



Anaerobic Oxidation of Methane

23 Aug ISME16 Montreal
13.30h Room 512

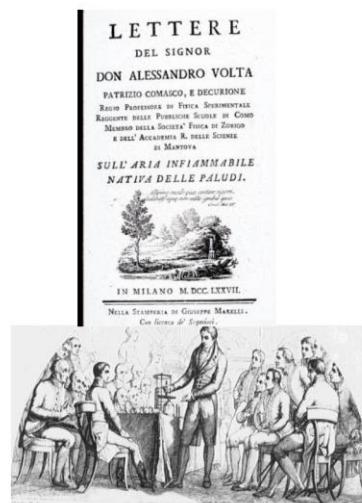
MIKE JETTEN

SOEHNGEN INSTITUTE OF ANAEROBIC MICROBIOLOGY

ERC AG 339880 EcoMoM 2014-2018
ERC PoC 713533 Initiator 2016-2017
STW 13184 CH₄ & N removal
Gravitation Grants NESSC & SIAM

Radboud University 
www.anaerobic-microbiology.eu

1777 Discovery of methane



www.anaerobic-microbiology.eu

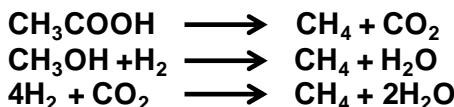
European Research Council



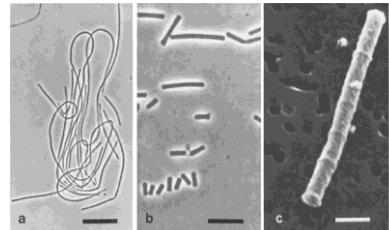
Source: methane-producing archaea



**“fat rod” *Methanotherix soehngenii*
first observed by
Nicolas Soehngen in 1906**



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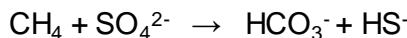


Methane Sinks:

Aerobic oxidation of methane (1906)



Sulfate dependent AOM (2000)



Quest for nitrite/nitrate/iron AOM

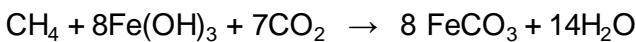
Nitrite dependent anaerobic oxidation of methane



Nitrate dependent anaerobic oxidation of methane



Iron dependent anaerobic oxidation of methane



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(Inter)national Collaboration & Funding

Soehngen Institute of Anaerobic Microbiology
www.anaerobic-microbiology.eu



Talent Grants in & out bound up to 3 months
 Visiting scientist program 3-6 months at a SIAM host

Post doc position 2 + 3 y



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Excellently educated & enthusiastic team members



Lab day out 23 june 2016 including AOM crew:
 Arshad, Gambelli, Ghasghavi, Guerrero Cruz, de Jong, Kox, Lueke, Maalcke-Luesken,
 van Niftrik, Op den Camp, Rasigraf, Slegers, Stultiens, Versantvoort & Welte



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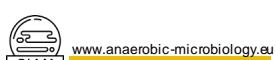
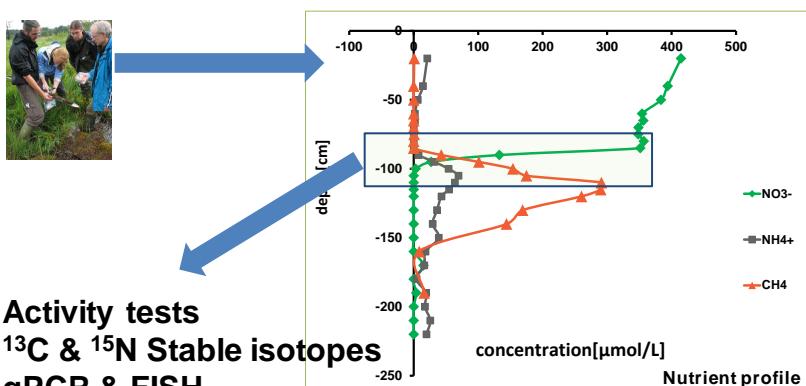
Where do we find nitrate/nitrite/iron-AOM?



HIGH NO_3^- due to agricultural run-off /ground water
 HIGH reactive iron in sediment
 HIGH CH_4 production in sediment



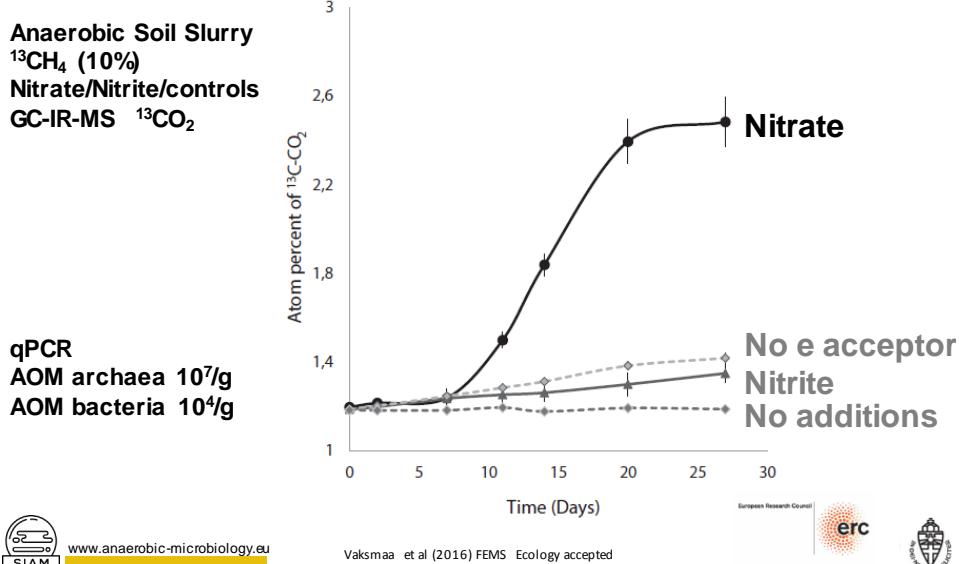
Depth Profile of Nitrate & Methane



$^{13}\text{CH}_4$ activity test Paddy field soil Italy



Annika Vaksmaa



www.anaerobic-microbiology.eu

Vaksmaa et al (2016) FEMS Ecology accepted

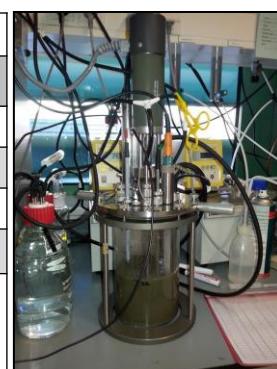


Enrichment Culture CH_4 & Nitrate



Annika Vaksmaa

Vercelli	
Inoculum	Paddy field soil
Electron donor	CH_4
Electron acceptor	NaNO_3
Type	Continuous SBR
In operation	A very long time
Monitoring	qPCR batch experiments nitrate, nitrite measurements Metagenome analysis



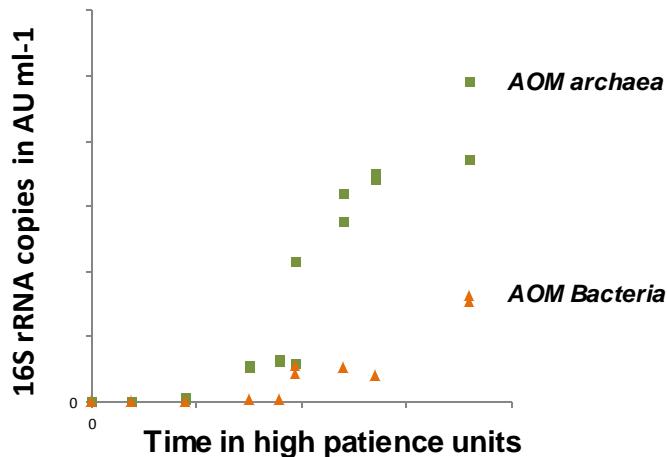
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Vaksmaa et al (2016) in prep

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qPCR Monitoring of enrichment culture for AOM archaea & bacteria



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Vaksmaa et al (2016) in prep

European Research Council



FISH analysis

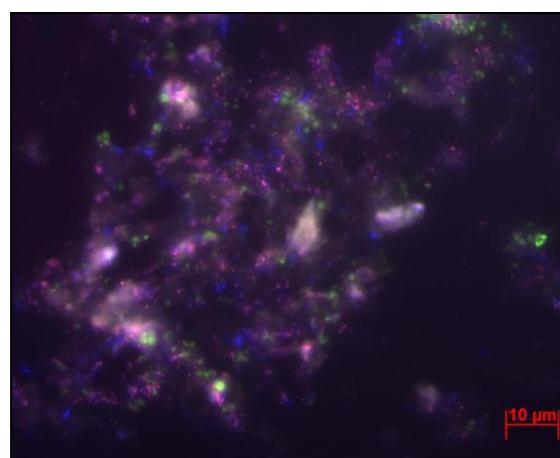
M. oxyfera bacteria

M. nitroreducens archaea

Metagenome analysis

M. oxyfera bacteria 6%

M. nitroreducens archaea 15%



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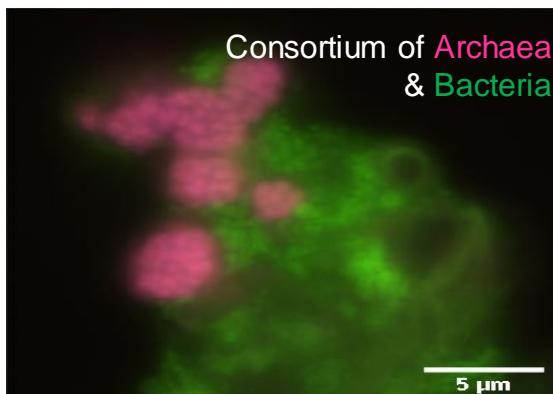


nature Vol 440(13 April 2006) doi:10.1038/nature04617

LETTERS

A microbial consortium couples anaerobic methane oxidation to denitrification

Ashna A. Raghoebarsing¹, Arjan Pol¹, Katinka T. van de Pas-Schoonen¹, Alfons J. P. Smolders², Katharina F. Ettwig¹, W. Irene C. Ripsstra³, Stefan Schouten³, Jaap S. Sinninghe Damsté³, Huub J. M. Op den Camp¹, Mike S. M. Jetten¹ & Marc Strous¹



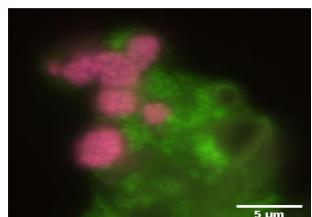
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Raghoebarsing et al 2006 Nature

European Research Council



+ Nitrate



+ Nitrite

Archaea
Methanoperedens
Mechanism?

Haroon et al 2013 Nature

Bacteria
Methylomirabilis
NO dismutase?

Physiology
Genome
Stable isotopes

Raghoebarsing et al 2006 Nature
Ettwig et al 2010 Nature



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Methylomirabilis oxyfera



Courtesy of Gambell

Visit me at
poster 072A



Karin Stultiens

Some strains only have *xoxF*

Rubisco

K_s values ?

hunting for putative NO dismutase

by membrane proteomics

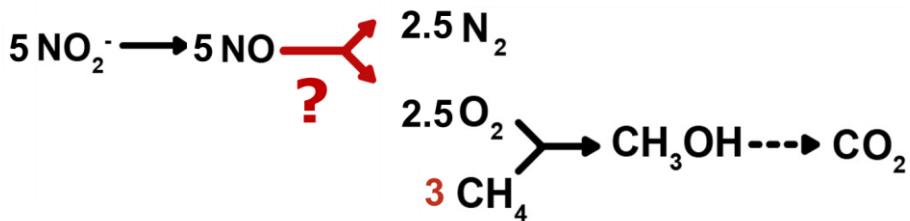
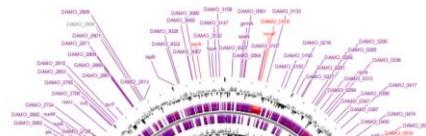


www.anaerobic-microbiology.eu

Stultiens et al (2016) in prep



Genome of *Methylomirabilis oxyfera*



pathway of (aerobic) methane oxidation
Incomplete denitrification
Putative NO dismutase



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Ettwig et al 2010 Nature

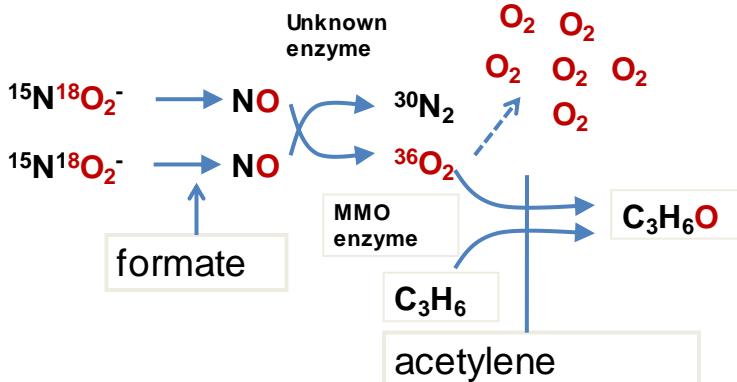
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Possible mechanisms of *M. oxyfera*

Can oxygen be released?

Tested with suicide substrates, inhibitors and $^{15}\text{N}^{18}\text{O}$ nitrite



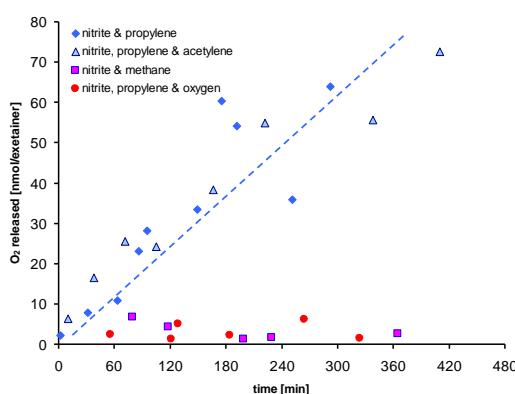
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Ettwig et al 2010 Nature

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Methylomirabilis oxyfera putative NO dismutase $2\text{NO} \rightarrow \text{N}_2 + \text{O}_2$



doi:10.1038/nature09880
nature
ARTICLES

Nitrite-driven anaerobic methane oxidation by oxygenic bacteria

Katharina F. Ettwig^{1,*}, Margaret K. Butler^{1,3}, Denis Le Paslier^{2,3,4}, Eric Pelletier^{2,3,4}, Sophie Mangenot⁵, Marcel M. M. Kuyper⁶, Frank Schreiter³, Bas E. Dutilh³, Johannes Zedelius⁷, Dirk de Beer⁷, Janin Giesbers⁸, Hans J. M. Op den Camp⁹, Eva M. Janssen-Megens⁹, Kees-Jan Frenken⁹, Henk Hamelink⁹, Jean Weissbuch^{10,11}, Mike S. M. Jetten⁶ & Marc Strous^{1,2}



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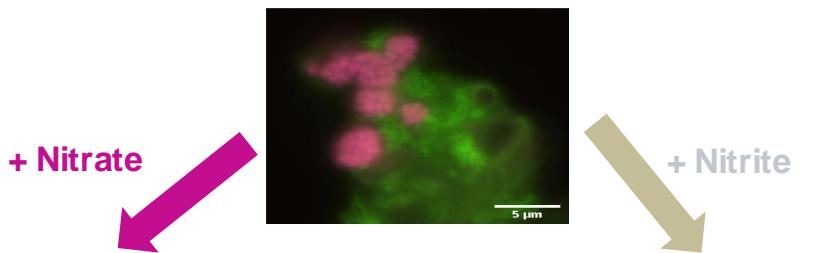
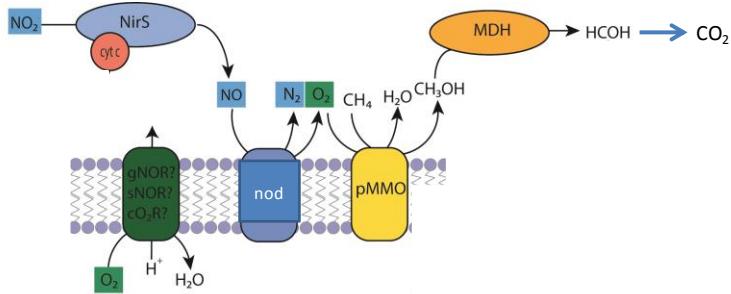
Ettwig et al 2010 Nature

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$^{15}\text{N}^{18}\text{O}$ experiments show: Oxygen Production

Proposed mechanism for nitrite-dependent methane oxidation



+ Nitrate

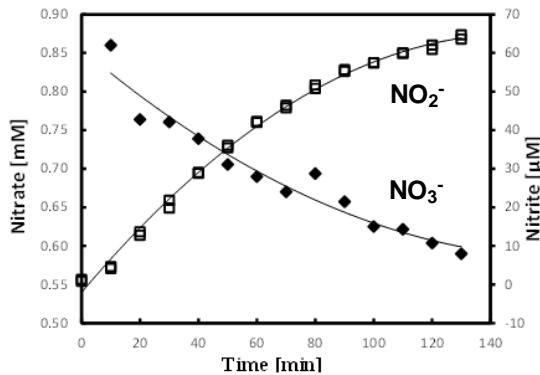
+ Nitrite

Archaea
Methanoperedens?
Mechanism?

metagenomics
Physiology & Biochemistry

Bacteria
Methylomirabilis
NO dismutase
pmoCAB

Nitrate-AOM by Methanoperedens Archaea



In co culture with nitrite scavengers *M. oxyfera* or anammox
Up to 2 mM NH_4^+ (10% of total N)



www.anaerobic-microbiology.eu

Zhu et al 2014



Genome inventory Methanoperedens



Arslan Arshad Cornelia Welte

Complete reverse CH_4 pathway

HDR complex

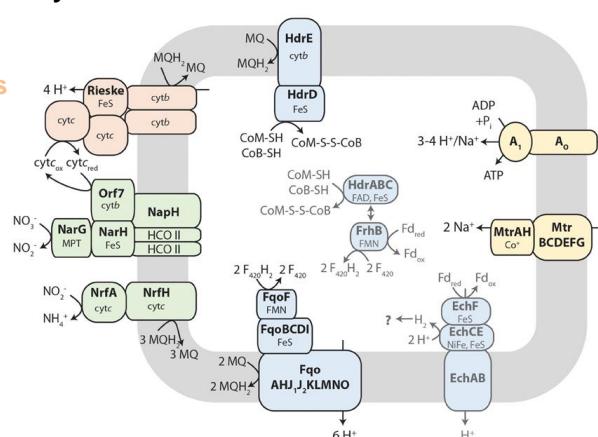
FQO complex

Many cytochrome c genes

bc_1 complex

Nitrate reductase *narGH*

Nitrite reductase *nrfAH*



Arshad et al 2015 *Frontier in Microbiology*
<http://journal.frontiersin.org/article/10.3389/fmicb.2015.01423/full>

SIAM PhD vacancy
Biochemistry of AOM



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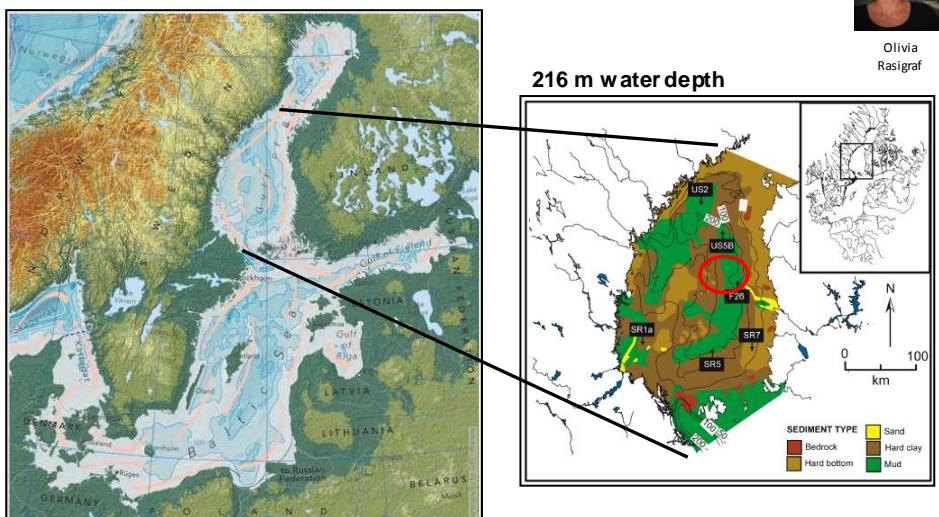


Anaerobic Oxidation of Methane

- Nitrite selects for *M oxyfera*
- *M oxyfera* makes O₂ from NO
- Novel nitrate reducing AOM archaea
- AOM present in many ecosystems
- Remaining : The Quest for iron-AOM



Sampling site: Bothnian Sea



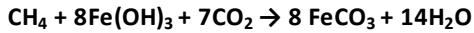
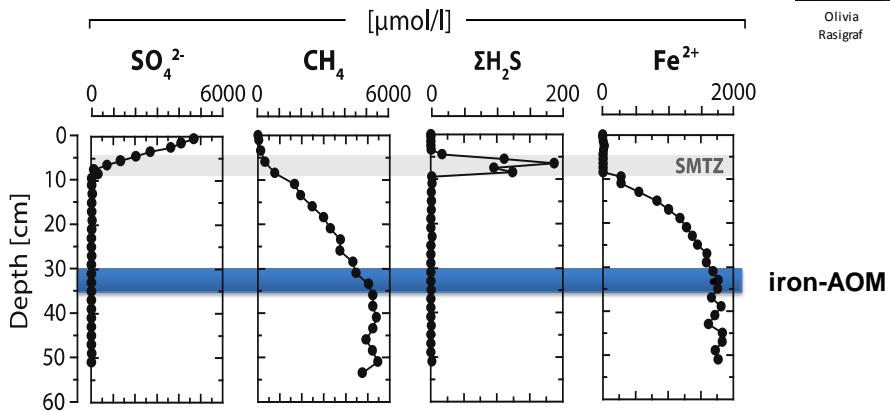
Matthias Egger & Caroline Slomp



Sediment biogeochemistry



Olivia
Rasigraf



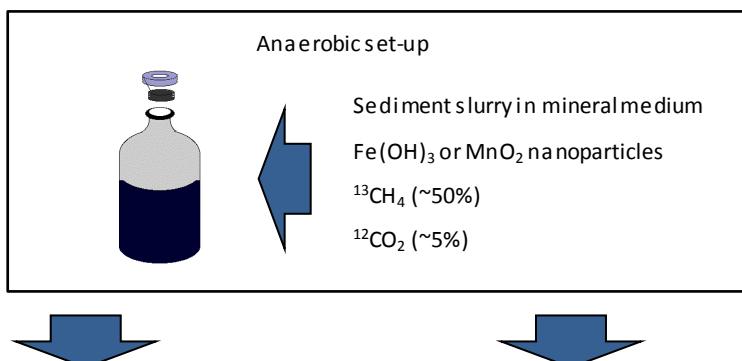
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Universiteit Utrecht



Incubation set up



Headspace gas analysis: GC-MS



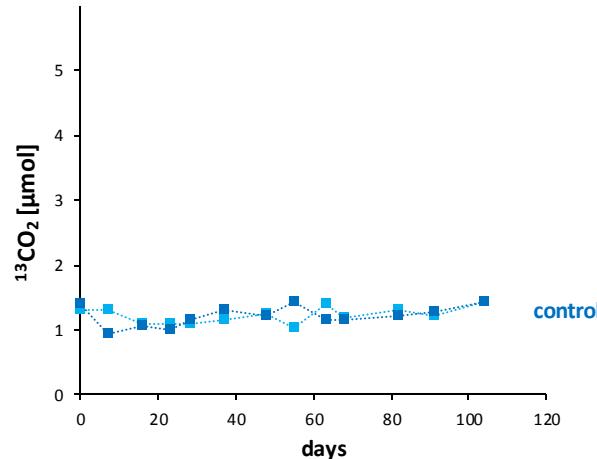
Ion measurements: ICP-OES



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$^{13}\text{CH}_4$ oxidation to $^{13}\text{CO}_2$ coupled to Fe^{3+} reduction



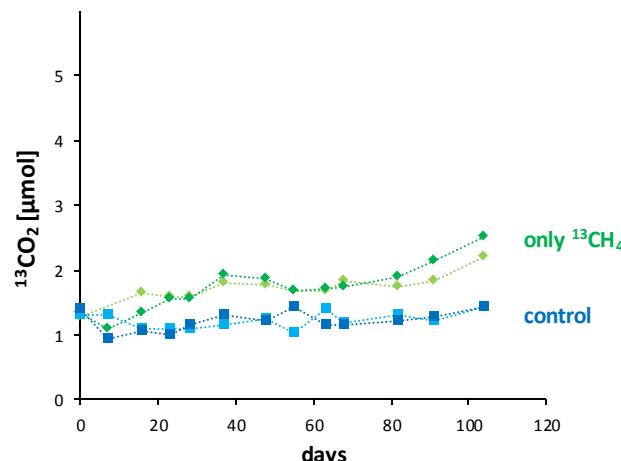
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Egger, Rasigraf et al 2015 EST



$^{13}\text{CH}_4$ oxidation to $^{13}\text{CO}_2$ coupled to Fe^{3+} reduction



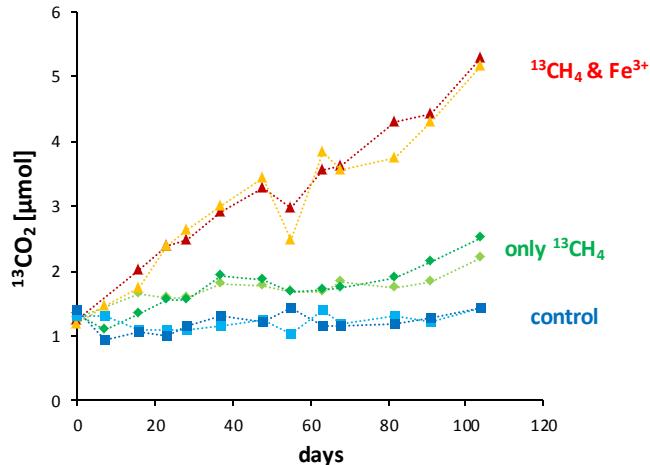
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Egger, Rasigraf et al 2015 EST



$^{13}\text{CH}_4$ oxidation to $^{13}\text{CO}_2$ coupled to Fe^{3+} reduction



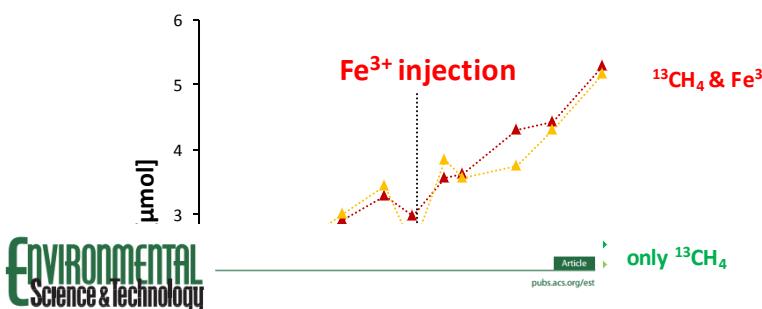
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Egger, Rasigraf et al 2015 EST



$^{13}\text{CH}_4$ oxidation to $^{13}\text{CO}_2$ coupled to Fe^{3+} reduction



Iron-Mediated Anaerobic Oxidation of Methane in Brackish Coastal Sediments

Matthias Egger,^{a,†} Olivia Rasigraf,[‡] Célia J. Sapart,^{§,||} Tom Jilbert,^{†,¶} Mike S. M. Jetten,[‡] Thomas Röckmann,[§] Carina van der Veen,[§] Narcisa Bândă,[‡],[¶] Boran Kartal,^{‡,#} Katharina F. Ettwig,[‡] and Caroline P. Slopen[†]

^aDepartment of Earth Sciences - Geochemistry, Faculty of Geosciences, Utrecht University, Budapestlaan 4, 3584 CD Utrecht, The Netherlands

[†]Department of Microbiology, Institute for Water and Wetland Research, Faculty of Science, Radboud University Nijmegen, Heyendaalsweg 135, 6525 AJ Nijmegen, The Netherlands

[‡]Institute for Marine and Atmospheric Research Utrecht (IMAU), Utrecht University, Princetonplein 5, 3584 CC Utrecht, The Netherlands

[§]Laboratoire de Glaciologie, Université Libre de Bruxelles, 50 Avenue F. D. Roosevelt, B-1050 Bruxelles, Belgium

^{||}Department of Biochemistry and Microbiology, Laboratory of Microbiology, Ghent University, K. L. Ledeganckstraat 35, 9000 Gent, Belgium

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More Evidence for iron AOM

Lee et al. *Microbiol Perspect* 2016;12:77

Eduardo Lee, Michael S. Saito, and Howard M. Saito

Microbial Cell Factories

RESEARCH

Open Access

Reversing methanogenesis to capture methane for liquid biofuel precursors

Valerie W. C. Soo¹, Michael J. McNulty², Arti Upadhye¹, Yaqin Zhu¹, Lijun Zhang¹, Emmanuel Hatzelau², Philip G. Sharp², Saurabh Agarwal², Fred Raison-Rikasse², Sarathram Gopalakrishnan², Howard M. Saito¹, James G. Perry¹, Curtis D. Marhan¹, Andrew B. Paluszewski¹ and Thomas A. Wood^{1,2}

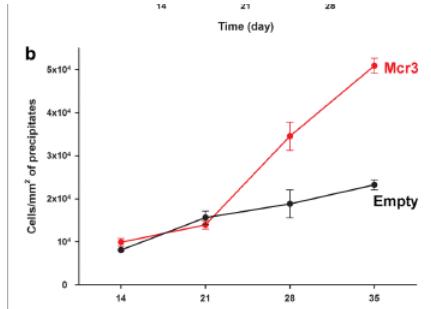
¹Department of Biological Sciences, University at Albany, State University of New York, Albany, NY, USA

²Department of Earth and Atmospheric Sciences, University at Albany, State University of New York, Albany, NY, USA

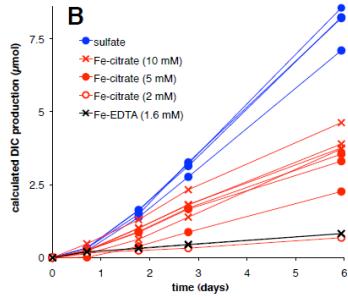
MICROBIAL PHYSIOLOGY

Artificial electron acceptors decouple archaeal methane oxidation from sulfate reduction

Silvia Scheller,¹ Hang Yu,¹ Grayson L. Chadwick,¹ Shawn E. McGlynn,¹ Victoria J. Orphan¹



Growth of engineered Methanosaerica
On Methane & 10 mM Fe Cl₃



Activity of AOM
On Methane & 10 mM Fe-Citrate



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New (an)aerobic C1 microbes could save the world

Wednesday, November 14, 2007

By Jeanna Bryner



Unique bacteria hiding out in a witches' brew of anoxic water not only thrive in cold wetlands and oceans but also chow down its C1 compounds

Thank you !

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