

Supplemental Material

Anaerobic methane oxidation driven by microbial reduction of natural organic matter in a tropical wetland

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Running title: Anaerobic methane oxidation linked to humus reduction

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Methods

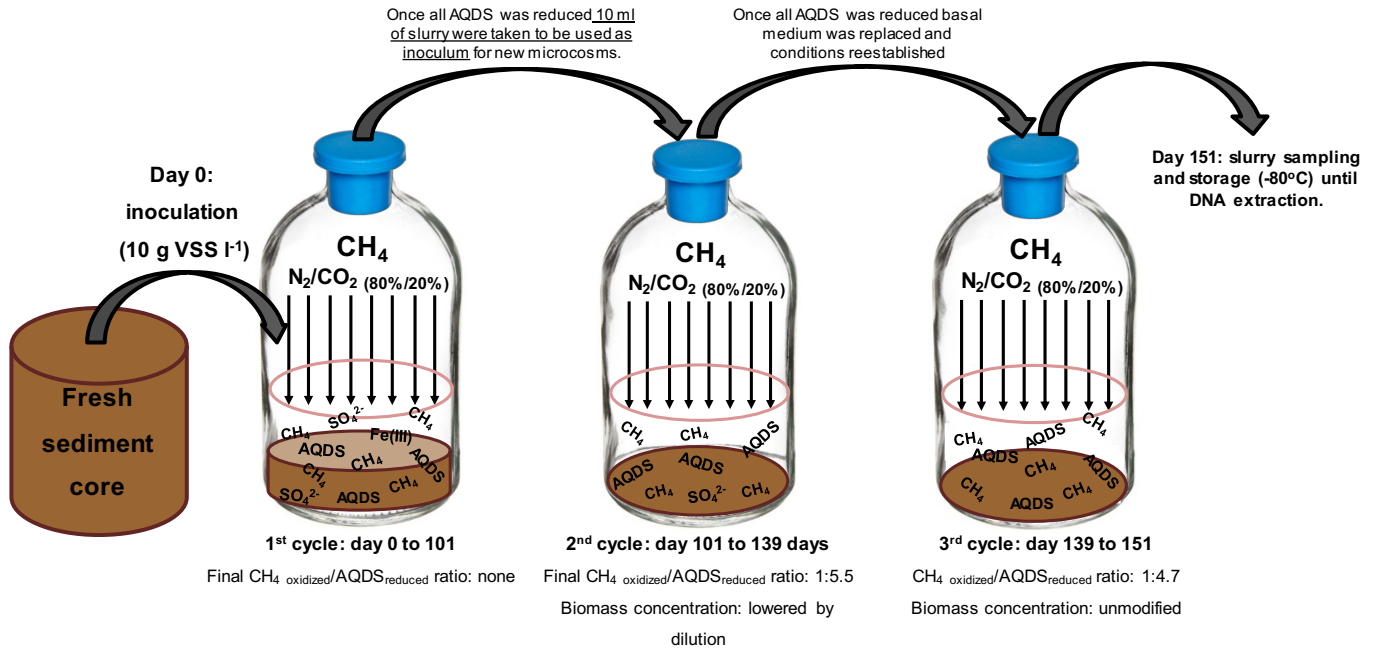


Fig. S1. Schematic representation of sequential incubation cycles for AQDS dependent methanotrophic activity.

Results

Table S1. Sediment and water characterization

	Column water	Pore water	Sediment
Sulfate (mM)	2.81±0.4	6.7±0.8	NA
Sulfide (mM)	2.07±0.19	6.18±0.22	NA
Nitrate (mM)	UD	1.75±0.06	NA
Nitrite (mM)	UD	UD	NA
Total iron (mg g_{dry sediment}⁻¹)	UD	UD	0.26±0.02
Total manganese (mg g_{dry sediment}⁻¹)	UD	UD	0.0067
TOC* (mg l⁻¹)	35.45±0.72	148.73±2.26	NA
TIC** (mg l⁻¹)	39.85±1.05	185.07±0.68	NA
TC (mg_{Carbon} g_{dry sediment}⁻¹)	NA	NA	80.35±4
TIC (mg_{Inorg. Carbon.} g_{dry sediment}⁻¹)	NA	NA	43.97±2.1
Volatile solids (%)	NA	NA	11%
Sodium (mg l⁻¹)	2006.7±11.5	3914.37±37	NA
Chloride (mg l⁻¹)	4361.11± 642.1	7506.24±607.9	NA

UD, undetectable; NA, not applicable. *total organic carbon measured in liquid phase,

**total inorganic carbon measured in liquid phase.

Table S2. Contribution of different electron acceptors on anaerobic oxidation of methane in wetland sediment incubations at the end of exponential phase (20 days of incubation)

Treatments	% of AOM linked to SO₄²⁻ reduction	% of AOM linked to Fe³⁺ reduction	% of AOM linked to HS or NOM reduction
With Pahokee Peat HS	-	-	7±0.4
With Pahokee Peat HS and molybdate	-	-	10±0.5
sediment only	42.5±2	0.46±0.05	-
sediment and molybdate	16±0.8	-	-

Percentage of ¹³C-methane oxidation linked to the reduction of different electron acceptors in sediment were quantified by dividing the amount of reduced terminal electron acceptors by the amount of ¹³CH₄ oxidized (calculated from ¹³CO₂ production), both corrected for endogenous controls. Stoichiometric relationships (1:1 for sulfate, and 1:8 for iron and HS or NOM) were considered.

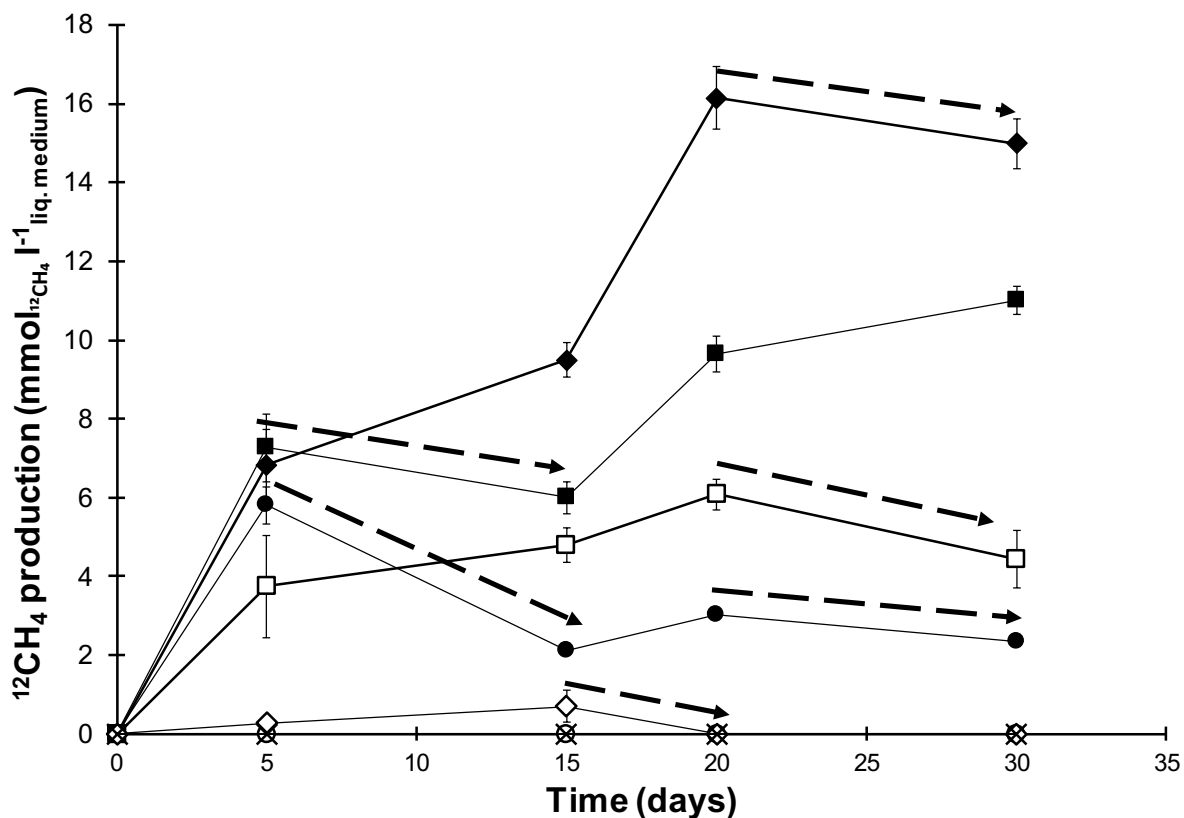



Fig S2. Produced $^{12}\text{CH}_4$ in wetland sediment microcosms. Filled symbols represent experiments amended with external NOM as Pahokee Peat HS: squares (■) represent microcosms amended with $^{13}\text{CH}_4$ (n=4), circles (●) represent $^{13}\text{CH}_4$ free microcosms (endogenous controls, n=4) and diamonds (◆) represent sulfate-reduction inhibited controls (n=4). In the same way, open symbols represent $^{13}\text{CH}_4$ added (□, n=4), endogenous (○, n=3) and sulfate-reduction inhibited controls (◇, n=3) without addition of external HS. Sterile controls are represented by crosses (×). Dashed arrows emphasize negative slopes, implying consumption of produced $^{12}\text{CH}_4$ eventually contributing to substantial reduction of intrinsic and added electron acceptors. Error bars represent the standard error among replicates.

Phylogenetic characterization of wetland sediment samples performing AOM

Taxonomy			Treatment				Capabilities			
Phylum	Order	Family	¹³ C-methane	HS enriched	HS + ¹³ C-methane	HS + ¹³ C-methane + SR-INH	HS or quinones reduction	Sulfate reduction	Iron reduction	Fermentative
<i>Actinobacteria</i>	<i>Acidimicrobia</i>	OM1_clade	0.3	1.0	0.4	0.5			• ³	
<i>Aminicenantes</i>	unclassified	unclassified	0.5	2.0	0.9	0.9				
<i>Atribacteria</i>	unclassified	unclassified	0.2	1.2	0.5	0.7				• ⁴
<i>Bacteroidetes</i>	<i>Bacteroidetes_BD2-2</i>	unclassified	0.5	2.3	0.9	1.1				
	<i>Bacteroidia</i>	<i>Draconibacteriaceae</i>	0.1	5.6	1.9	0.3				
<i>Bacteroidetes</i>	<i>Cytophagia</i>	<i>Cytophagaceae</i>	0.0	1.1	0.5	0.3				
<i>Chloroflexi</i>	<i>Anaerolineae</i>	<i>Anaerolineaceae</i>	2.3	4.8	2.6	3.3				• ⁵
	<i>Dehalococcoidia</i>	unclassified	0.7	2.4	1.0	0.9				
<i>Deferribacteres</i>	<i>Deferribacteres_Incertae_Sedis</i>	Unknown_Family	0.4	1.1	0.4	0.4			• ⁶	
<i>Firmicutes</i>	<i>Bacilli</i>	<i>Bacillaceae</i>	16.3	0.8	0.3	2.3	• ^{1,2}			• ⁷
		Family_XII	0.4	5.3	1.9	2.1	• ¹			• ⁷
		<i>Planococcaceae</i>	23.9	0.1	0.1	20.0	• ¹			• ⁷
	<i>Clostridia</i>	<i>Clostridiaceae_4</i>	0.1	0.9	0.4	1.6	• ^{1,2}			• ⁷
		Family_XII	0.2	14.8	6.9	28.6	• ^{1,2}			• ⁷
		<i>Ruminococcaceae</i>	0.0	1.6	0.4	0.3	• ^{1,2}			• ⁷
<i>Gemmatimonadetes</i>	<i>Gemmatimonadetes</i>	unclassified	0.3	1.3	0.4	0.4				
<i>Latescibacteria</i>	unclassified	unclassified	0.3	1.0	0.4	0.4			• ⁸	
<i>Proteobacteria</i>	<i>Betaproteobacteria</i>	<i>Alcaligenaceae</i>	0.7	6.6	1.3	2.5	• ¹			
	<i>Deltaproteobacteria</i>	<i>Desulfarculaceae</i>	0.4	1.6	0.5	0.5		• ⁹		
		<i>Desulfobacteraceae</i>	0.2	1.1	0.3	0.3		• ⁹		
	<i>Gammaproteobacteria</i>	<i>Aeromonadaceae</i>	42.8	0.4	62.4	0.2	• ²			
		<i>Oceanospirillaceae</i>	0.2	0.2	0.2	3.0				
		<i>Pseudomonadaceae</i>	0.8	10.1	1.9	10.8	• ¹			
<i>Spirochaetae</i>	<i>Spirochaetes</i>	<i>Spirochaetaceae</i>	0.6	2.3	1.0	1.2				
Others	Others	Others	7.7	30.4	12.4	17.3				



Abundance code

Fig. S3. Bacterial community composition, and associated respiring capabilities, in wetland sediment samples performing AOM based on 16S rRNA gene libraries obtained by MiSeq ILLUMINA technology. HS stands for *humic substances*, as reference for added *Pahokee Peat* enriched microcosms, SR-INH stands for sulfate-reduction inhibited, as reference for those treatments spiked with sodium molybdate.

Estimation of suppression of methanogenesis by microbial reduction of NOM

The maximum measured AOM activity linked to microbial reduction of NOM was 100 nmol $^{13}\text{C-CH}_4$ oxidized $\text{cm}^{-3} \text{d}^{-1}$ (Fig. 1 and Fig. 2). This activity was calculated based on quantified NOM reduction linked to AOM (corrected for the endogenous control lacking $^{13}\text{CH}_4$), which was then divided by 8 (number of electrons derived from the oxidation of $^{13}\text{CH}_4$ to $^{13}\text{CO}_2$). Considering that AOM activities prevail within the top 40 cm from wetland sediments (10) and a global coastal wetland area of 48.9 Mha (11), the total coastal wetland volume in which AOM occurs is:

$$\text{Total coastal wetland volume for AOM} = 40 \text{ cm} \times 48.9 \times 10^6 \text{ ha} \times (1 \text{ cm}^2/10^{-8} \text{ ha})$$

Therefore,

$$\text{Total coastal wetland volume for AOM} = 1.956 \times 10^{17} \text{ cm}^3$$

Taking into account this total volume of coastal wetland, methane consumption by microbial reduction of NOM in coastal ecosystems is:

$$100 \text{ nmol CH}_4 \text{ cm}^{-3} \text{ d}^{-1} \times (16 \text{ ng CH}_4/1 \text{ nmol CH}_4) \times (1 \text{ Tg CH}_4/ 10^{21} \text{ ng CH}_4) \times 1.956 \times 10^{17} \text{ cm}^3 \times (365 \text{ d/yr})$$

Therefore,

$$\text{Total methane consumption in coastal wetlands by NOM reduction} = 114.23 \text{ Tg CH}_4 \text{ yr}^{-1}$$

Considering the global wetlands area of 570 Mha (12), the calculated methane suppression by this microbial process can be as follows:

$$\text{Global wetlands volume for AOM} = 40 \text{ cm} \times 570 \times 10^6 \text{ ha} \times (1 \text{ cm}^2/10^{-8} \text{ ha})$$

$$\text{Global wetlands volume for AOM} = 2.28 \times 10^{18} \text{ cm}^3$$

Taking into account this volume for global wetlands, methane consumption by microbial reduction of NOM in wetlands is:

$$100 \text{ nmol CH}_4 \text{ cm}^{-3} \text{ d}^{-1} \times (16 \text{ ng CH}_4/1 \text{ nmol CH}_4) \times (1 \text{ Tg CH}_4/ 10^{21} \text{ ng CH}_4) \times 2.28 \times 10^{18} \text{ cm}^3 \times (365 \text{ d/yr})$$

Therefore,

$$\text{Total methane consumption in wetlands by NOM reduction} = 1,331.5 \text{ Tg CH}_4 \text{ yr}^{-1}$$

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