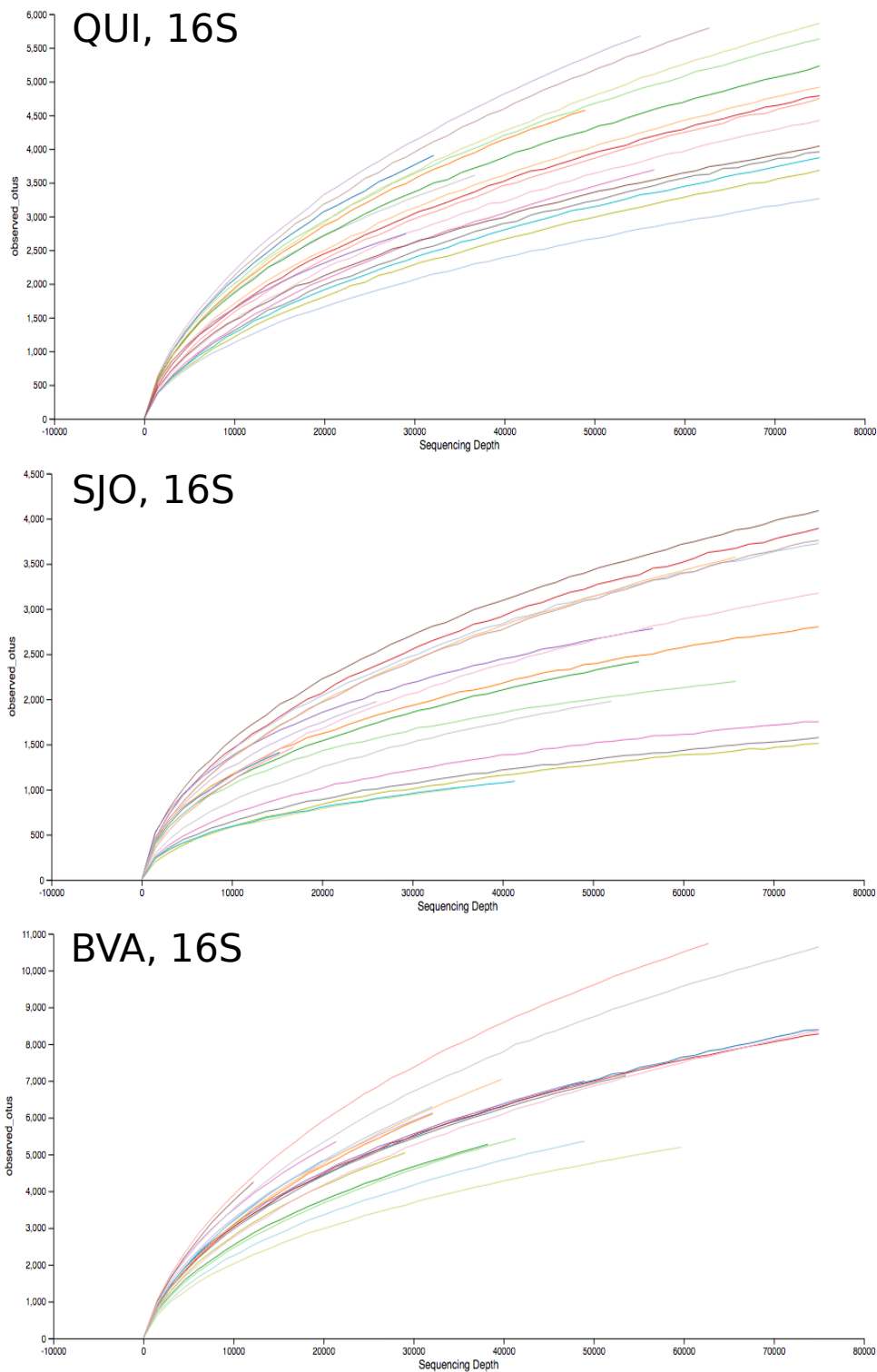
Supplementary Figure 1. Left panel: Ca/Mg ratio in lines for QUI (dashed), BVA (solid), and SJO (point-dashed). pH in symbols for QUI (circles), SJO (triangles), and BVA (squares). Each point is derived from one replicate soil core. **Middle panel:** PO₄³⁻ concentration with data points representing the mean of values from replicate soil cores. **Right panel:** SO₄²⁻ concentration with data points representing the mean of values from replicate soil cores. Error bars denote one *SD* and symbols are consistent with left panel.

Pore water of SJO strongly resembles rainwater composition (Ca/Mg mean of ~8.8, Honório et al., 2010). BVA is likely influenced by groundwater (vertical or lateral water movements) that provides the peat body with minerals. Minerotrophic peatlands have a mineral-derived alkalinity that counteracts acidification caused by the degradation of plant litter and the release of organic acids. Ombrotrophic peatlands are characterized by a low basic cation content that lacks buffer capacity. BVA is a minerotrophic site, where as QUI is transitional minerotrophic-ombrotrophic. SJO is strictly ombrotrophic and the convex shape of this peatland further induces a barrier for groundwater to reach top soil layers (Lähteenoja et al., 2009).

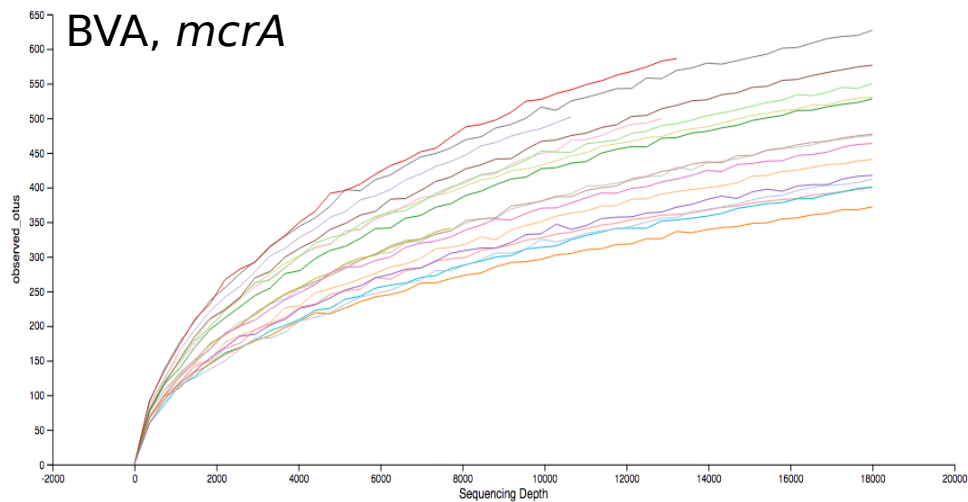
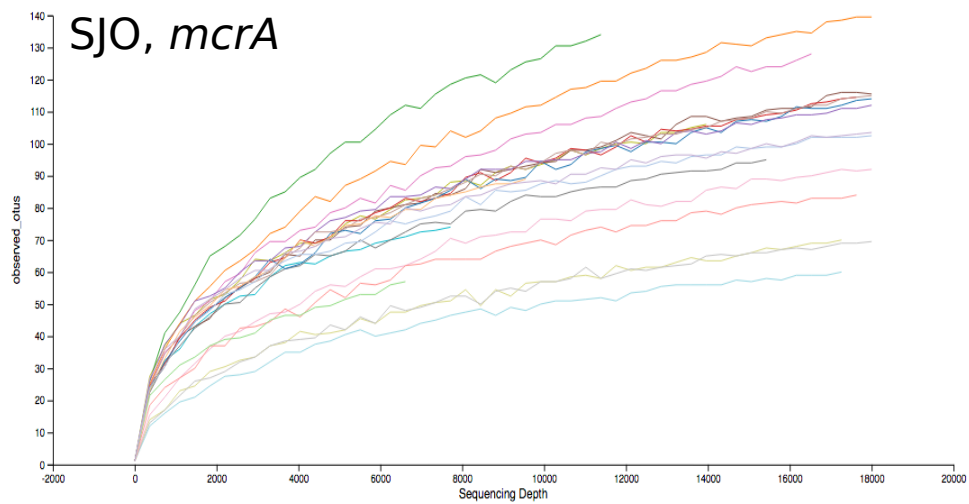
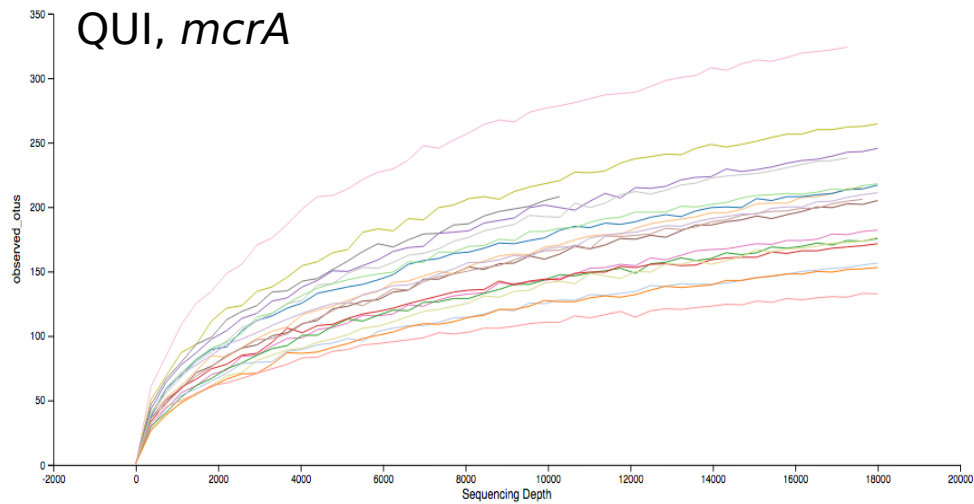


Supplementary Figure 2. Dissolved oxygen concentration in water of saturated soil.

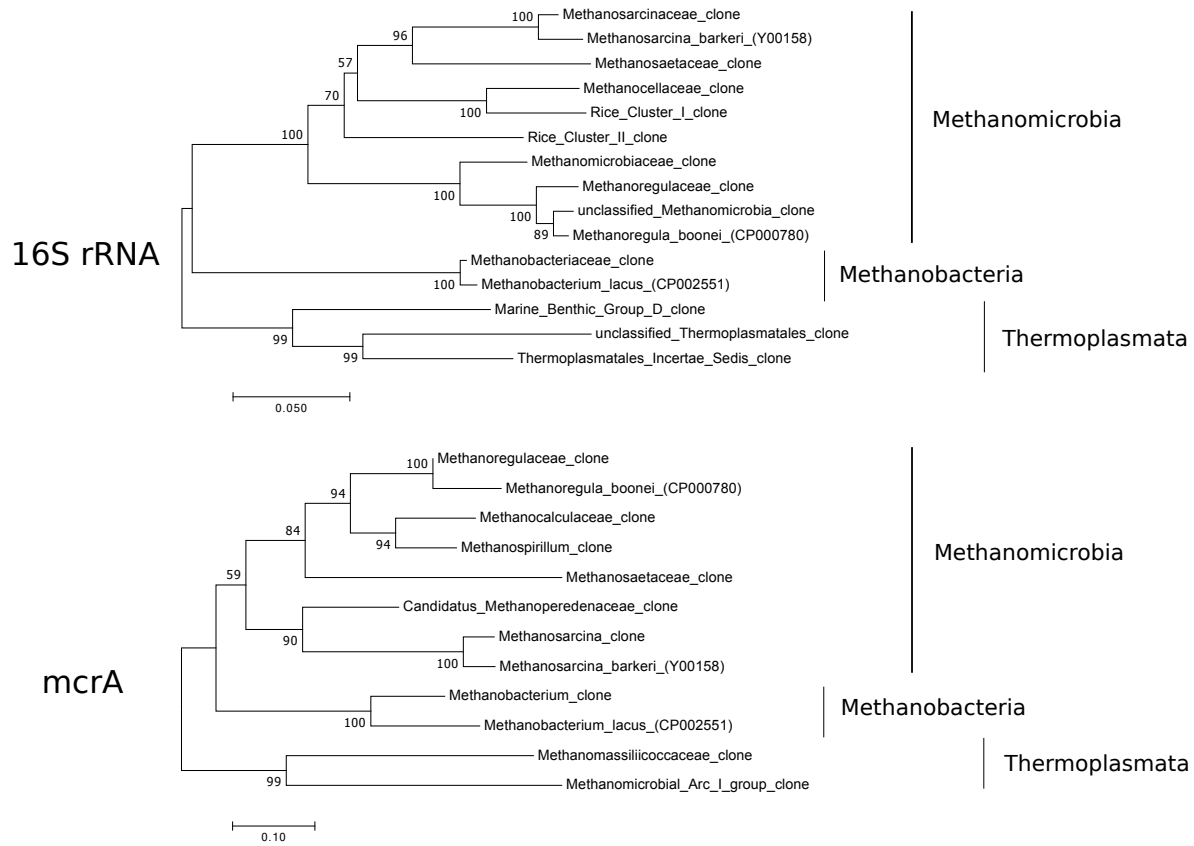
Measurements were done using a microelectrode, which was either inserted directly into soft peat (red symbols) or into a shallow (~3 cm) water pond (blue symbols). Measurements were conducted in QUI peatland showing O₂ concentrations drop below 1 µM detection levels after 1 or 2.7 cm depths.



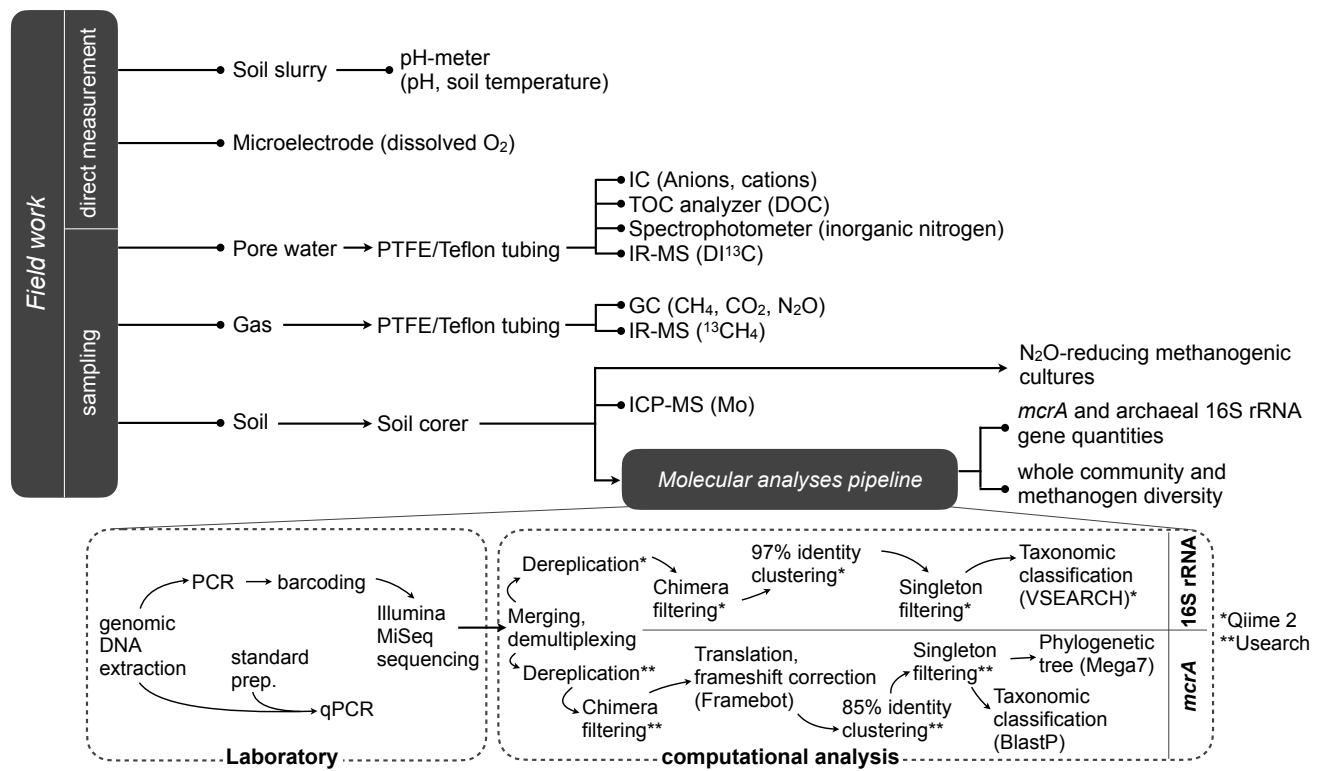
Supplementary Figure 3. Alpha-rarefaction curves for 16S rRNA sequences, used to adjust for variability in library size across samples. Rarefaction curves were used to determine the minimum number of reads per sample used for alpha diversity metrics.



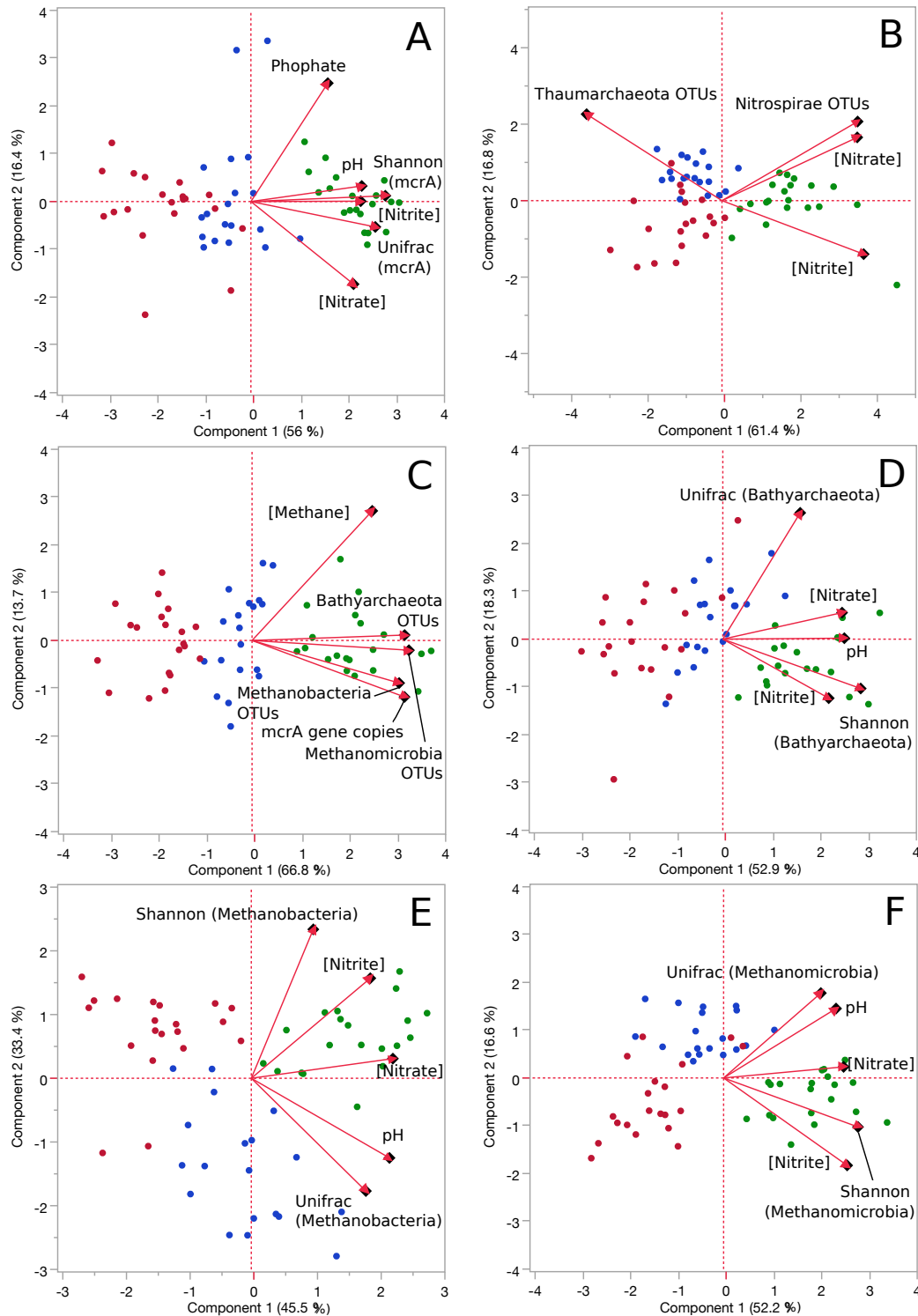
Supplementary Figure 3 continued. Alpha-rarefaction curves for *mcrA* sequences, used to adjust for variability in library size across samples. Rarefaction curves were used to determine the minimum number of reads per sample used for alpha diversity metrics. Samples were rarefied to 20,000 reads per sample (Yang et al., 2017).



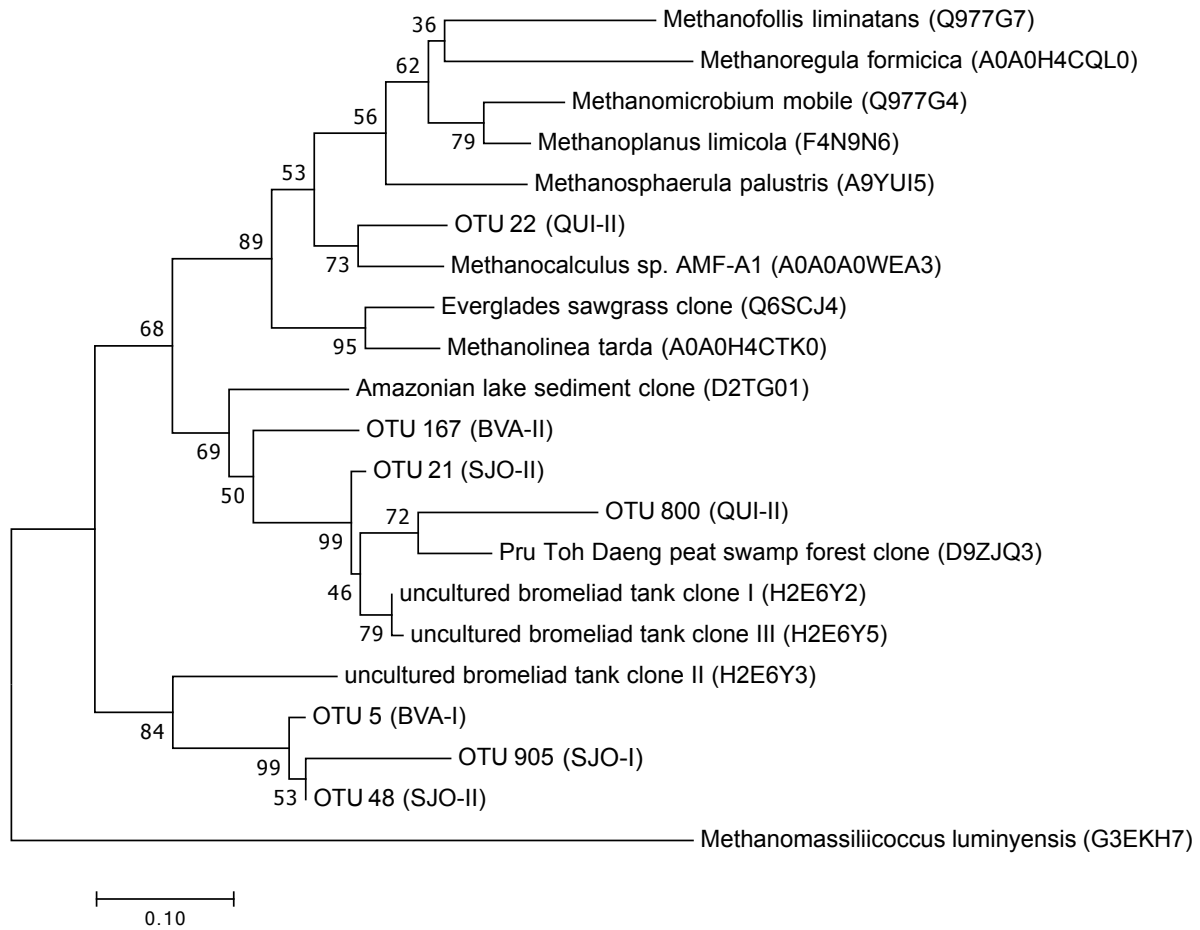
Supplementary Figure 4. 16S rRNA and *mcrA* phylogenetic trees based on the neighbor-joining method and 100 iterations. The trees reflect congruency of classified sequences from 16S and *mcrA* amplicon sequencing.



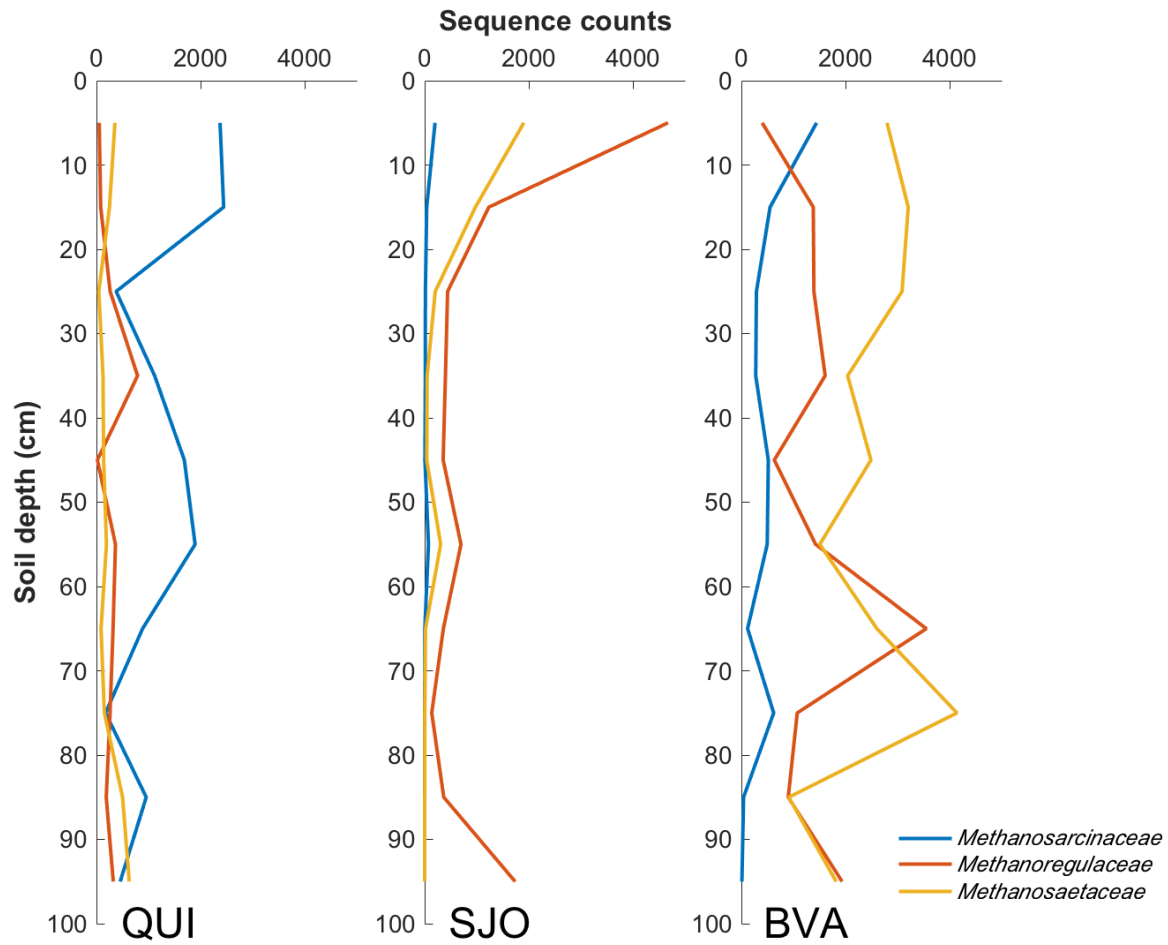
Supplementary Figure 5. Workflow overview including the molecular analysis pipeline.



Supplementary Figure 6. Principal component Analysis (PCA) ordination plots of microbial and environmental data from three soil profiles of contrasting Amazon peatlands. Samples were derived from QUI (triangles), SJO (circles) and BVA (squares) with abbreviations as in Fig. 1. Extended environmental variables or microbial groups were evaluated against overall microbial communities variation. Figure 3 in main text is identical to panels C and D.



Supplementary Figure 7. Neighbor-joining (1000 iterations) phylogenetic analysis of selected *Methanomicrobiales mcrA* sequences from three Amazon peatlands. Reference sequences were picked from environmental data derived from tropical peatlands, Amazon lakes (Conrad et al., 2010), and tropical vegetation (Goffredi et al., 2011), and from isolates for which the accession number is provided.



Supplementary Figure 8. Distribution of raw *mcrA* sequence counts along soil profiles. QUI: Quistococha, BVA: Buena Vista and SJO: San Jorge

2.2 Supplementary Tables

Supplementary Table 1. OTU abundances and microbial family metabolic capacity and traits.

Taxon	Core	Mean relative abundance along depth (%)	N metabolic reaction	Potential syntrophy with methanogen	Reference
<i>Acido-bacteriaceae</i>	QUI-1	5.0	N respiration not a dominant feature of this family.		Rosenberg et al., 2014
	QUI-2	3.5			
	SJO-1	8.3			
	SJO-2	6.0			
	BVA-1	0.7			
	BVA-2	0.7			
<i>Solibacteraceae</i>	QUI-1	1.3	N respiration not a dominant feature of this family.		Eichorst et al., 2011
	QUI-2	0.8			
	SJO-1	2.3			
	SJO-2	1.3			
	BVA-1	0.3			
	BVA-2	0.2			
<i>Acido-thermaceae</i>	QUI-1	1.3	Denitrification (<i>Acidothermus</i>) not verified in culture.		Barta et al., 2017 Mohagheghi et al., 1986
	QUI-2	0.5			
	SJO-1	1.6			
	SJO-2	1.4			
	BVA-1	0.1			
	BVA-2	0.1			
<i>Bacillaceae</i>	QUI-1	8.4	Denitrification, DNRA and sole N ₂ O reduction (<i>Bacillus</i> and <i>Geobacillus</i>). Nitrate reduction verified in culture.		Mandić-Mulec et al., 2015
	QUI-2	2.1			
	SJO-1	0.0			
	SJO-2	0.0			
	BVA-1	0.2			
	BVA-2	0.2			
<i>Paeni-bacillaceae</i>	QUI-1	2.7	Nitrate reduction and N fixation (<i>Paenibacillus</i>). Nitrate reduction verified in culture.		Yoon, 2003 Beneduzi et al., 2010
	QUI-2	0.1			
	SJO-1	0.0			
	SJO-2	0.0			
	BVA-1	0.9			
	BVA-2	0.6			

Supplementary Table 1 continued

Taxon	Core	Mean relative abundance along depth (%)	N metabolic reaction	Potential syntrophy with methanogen	Reference
<i>Nitrospiraceae</i>	QUI-1	0.5	Nitrite oxidation (<i>Nitrospira</i>), Fe ²⁺ oxidation (<i>Leptosprillum</i>). Nitrite oxidation verified in culture.		Rosenberg et al., 2014
	QUI-2	0.5			
	SJO-1	0.7			
	SJO-2	0.9			
	BVA-1	3.7			
	BVA-2	3.3			
<i>Planctomycetaceae</i>	QUI-1	3.0	Anammox (Cand. Anammoximicrobium), Nitrate reduction (<i>Planctomyces</i> , <i>Rhodopirellula</i>). Nitrate reduction verified in culture.		Khramenkov et al., 2013
	QUI-2	1.6			
	SJO-1	1.9			
	SJO-2	1.9			Bondoso et al., 2014
	BVA-1	2.2			
	BVA-2	2.1			
<i>Bradyrhizobiaceae</i>	QUI-1	0.1	Nitrite oxidation (<i>Nitrobacter</i>), N fixation (<i>Bradyrhizobium</i>). N mineralization and nitrate production activity verified in culture.		Zahran, 1999 Rosenberg et al., 2014
	QUI-2	0.1			
	SJO-1	0.2			
	SJO-2	0.1			
	BVA-1	0.2			
	BVA-2	0.1			
<i>Hyphomicrobiaceae</i>	QUI-1	0.5	Denitrification (<i>Hyphomicrobium</i>). Nitrate reduction verified in culture.		Martineau et al., 2015
	QUI-2	0.3			
	SJO-1	0.1			
	SJO-2	0.6			
	BVA-1	0.9			
	BVA-2	0.7			
<i>Xanthobacteraceae</i>	QUI-1	2.3	N fixation.		Rosenberg et al., 2014
	QUI-2	1.7			
	SJO-1	4.1			
	SJO-2	5.4			
	BVA-1	2.0			
	BVA-2	1.9			

Supplementary Table 1 continued

Taxon	Core	Mean relative abundance along depth (%)	N metabolic reaction	Potential syntrophy with methanogen	Reference
<i>Rhodo-spirillaceae</i>	QUI-1	0.5	N fixation (<i>Azospirillum</i>),		Rosenberg et al., 2014
	QUI-2	0.4	Nitrate reduction		
	SJO-1	0.6	(<i>Azospirillum</i> ,		
	SJO-2	0.5	<i>Magneto-spirillum</i>), N ₂ O		
	BVA-1	0.2	reduction (<i>Azospirillum</i>).		
	BVA-2	0.2	Nitrate reduction verified in culture.		
<i>Alcaligenaceae</i>	QUI-1	0.2	Nitrate reduction (<i>Candidimonas</i>),		Rosenberg et al., 2014 Vaz-Moreira et al., 2011
	QUI-2	0.2	Nitrite reduction (<i>Alcaligenes</i>).		
	SJO-1	0.5	Nitrate reduction		
	SJO-2	0.4	verified in culture.		
	BVA-1	0.0			
	BVA-2	0.0			
<i>Burkholderiaceae</i>	QUI-1	0.3	Denitrification.		Rosenberg et al., 2014
	QUI-2	1.6	Nitrate reduction verified in culture.		
	SJO-1	18.6			
	SJO-2	11.1			
	BVA-1	0.2			
	BVA-2	0.2			
<i>Comamonadaceae</i>	QUI-1	0.0	Nitrate reduction (<i>Comamonas</i> ,		Rosenberg et al., 2014
	QUI-2	0.0	<i>Brachymonas</i> ,		
	SJO-1	0.4	<i>Diaphorobacter</i>).		
	SJO-2	0.0	Nitrate reduction		
	BVA-1	1.1	verified in culture.		
	BVA-2	1.2			
<i>Neisseriaceae</i>	QUI-1	0.0	Nitrite and NO		Rock et al., 2005
	QUI-2	0.0	reduction (<i>Neisseria</i>).		
	SJO-1	1.9	Nitrite reduction		
	SJO-2	0.0	verified in culture.		
	BVA-1	0.2			
	BVA-2	0.3			

Supplementary Table 1 continued

Taxon	Core	Mean relative abundance along depth (%)	N metabolic reaction	Potential syntrophy with methanogen	Reference
<i>Nitroso-monadaceae</i>	QUI-1	0.1	Ammonia oxidation. Verified in culture.		Rosenberg et al., 2014
	QUI-2	0.0			
	SJO-1	0.0			
	SJO-2	0.0			
	BVA-1	0.8			
	BVA-2	0.7			
<i>Desulfurellaceae</i>	QUI-1	0.5		H ₂ consumption and syntrophic association with methanogen? Competition possible. Propionate oxidation and syntrophy with methanogens. Syntrophy studied in cultures.	Miroshnichenko et al., 1998
	QUI-2	0.4			
	SJO-1	0.9			
	SJO-2	0.5			
	BVA-1	0.8			
	BVA-2	0.7			
<i>Syntrophaceae</i>	QUI-1	1.0		Fermenter or syntrophic association with H ₂ /formate-utilizing partners. Syntrophy studied in cultures.	Lueders et al., 2003
	QUI-2	1.2			
	SJO-1	0.6			
	SJO-2	0.8			
	BVA-1	2.1			
<i>Syntrophobacteraceae</i>	BVA-2	1.7			Rosenberg et al., 2014
	QUI-1	0.8			
	QUI-2	0.6			
	SJO-1	1.1			
	SJO-2	0.8			
	BVA-1	0.4			
<i>Pseudomonadaceae</i>	BVA-2	0.3	Denitrification (<i>Pseudomonas</i>). Nitrate and nitrite reduction verified in culture.		Rosenberg et al., 2014
	QUI-1	2.2			
	QUI-2	1.9			
	SJO-1	0.0			
	SJO-2	0.0			
	BVA-1	1.0			
BVA-2	1.3				

Supplementary Table 1 continued

Taxon	Core	Mean relative abundance along depth (%)	N metabolic reaction	Potential syntrophy with methanogen	Reference
<i>Spirochaetaceae</i>	QUI-1	0.8		Fermenter with known	Troshina et al., 2015
	QUI-2	0.7		syntrophic association	
	SJO-1	0.5		with	
	SJO-2	0.6		methano-	
	BVA-1	1.0		gens (<i>Sphaerochaeta</i>). Syntrophy studied in cultures.	
<i>Opitutaceae</i>	BVA-2	1.0		Propionate production	Chin et al., 2001
	QUI-1	0.1	N fixation	(<i>Diplosphaera</i>)	
	QUI-2	0.1	and nitrate	reduction	
	SJO-1	0.1	(<i>Opitutus</i>).	Syntrophy not assessed	
	SJO-2	0.1	Nitrate reduction	in culture.	
	BVA-1	0.0	verified in	culture.	
BVA-2	0.1				

Supplementary Table 2. Spearman's rank multivariate analysis of geochemical parameters (pH and concentration of different species) and selected methanogen taxa. Increasing positive correlation is indicated by blue text color and increasing negative correlation is indicated by red text color. Correlation of a parameter with itself = 1.0.

<i>Quistococha (QUJ)</i>	pH	Phosphate	Nitrite	Nitrate	Ammonium	Sulfate	Methanobacteriaceae	Methanoregulaceae	Methanosaeataceae	Methanosarcinaceae	Methanocellaceae	Methanomassiliococcaceae
pH	1.0000	-0.0301	-0.0840	0.4560	-0.4968	-0.3888	0.1907	-0.3200	-0.4336	0.4939	-0.3586	0.3818
Phosphate	-0.0301	1.0000	-0.0118	-0.2441	0.3215	-0.0392	-0.0514	0.1474	0.3532	-0.0640	-0.0196	-0.2264
Nitrite	-0.0840	0.0118	1.0000	0.0845	0.1631	-0.1345	0.4523	-0.1629	0.0762	0.3139	-0.0765	0.3098
Nitrate	0.4560	-0.2441	0.0845	1.0000	-0.3187	-0.3320	0.0627	-0.1910	-0.1808	0.0347	-0.2097	0.0853
Ammonium	-0.4968	0.3215	0.1631	-0.3187	1.0000	0.5091	-0.3472	0.3686	0.4532	-0.3578	-0.0966	-0.4121
Sulfate	-0.3888	-0.0392	-0.1345	-0.3320	0.5091	1.0000	-0.0674	0.0874	0.4966	-0.1086	-0.0606	-0.0065
Methanobacteriaceae	0.1907	-0.0514	0.4523	0.0627	-0.3472	-0.0674	1.0000	-0.3189	0.2621	0.7457	-0.0584	0.8491
Methanoregulaceae	-0.3200	0.1474	-0.1629	-0.1910	0.3686	0.1930	-0.3189	1.0000	0.1815	-0.2867	0.1087	-0.4746
Methanosaeataceae	-0.4336	0.3532	0.0762	-0.1808	0.4532	0.4966	0.2621	0.1815	1.0000	0.0793	-0.1805	-0.0388
Methanosarcinaceae	0.4939	-0.0640	0.3139	0.0347	-0.3578	-0.1086	0.7457	-0.2867	-0.1805	1.0000	-0.3540	0.7585
Methanocellaceae	-0.3586	-0.0196	-0.0765	-0.2097	-0.0966	-0.0606	-0.0584	0.1087	-0.3540	0.0793	1.0000	-0.1510
Methanomassiliococcaceae	0.3818	-0.2264	0.3098	0.0853	-0.4121	-0.0065	0.8491	-0.4746	-0.0388	0.7585	-0.1510	1.0000
<i>San Jorge (SJO)</i>												
pH	1.0000	0.3968	0.0302	-0.0287	-0.1371	0.2855	-0.1593	-0.3097	-0.0479	-0.2067	-0.1164	-0.0454
Phosphate	0.3968	1.0000	0.0339	-0.1553	-0.1813	0.4215	-0.3666	-0.3588	-0.1796	-0.1216	-0.0081	-0.1393
Nitrite	0.0302	0.0339	1.0000	0.0457	-0.4562	-0.6845	0.0284	0.0529	0.2279	0.0262	0.0845	0.0646
Nitrate	-0.0287	-0.1553	0.0457	1.0000	-0.4835	-0.2225	0.0737	0.2166	0.6195	0.7156	0.4142	0.6312
Ammonium	-0.1371	-0.1813	-0.4562	-0.4835	1.0000	0.0822	-0.0870	-0.2831	-0.4339	-0.4213	-0.3646	-0.3895
Sulfate	0.2855	0.4215	-0.6845	-0.2225	0.0822	1.0000	-0.2405	-0.0831	-0.2052	-0.1255	0.1085	0.1016
Methanobacteriaceae	-0.1593	-0.3666	0.0284	0.0737	-0.0870	-0.2405	1.0000	0.3381	0.1431	0.0788	0.0443	0.0312
Methanoregulaceae	-0.3097	-0.3588	0.0529	0.2166	-0.2831	-0.0831	0.3381	1.0000	0.8278	0.7137	0.8891	0.8934
Methanosaeataceae	-0.0479	-0.1796	0.2279	0.6195	-0.4339	-0.2052	0.1431	0.8278	1.0000	0.8978	0.9441	0.9523
Methanosarcinaceae	-0.2067	-0.1216	0.0262	0.7156	-0.4213	-0.1255	0.0788	0.7137	0.8978	1.0000	0.8211	0.9109
Methanocellaceae	-0.1164	-0.0081	0.0845	0.4142	-0.3646	0.1085	0.0443	0.8891	0.9441	0.8211	1.0000	0.8918
Methanomassiliococcaceae	-0.0454	-0.1393	0.0646	0.6312	-0.3895	0.0106	0.0312	0.8934	0.9523	0.9109	0.8918	1.0000
<i>Buena Vista (BVA)</i>												
pH	1.0000	0.4727	0.4142	0.1493	-0.5684	-0.1202	-0.2784	-0.2500	-0.0645	-0.2370	-0.3571	-0.0748
Phosphate	0.4727	1.0000	-0.2895	-0.2556	-0.2537	0.2779	-0.0417	0.5263	0.2637	-0.2523	-0.5575	0.3445
Nitrite	0.4142	-0.2895	1.0000	0.0650	-0.2061	-0.3836	0.0358	-0.7433	-0.3041	0.1434	0.2857	-0.3409
Nitrate	0.1493	-0.2556	0.0650	1.0000	-0.1896	-0.0061	0.4202	-0.0995	0.2631	0.5589	0.6293	0.2669
Ammonium	-0.5684	-0.2537	-0.2061	-0.1896	1.0000	-0.4134	-0.1979	-0.4331	-0.3100	-0.3100	-0.2903	-0.4689
Sulfate	-0.1202	0.2779	-0.3836	-0.0061	-0.4134	1.0000	-0.0491	0.3826	0.3345	-0.3075	-0.1694	-0.4689
Methanobacteriaceae	-0.2784	-0.0417	0.0358	0.4202	-0.1979	-0.0491	1.0000	-0.2560	0.7044	0.8408	-0.3427	0.6608
Methanoregulaceae	-0.2500	0.5263	-0.7433	-0.0995	0.1439	0.3826	-0.2560	1.0000	0.1582	-0.3427	-0.3952	0.1766
Methanosaeataceae	-0.0645	0.2637	-0.3041	0.2631	-0.4331	0.3345	0.7044	0.1582	1.0000	0.4764	0.3952	0.8377
Methanosarcinaceae	-0.2370	-0.2523	0.1434	0.5589	-0.3100	-0.3075	0.8408	-0.3427	0.4764	1.0000	0.8553	0.5254
Methanocellaceae	-0.3571	-0.5575	0.2857	0.6293	-0.2903	-0.1694	0.7744	-0.3395	0.3952	0.8553	1.0000	0.3838
Methanomassiliococcaceae	-0.0748	0.3445	-0.3409	0.2669	-0.4689	0.3492	0.6608	0.1766	0.8377	0.5254	0.3838	1.0000

3 Supplementary References

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