THE UNIVERSITY of York

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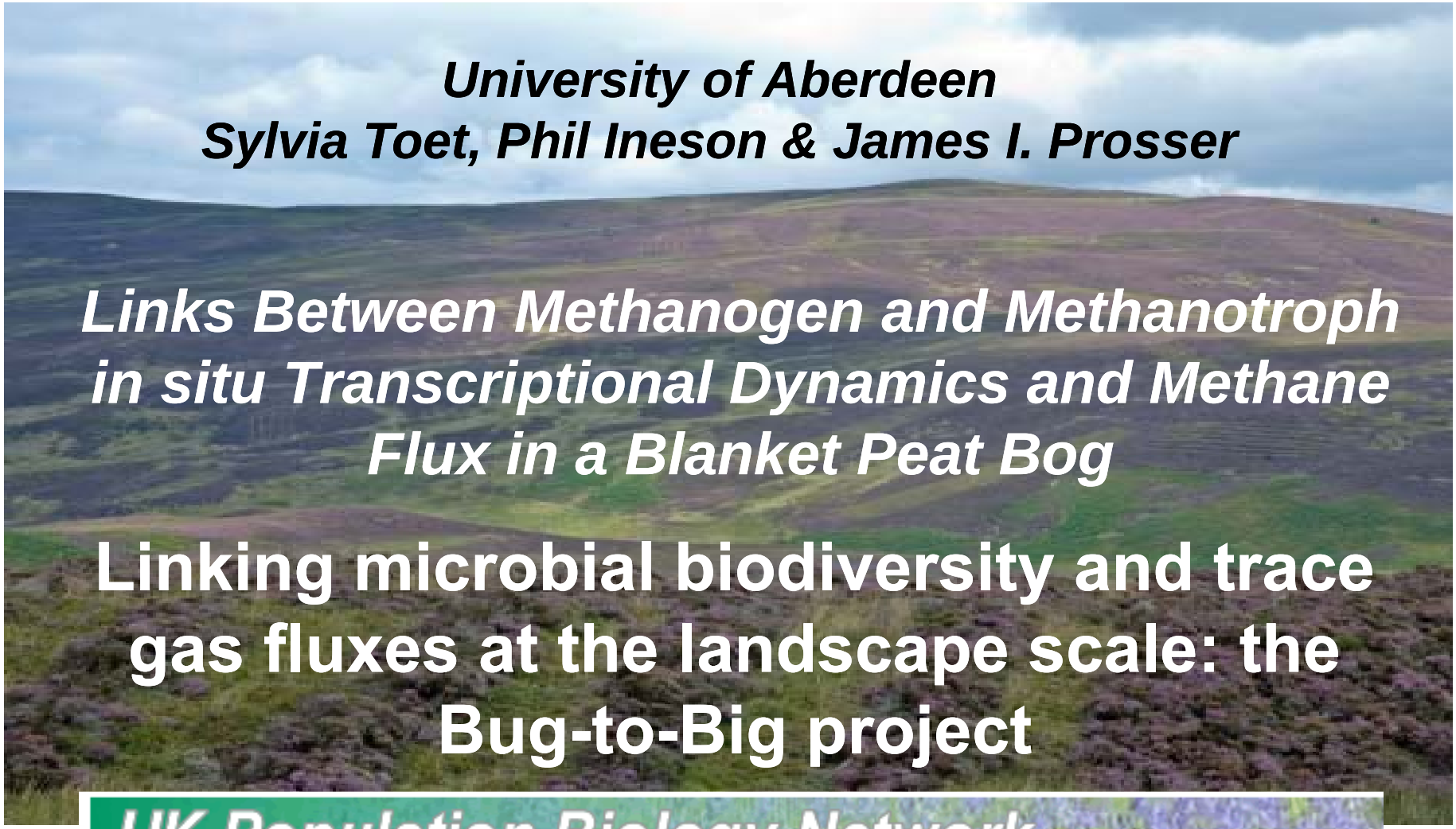
Thomas Freitag

University of Aberdeen

Sylvia Toet, Phil Ineson & James I. Prosser



Links Between Methanogen and Methanotroph in situ Transcriptional Dynamics and Methane Flux in a Blanket Peat Bog

Linking microbial biodiversity and trace gas fluxes at the landscape scale: the Bug-to-Big project

UK Population Biology Network

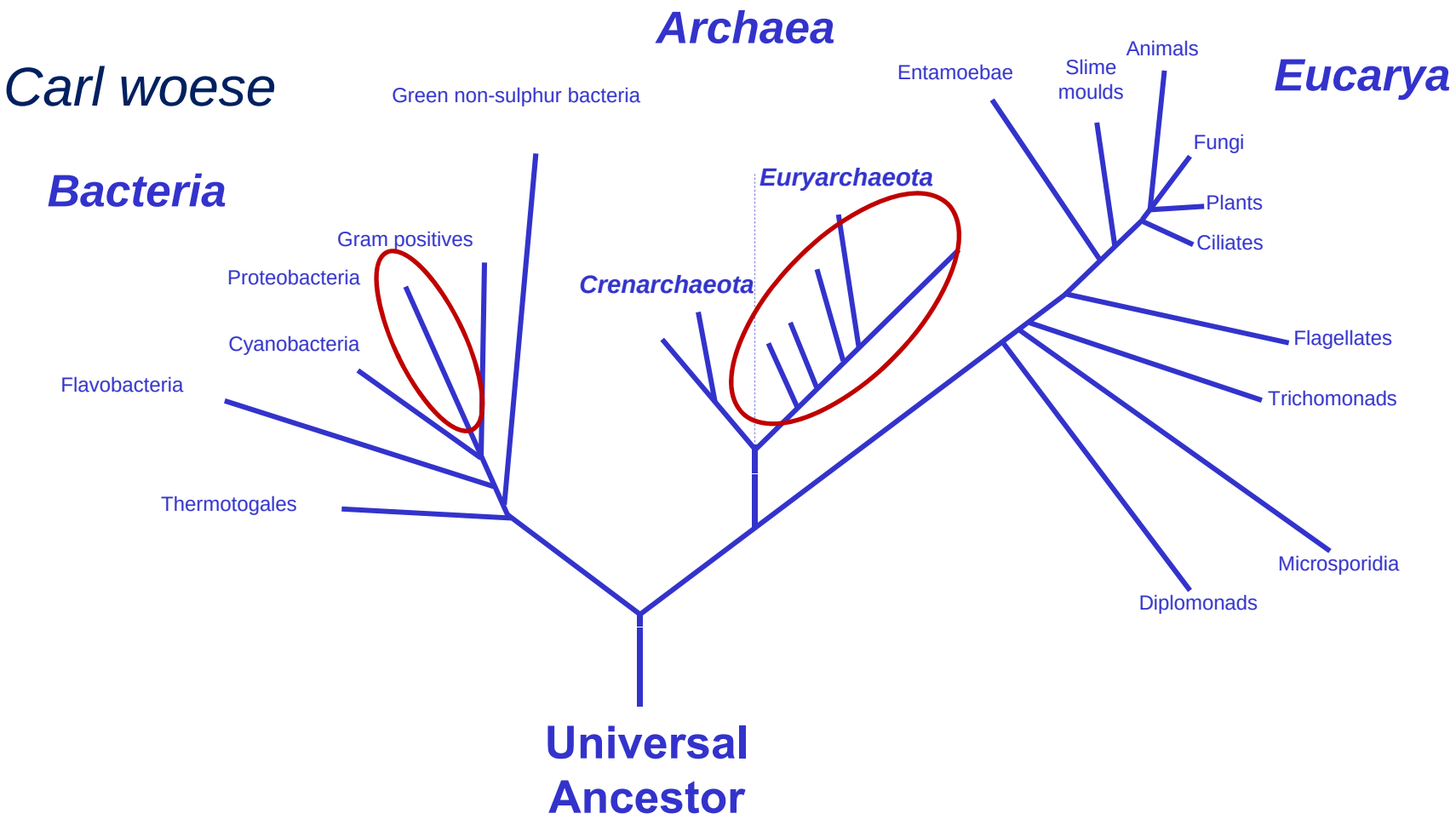
Opening the Black Box : What controls CH_4-emission in peat?

- **CH_4- fluxes are usually well correlated with temperature, water saturation (water table position) and to a lesser degree with humification (degree of decomposition of organic matter), vegetation type and cover.**

- **CH_4 "net flux to the atmosphere is a complex function of the processes that control the production, consumption, transport, and release of the gas" (Bartlett & Harriss, 1993).**

- **Is the *in situ* activity of CH_4- producers (methanogens) and oxidizers (methanotrophs) quantifiable? Is there a relationship of the *in situ* activity with CH_4 flux?**

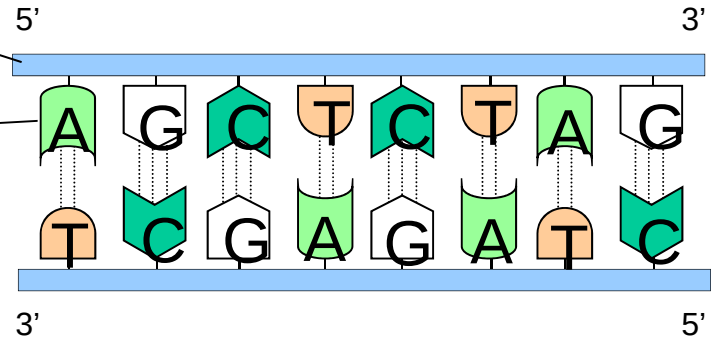
Universal Tree of life (rRNA genes)



sugar-phospate backbone

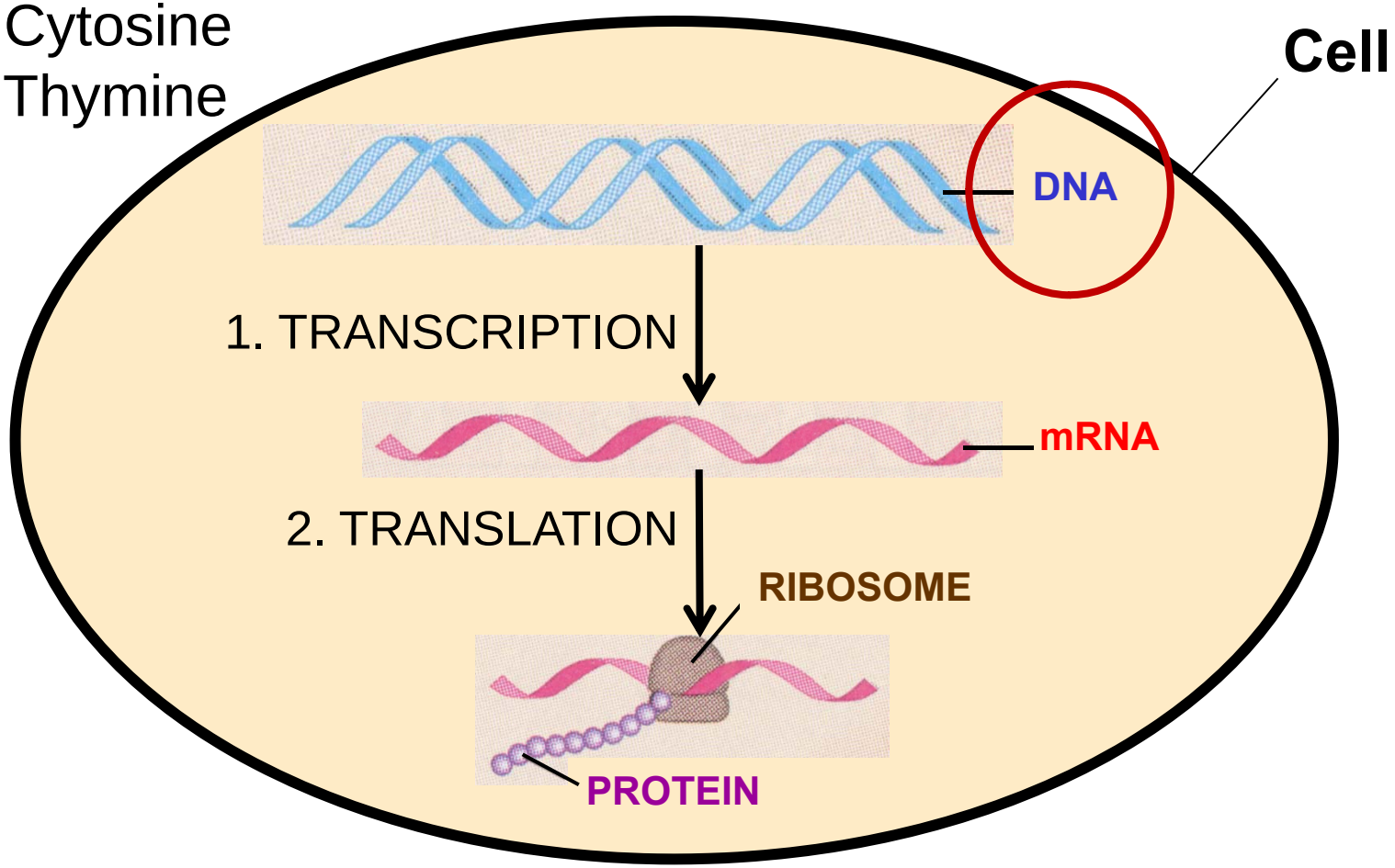
bases

- Adenine
- Guanine
- Cytosine
- Thymine

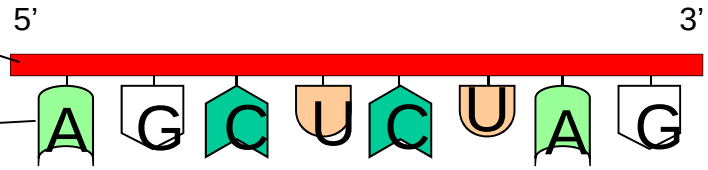


Proxy for abundance “standing stock”

DNA



sugar-phospate backbone

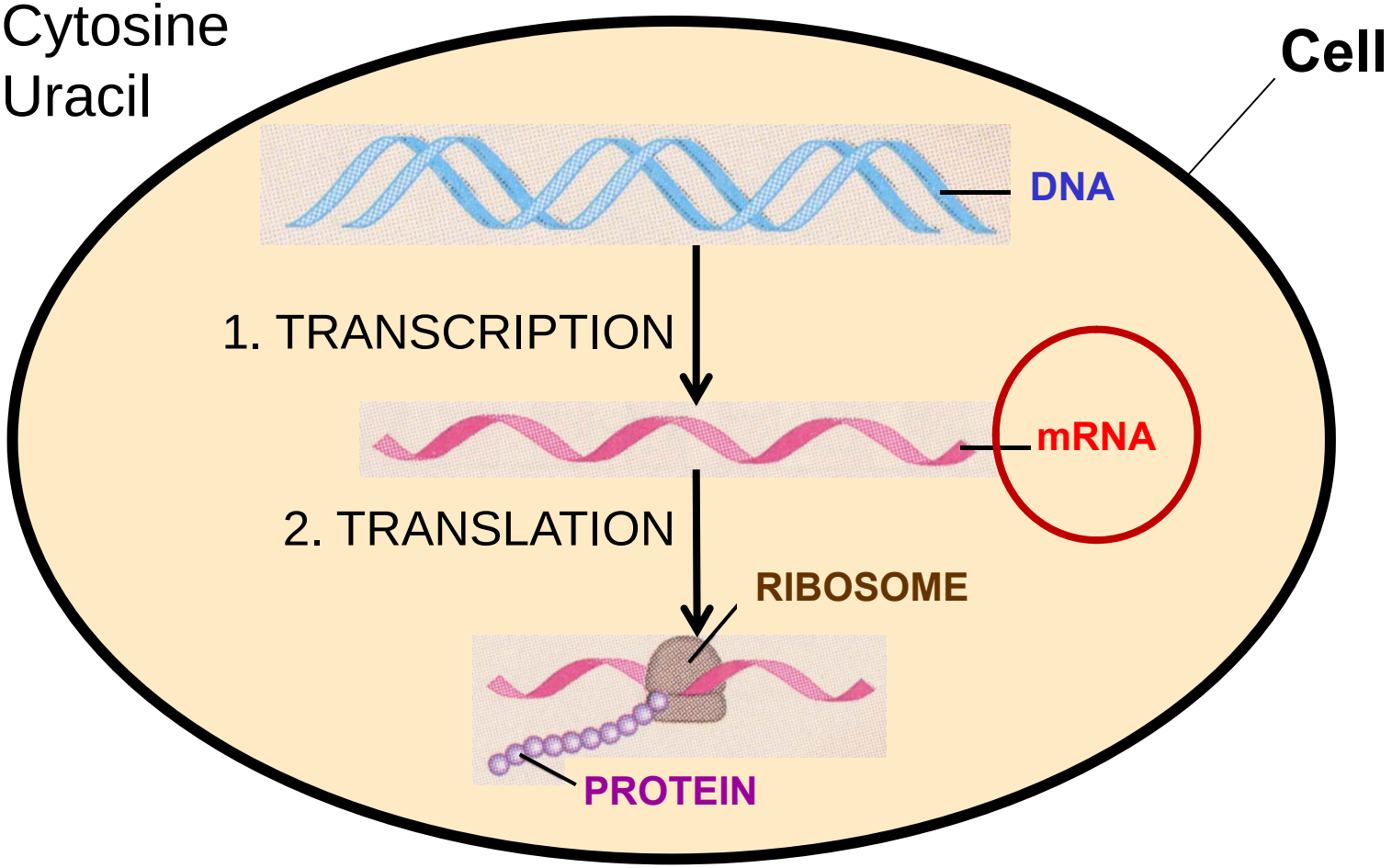


bases

- Adenine
- Guanine
- Cytosine
- Uracil

Proxy for activity

RNA



Extraction of nucleic acids in soil

0.5 g
Glass beads
Phenol
Chloroform
Buffer

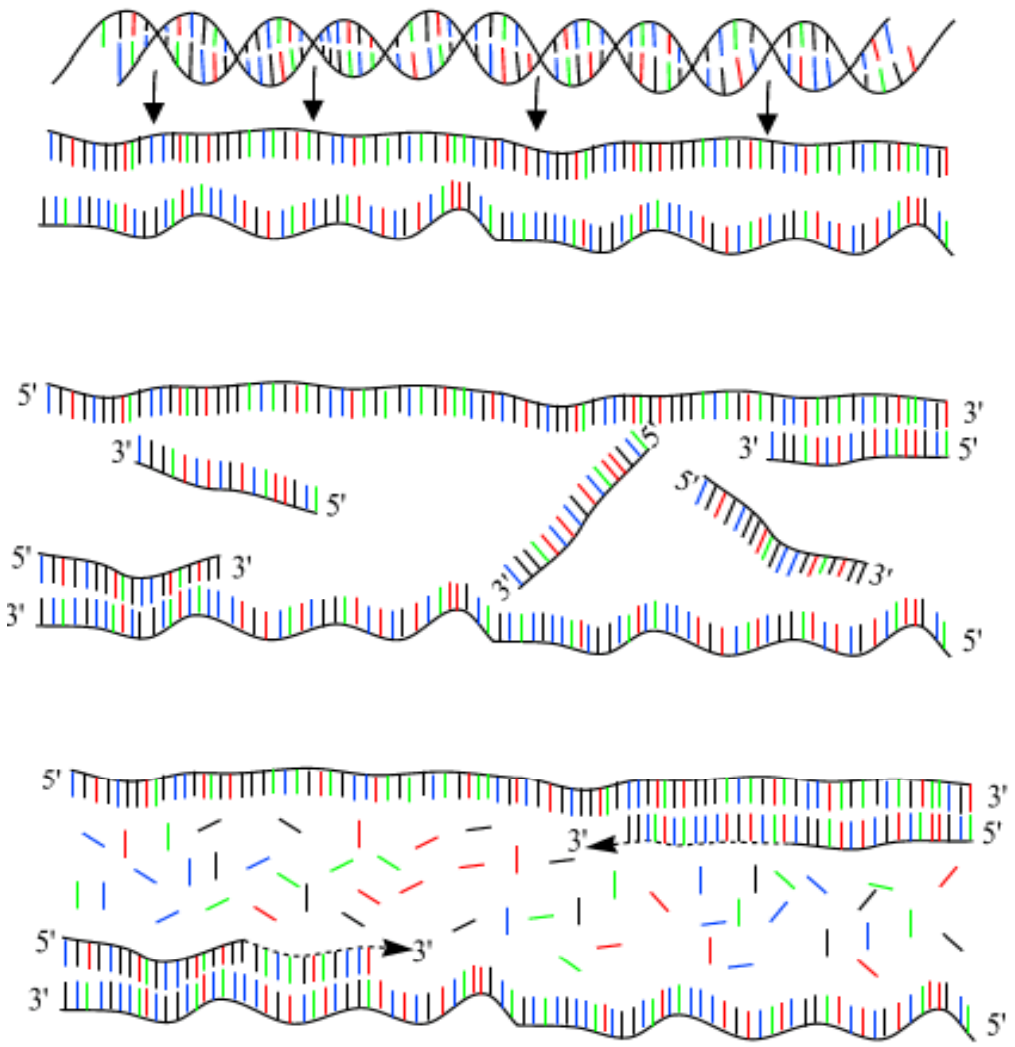
Shake/Vortex
then
centrifuge

Aqueous phase
Organic phase
Soil/glass beads debris

Remove
Aqueous
phase

Nucleic acids

PCR amplification of nucleic acids

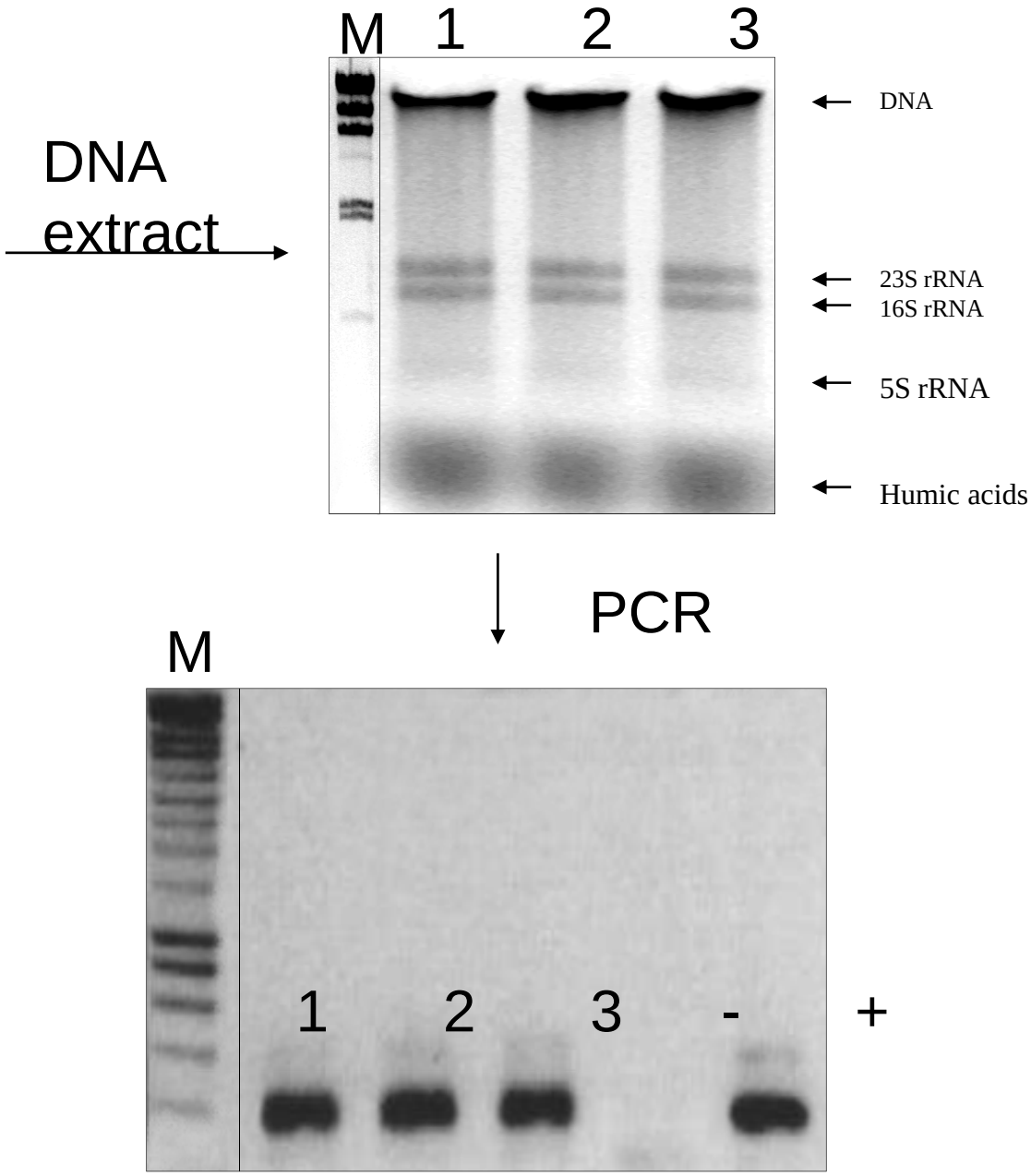


1. Denature DNA
(95°C – 30 s)

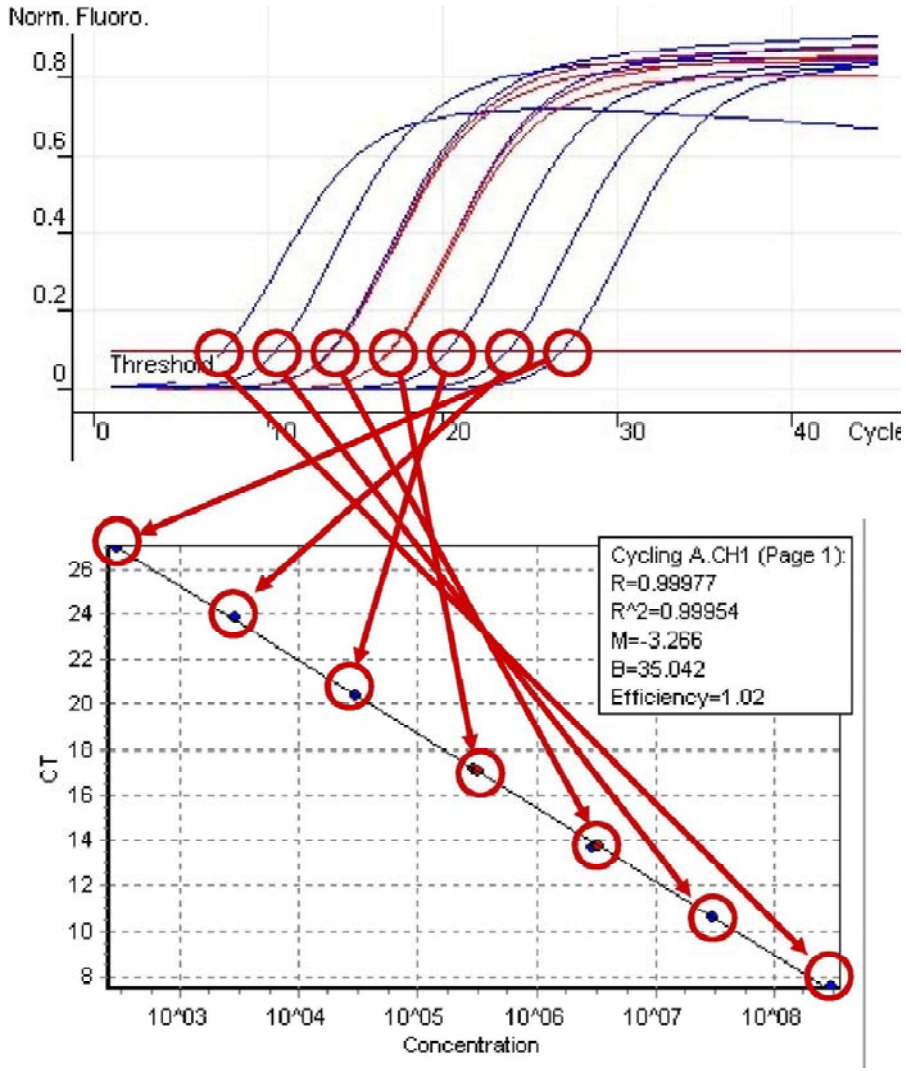
2. Anneal primers
(55°C – 30 s)

3. Primer extension
(72°C – 1 min)

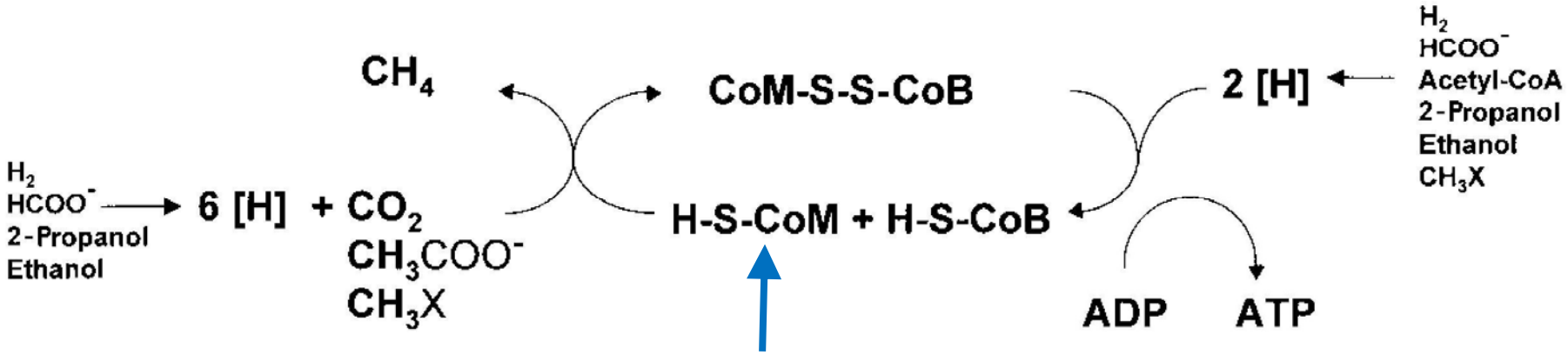
35x


M
1
2
3
DNA extract
DNA
23S rRNA
16S rRNA
5S rRNA
Humic acids
PCR
M
1
2
3
-
+

Quantitative PCR amplification of nucleic acids



Methanogens molecular marker *mcr*A mRNA



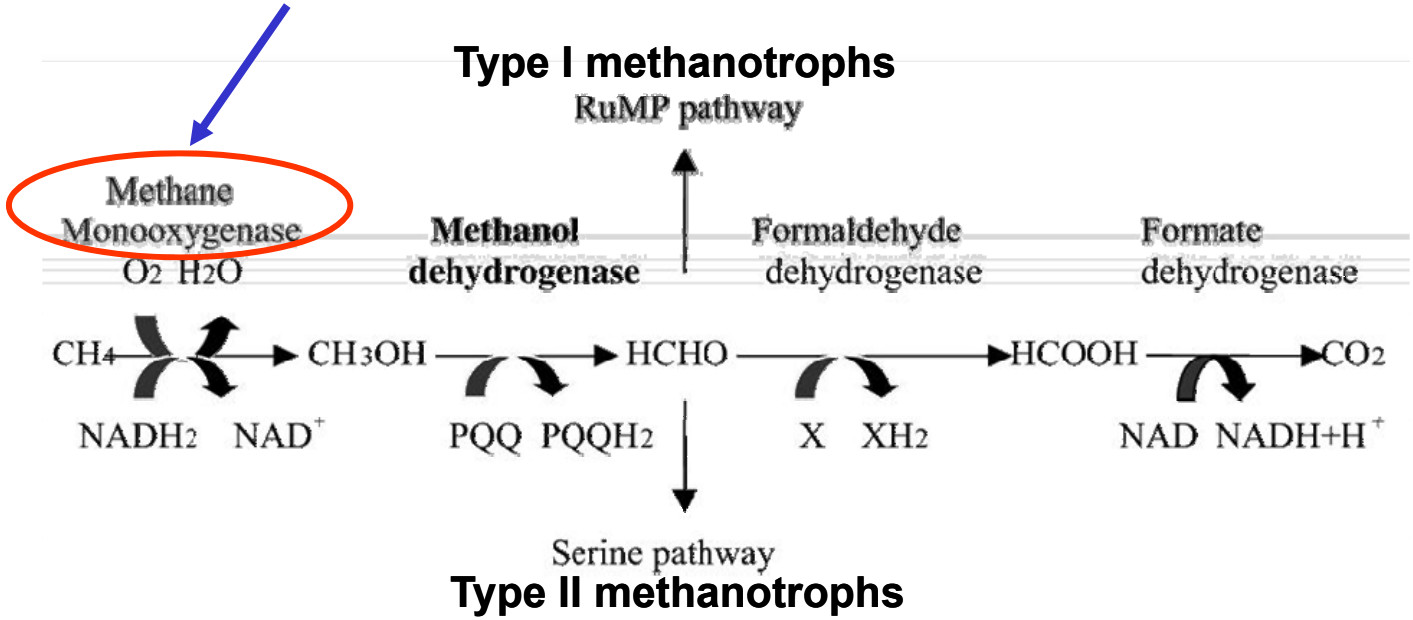
methyl coenzyme M reductase (MR)

- **Gene encoding MR subunit ά (*mcr*A)**
- **Present in all known methanogens**
- **qPCR & RT- qPCR with ML primers by Luton et al. 2002**

Thauer 1998

Molecular marker methane oxidation: *pmmo & smmo* mRNA

Particular (pmmo) or soluble (*smmo*) methane monooxygenase



- **Gene encoding pmmo subunit ά (*pmoA*)**
- **present in all methanotrophs, except *Methylocella* spp.**
- **qPCR & RT- qPCR with *pmoA* A189f–mb661r primers by Costello *et al*. 1999**

Murrell 2008

Relationship between transcriptional and physiological activity

Appl Microbiol Biotechnol (2003) 61:61–68
DOI 10.1007/s00253-002-1191-5

ORIGINAL PAPER

C. Glanemann · A. Loos · N. Gorret · L. B. Willis ·
X. M. O'Brien · P. A. Lessard · A. J. Sinskey

Disparity between changes in mRNA abundance and enzyme activity in *Corynebacterium glutamicum*: implications for DNA microarray analysis

"Clear differences were observed in the timing and magnitude of changes in mRNA abundance and their corresponding enzyme activities."

"..... it is difficult to generally predict protein activity from quantitative transcriptome data."

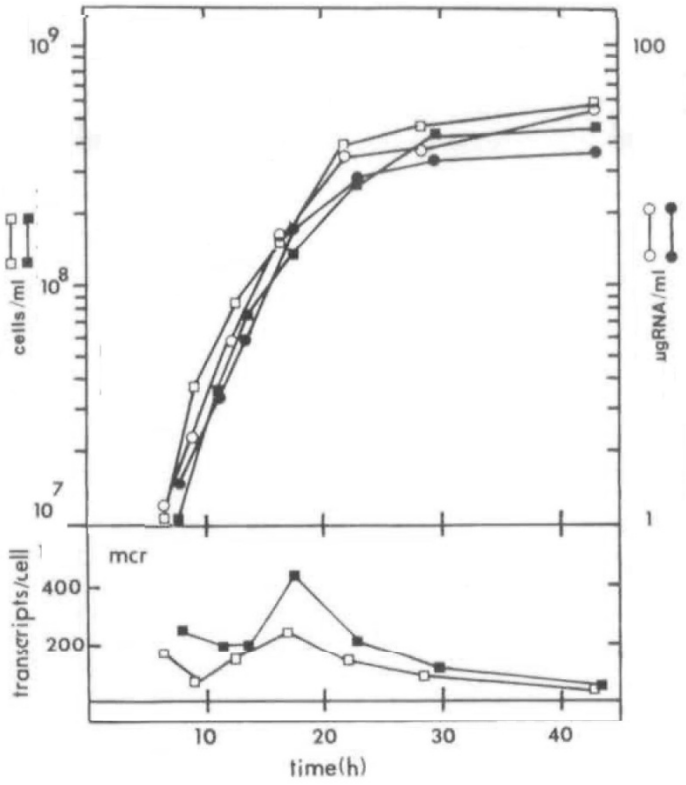
Relationship between transcriptional and physiological activity in methanogens

Molecular Microbiology (1994) 11(4), 655–670

mRNAs in the methanogenic archaeon *Methanococcus vannielii*: numbers, half-lives and processing

Aidan N. Hennigan† and John N. Reeve*
Department of Microbiology, The Ohio State University, Columbus, Ohio 43210, USA.

- Only 8-fold increase in *mcrA* mRNA numbers during growth curve in pure culture.
- 50 to 450 *mcrA* molecules per cell.
- half-life of *mcrA* mRNA at 37°C =15min.



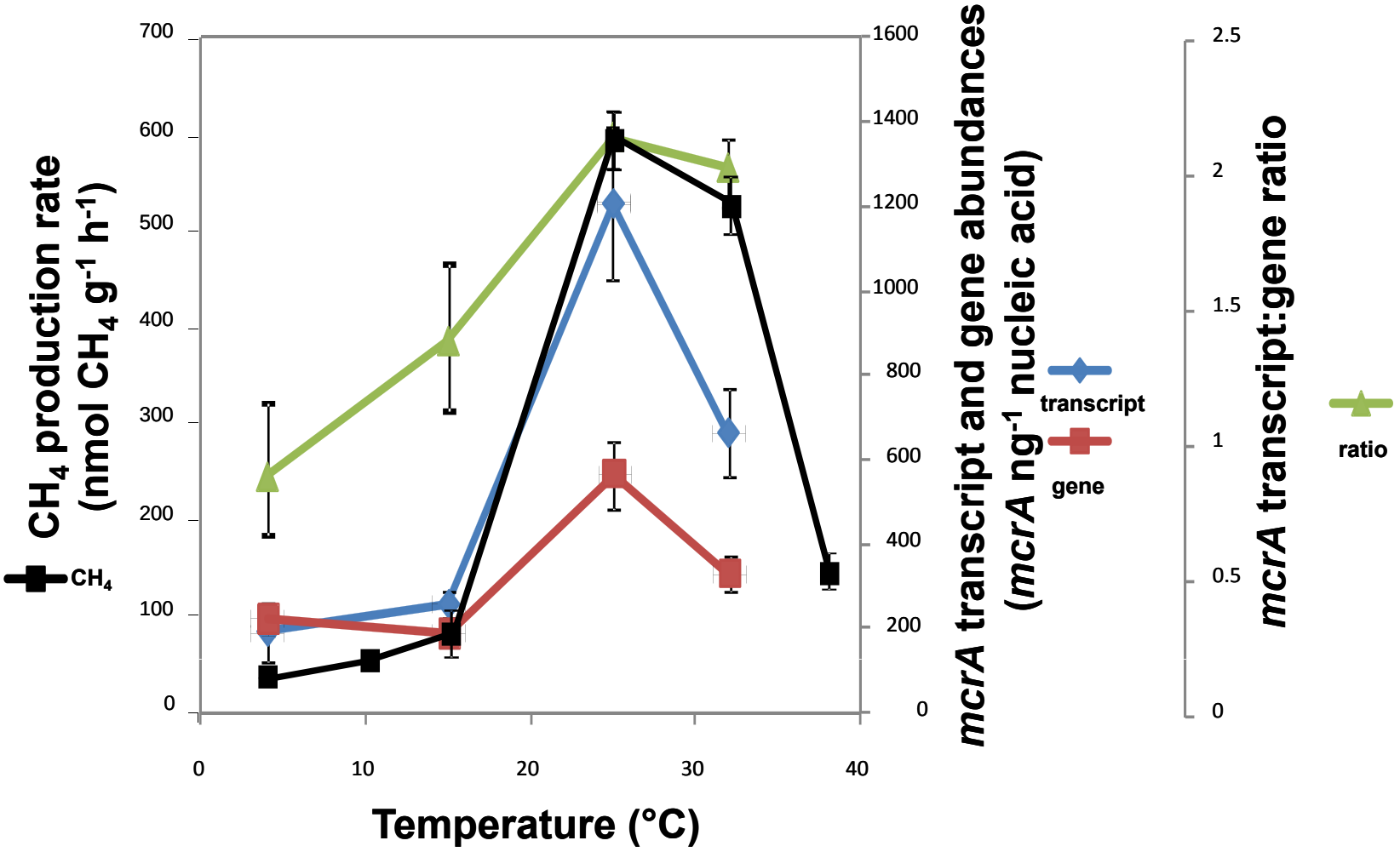
Relationship between transcriptional and physiological activity in methanogens

Peat soil from Lake Vyrnwy moorland, common heather with cottongrass

- Homogenised and incubated at temperatures from 4 to 37°C.
- CH_4 production analysed by FID-GC
- *mcrA* analysed by qPCR & RT-qPCR

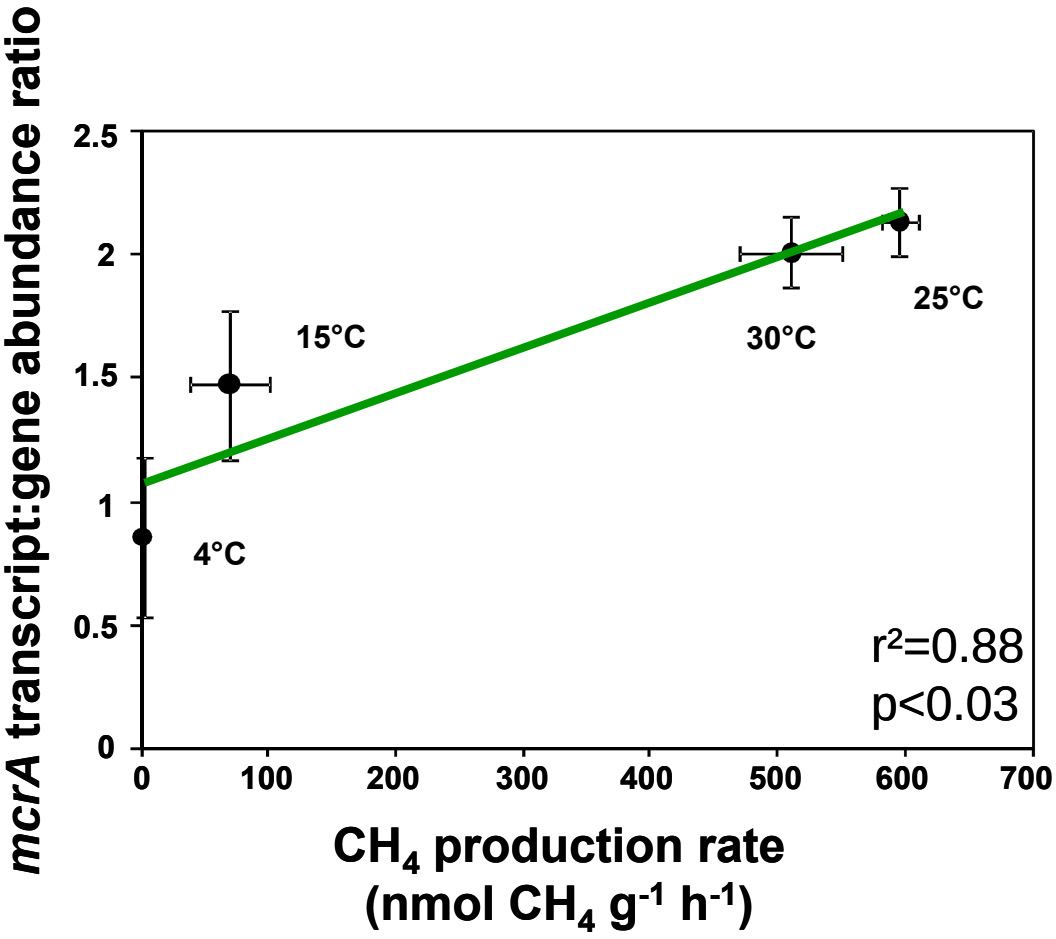
AEM 2009, 75:6679–6687

Methanogen temperature response: physiological rates and *mcrA* transcript :gene ratios



AEM 2009, 75:6679–6687

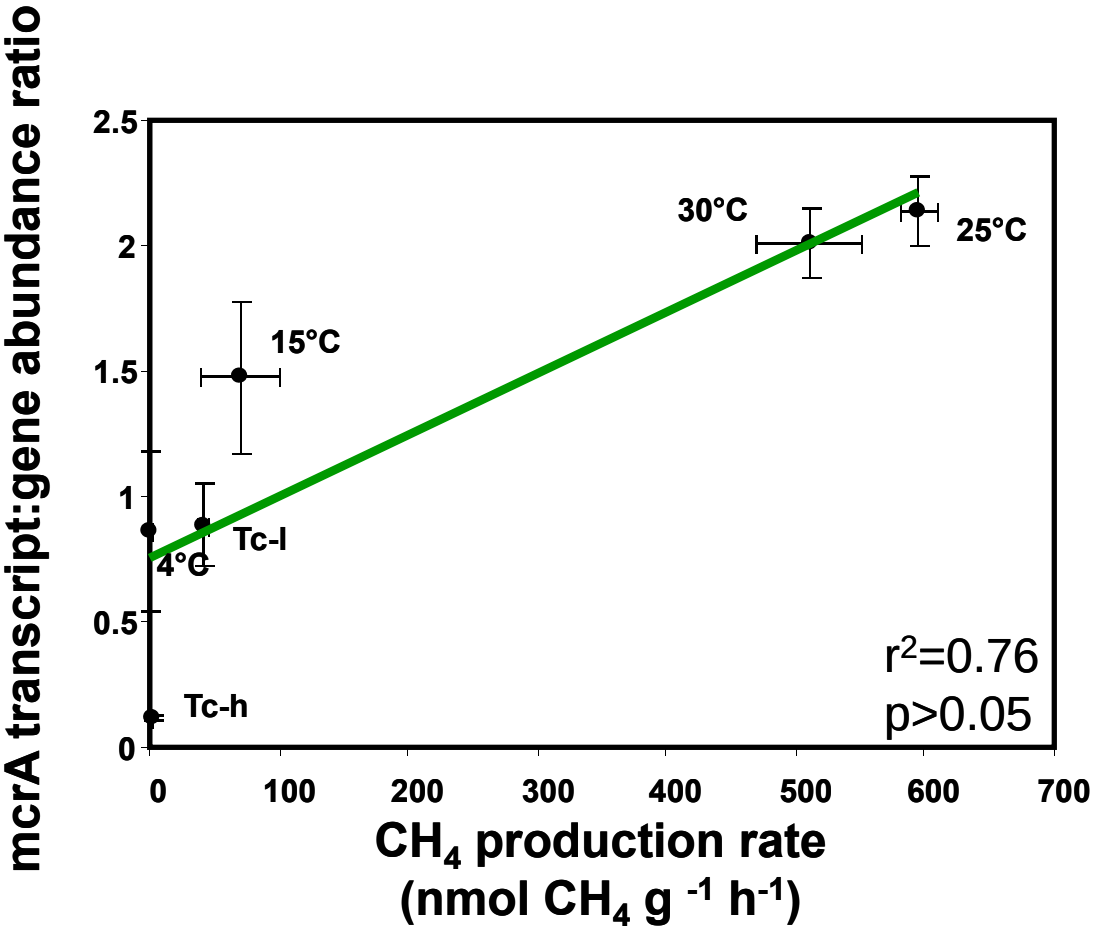
Methanogen physiological rates and corresponding *mcrA* transcript :gene ratios



Correspondence of *mcrA* transcript: gene abundance ratios with physiological rates

AEM 2009, 75:6679–6687

Methanogen physiological rates and corresponding *mcrA* transcript :gene ratios



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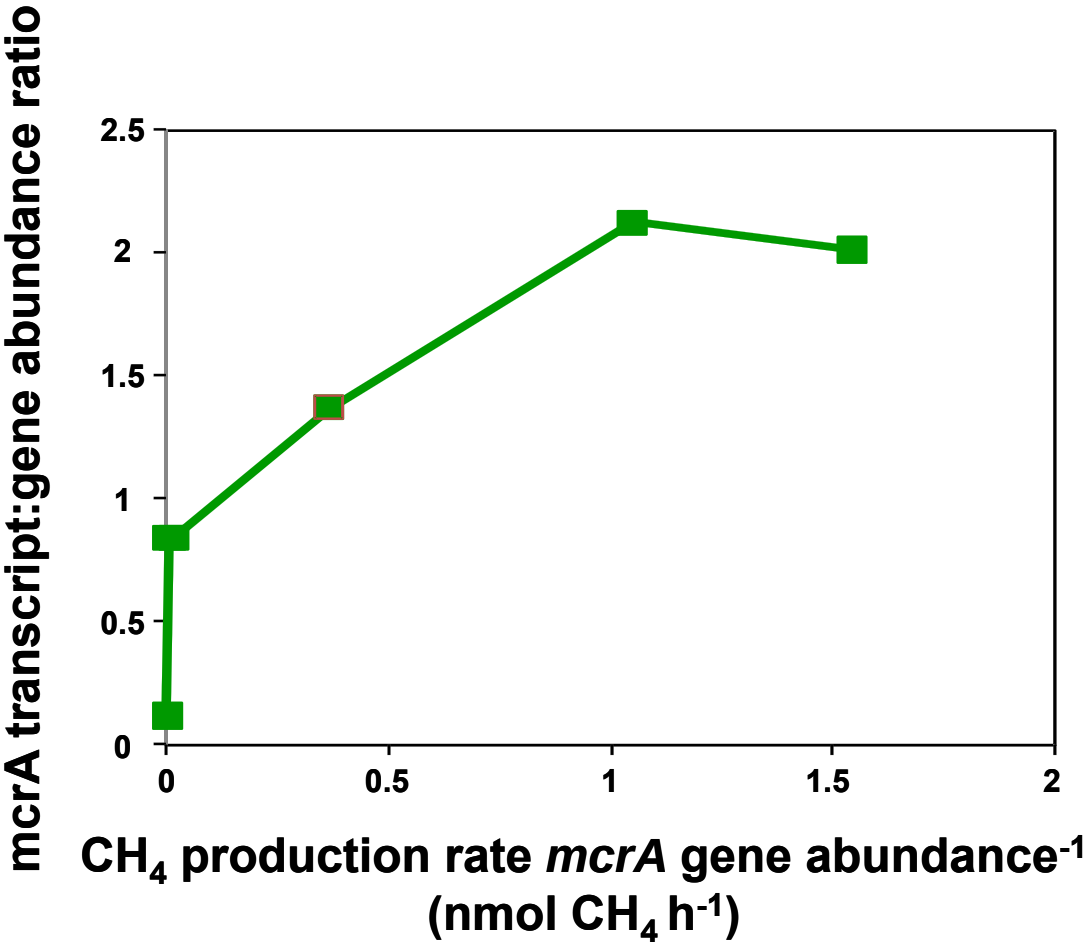
AEM 2009, 75:6679–6687

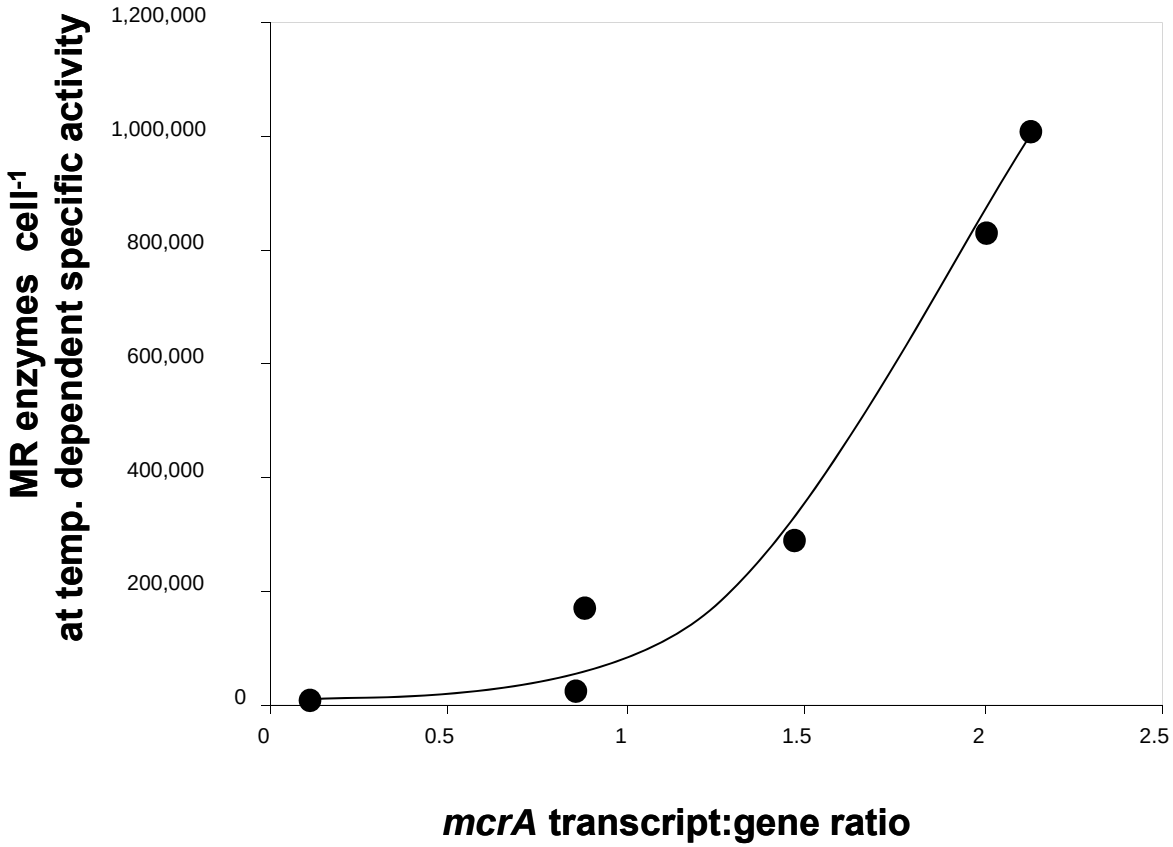
Methanogen physiological rates and corresponding *mcrA* transcript :gene ratios



Correspondence of *mcrA* transcript: gene abundance ratios with physiological rates per cell (*mcrA* gene copy)

AEM 2009, 75:6679–6687

MR enzyme projection



No saturation of methanogen activity

Underestimation of MR specific activity or cell abundance

Methanogen physiological rates and corresponding *mcrA* transcript :gene ratios

Summary:

- Transcripts were detectable in all samples, even when physiological rates were at analysis threshold.
- Transcript: gene ratios increased only 18- fold from minimum to maximum, corresponding to 1000- fold increase in CH_4- production per gene copy.
- Maximum transcript: gene ratios were low compared to pure culture studies – suggesting high numbers of inactive or dead cells or underestimation of transcripts.

AEM 2009, 75:6679–6687

Methanogen physiological rates and corresponding *mcrA* transcript :gene ratios

Summary:

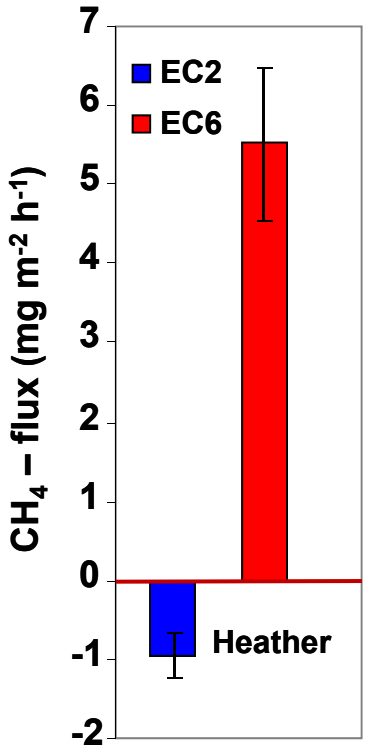
- **A significant relationship between *mcrA* transcript :gene ratios and CH_4- production rates was evident.**

- **We need more pure culture studies on the relationships between transcription of key functional genes and related biogeochemical process.**

AEM 2009, 75:6679–6687

Relationship between CH_4 flux rates and transcriptional activity of methanogens and methanotrophs

Lake Vyrnwy



Two neighbouring sites with similar characteristics (plant species composition, coverage, aspect, water content) but contrasting CH_4- flux rates:

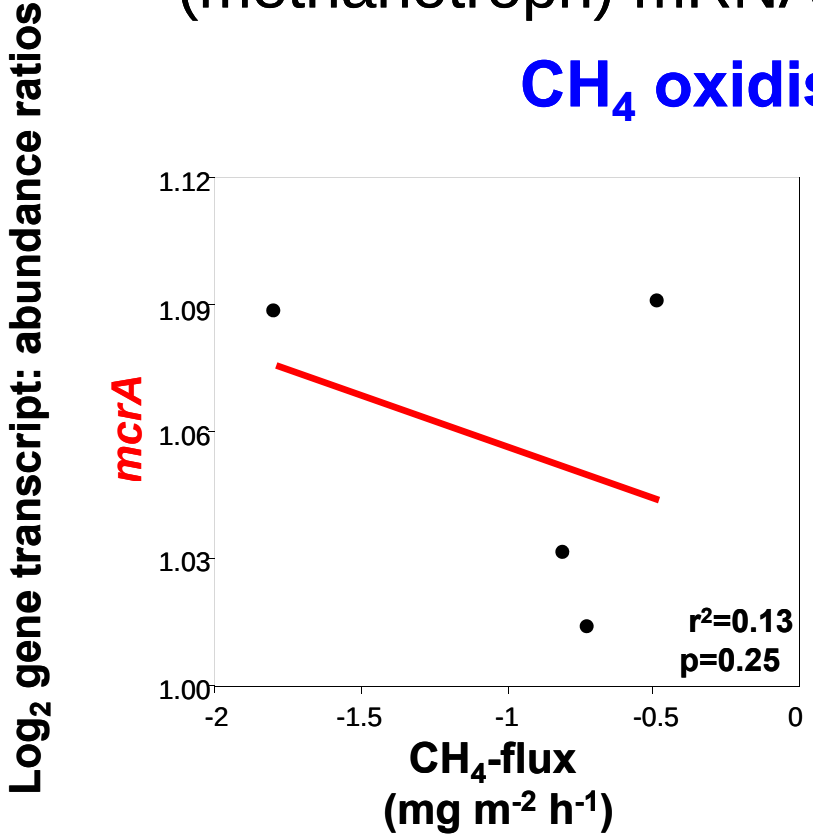
EC2 = CH_4 sink **EC6 = CH_4 source**

FEMS MicrobEcol 2010 ,73:157–165

Methane emission from Lake Vyrnwy peat soils

Field study: correlation of *mcrA* (methanogen) and *pmoA* (methanotroph) mRNA expression with methane flux

CH_4 oxidising heather site EC2 ↓



- **No relationship of *mcrA* gene transcript abundance ratios with CH_4- flux.**

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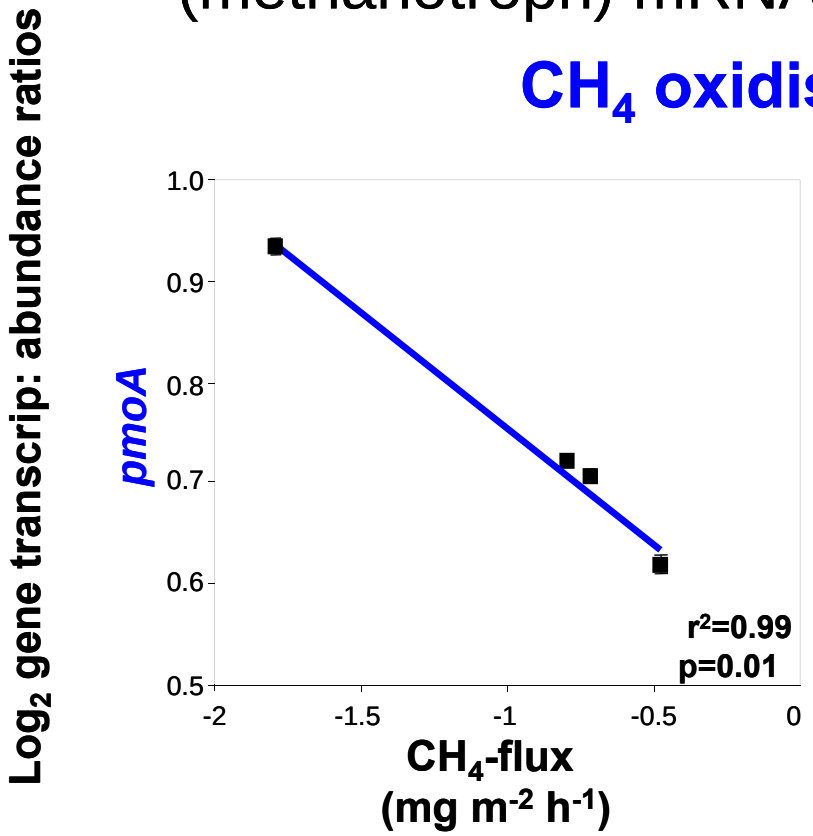


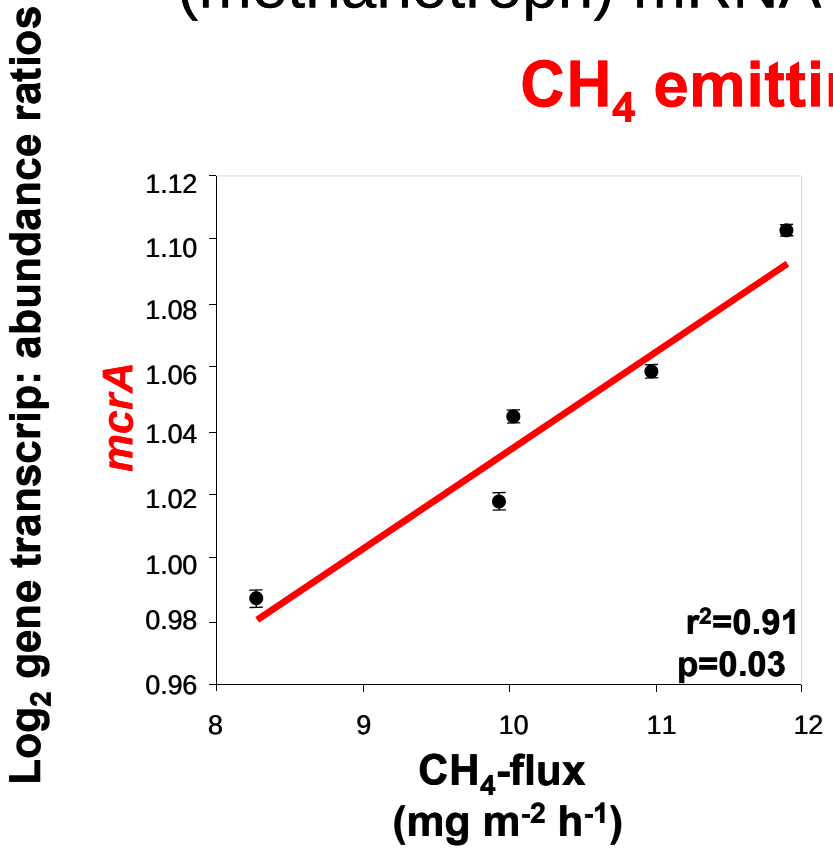
- **No relationship of *mcrA* gene transcript abundance ratios with CH_4- flux.**
- **Inverse relationship of *pmoA* gene with CH_4- flux: increasing ratios with increasing negative fluxes.**

FEMS MicrobEcol 2010 ,73:157–165

Methane emission from Lake Vyrnwy peat soils

Field study: correlation of *mcrA* (methanogen) and *pmoA* (methanotroph) mRNA expression with methane flux

CH_4 emitting heather site EC6 ↑



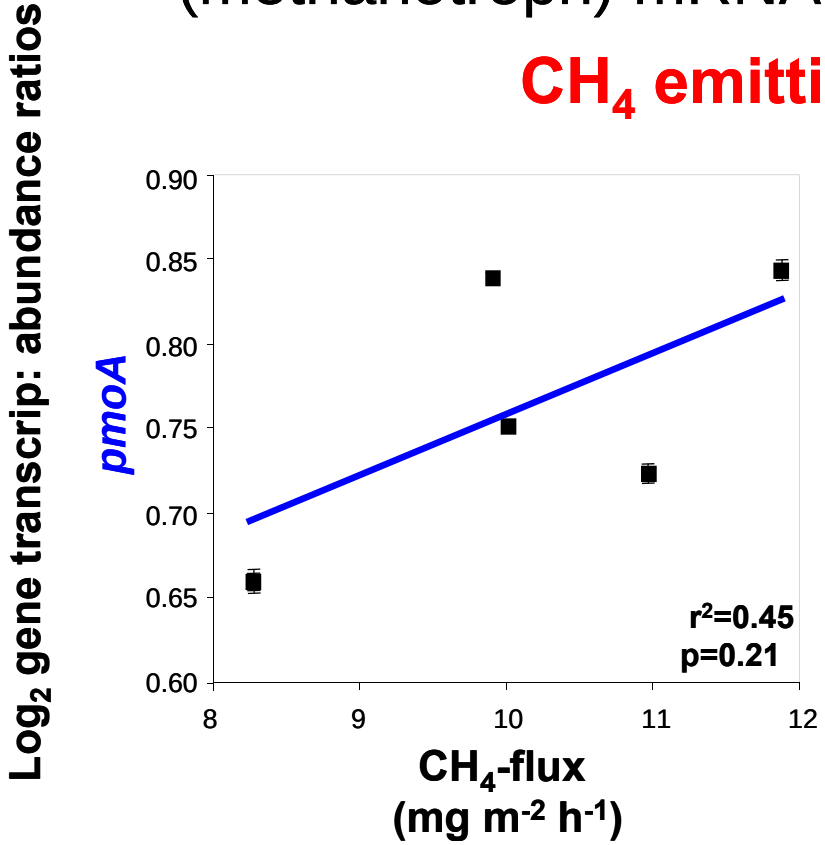
- **Relationship of *mcrA* gene transcript abundance ratios with CH_4- flux; increasing ratios with increasing flux.**

FEMS MicrobEcol 2010 ,73:157–165

Methane emission from Lake Vyrnwy peat soils

Field study: correlation of *mcrA* (methanogen) and *pmoA* (methanotroph) mRNA expression with methane flux

CH_4 emitting heather site EC6 ↑



- **Relationship of *mcrA* gene transcript abundance ratios with CH_4- flux; increasing ratios with increasing flux.**
- **No relationship of *pmoA* gene with CH_4- flux.**

FEMS MicrobEcol 2010 ,73:157–165

Methane emission from Lake Vyrnwy peat soils

Field study: correlation of *mcrA* (methanogen) and *pmoA* (methanotroph) mRNA expression with methane flux

Summary

- **At the CH_4 source site ↑, the correlation of methanogen activity and positive flux rates suggests, flux rates were mainly dependant of CH_4 production.**

- **Methanotroph activity was also high, but was not correlated to flux rates, suggesting that factors other than substrate availability limited methanotroph activity.**

Methane emission from Lake Vyrnwy peat soils

Field study: correlation of *mcrA* (methanogen) and *pmoA* (methanotroph) mRNA expression with methane flux

Summary

- **Conversely, at the CH_4 sink site ↓, the correlation between methanotroph activity and CH_4-flux rates suggests that all the CH_4 produced was also oxidised and that methanotroph activity was substrate-limited.**

- **In total; data suggest that methanotrophs are the major microbiological flux regulating factor: methane production is always high, only the modulation of methanotroph activity controls the surface flux.**

Acknowledgements

Sylvia Toet, Phil Ineson

THE UNIVERSITY of York

RSPB

NATURAL ENGLAND

Prosser Lab 1.01
Institute of Biological and Environmental Sciences

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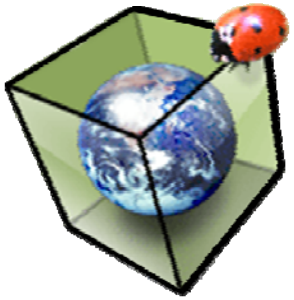


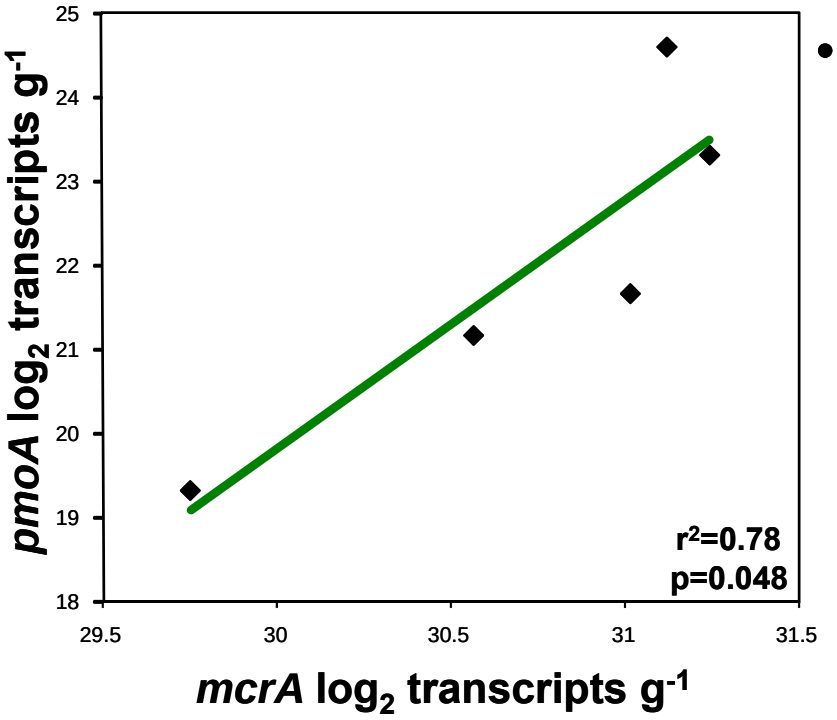
NATURAL ENVIRONMENT RESEARCH COUNCIL

UK Population Biology Network

Methane emission from Lake Vyrnwy peat soils

Field study: correlation of *mcrA* (methanogen) and *pmoA* (methanotroph) mRNA expression

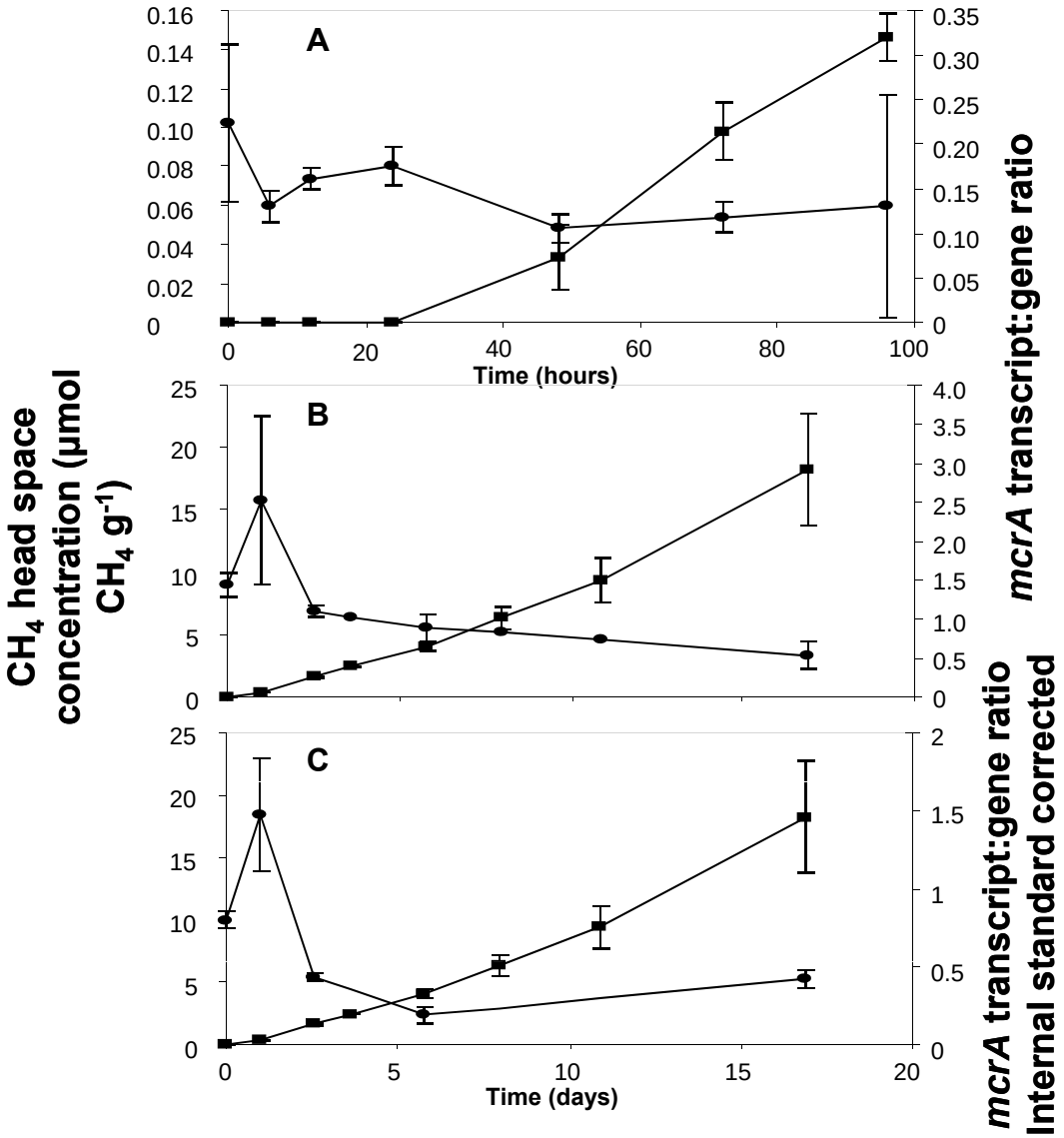
CH_4 emitting heather site EC6 ↑



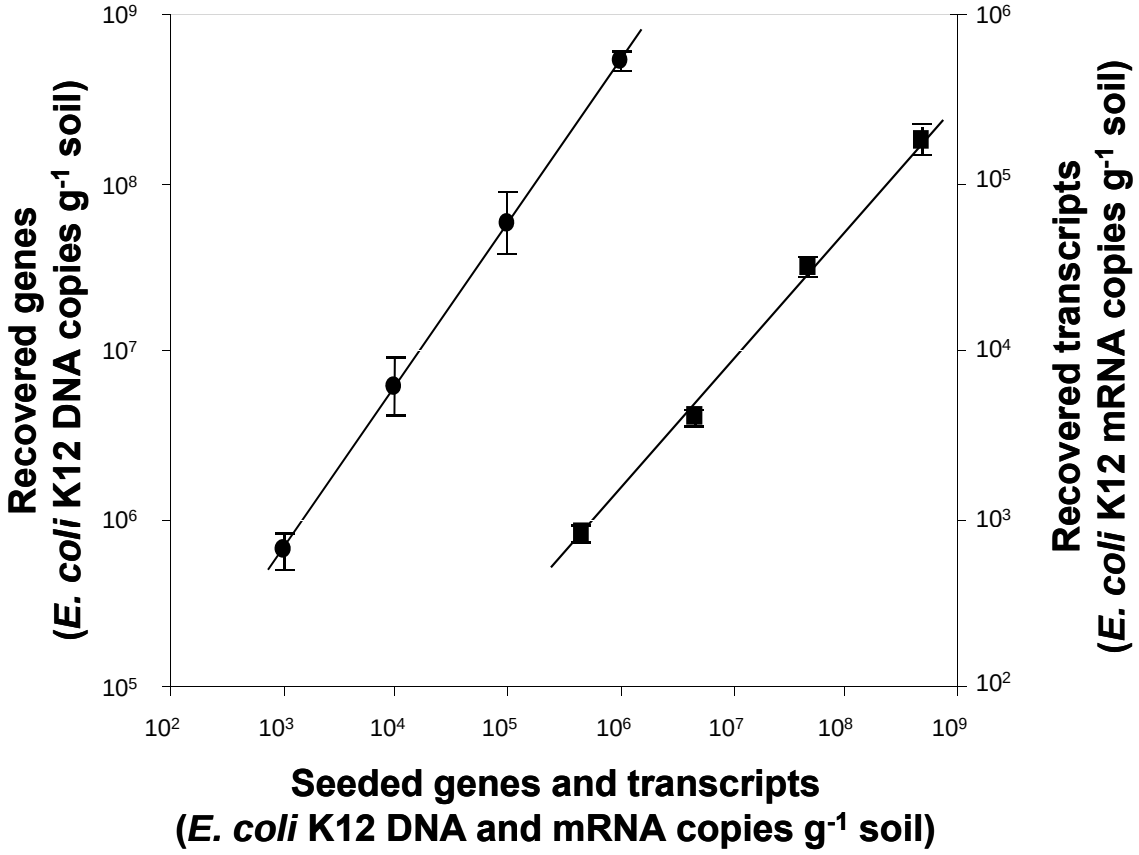
- **Significant relationship between *mcrA* and pmoA transcript abundances.**

FEMS MicrobEcol 2010 ,73:157–165

Methanogen response over time at constant temp.: physiological rates and *mcrA* transcript :gene ratios



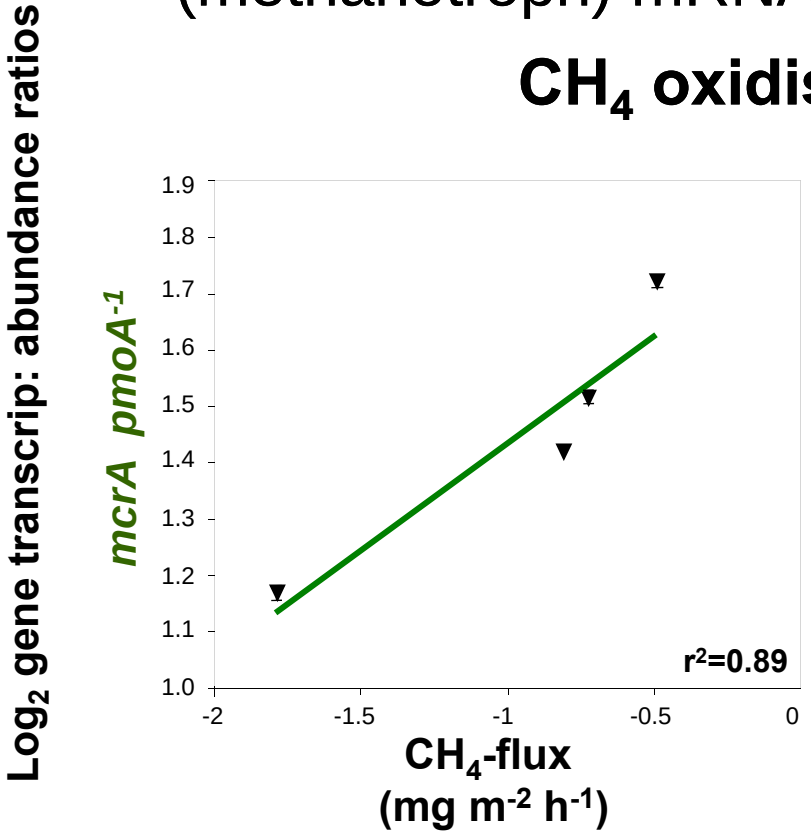
E.coli K12 cell seeding



Methane emission from Lake Vyrnwy peat soils

Field study: correlation of *mcrA* (methanogen) and *pmoA* (methanotroph) mRNA expression with methane flux

CH_4 oxidising heather site EC2 ↓

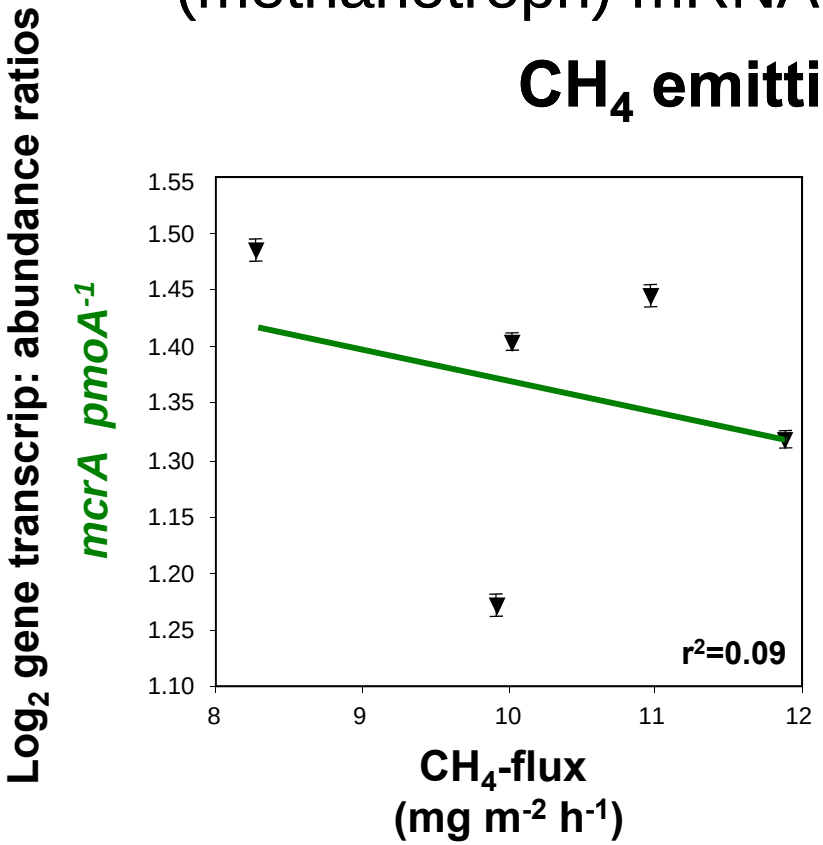


- **No linear relationship of *mcrA* gene transcript abundance ratios with CH_4- flux.**
- **Strong inverse linear relationship of *pmoA* gene with CH_4- flux: increasing ratios with increasing negative fluxes.**
- **Linear relationship of *mcrA: pmoA* ratio with CH_4- flux.**

Methane emission from Lake Vyrnwy peat soils

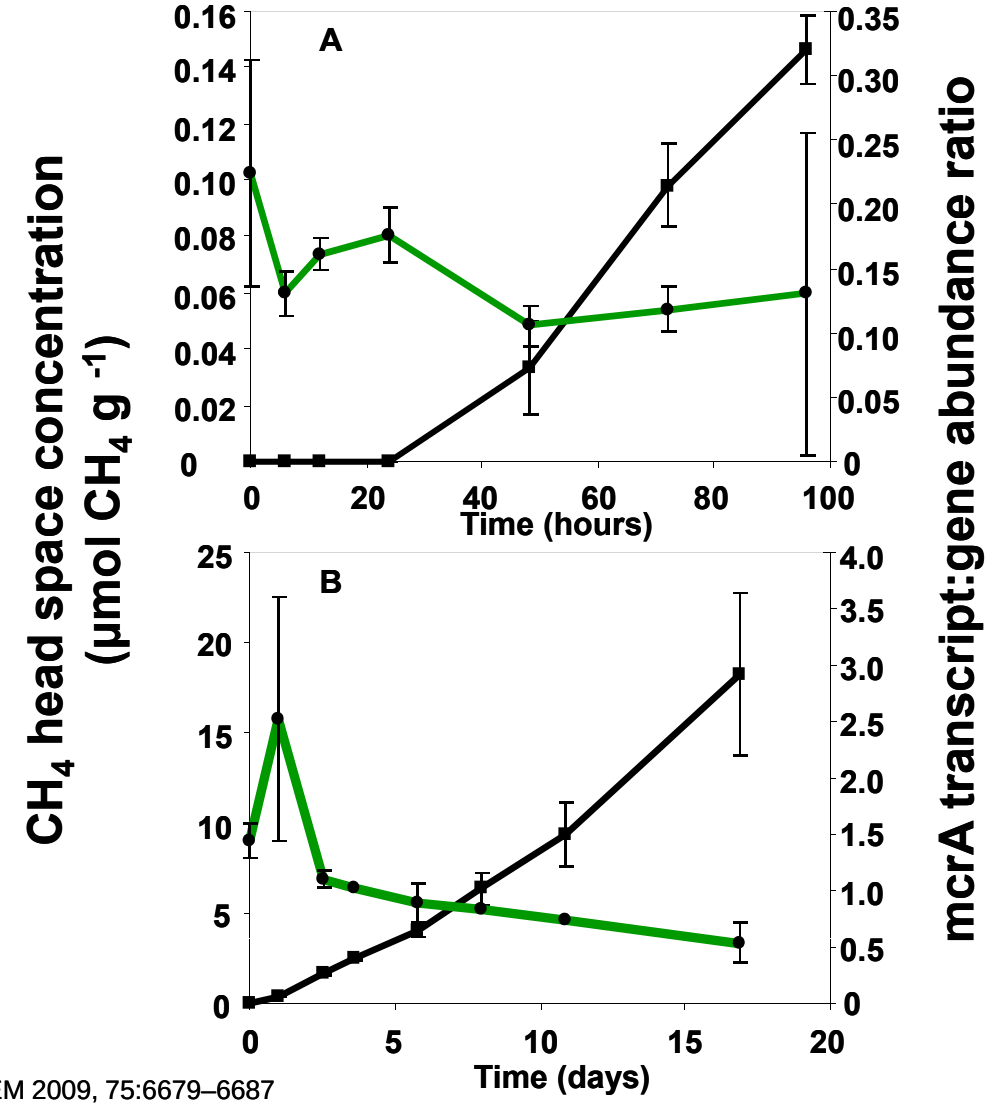
Field study: correlation of *mcrA* (methanogen) and *pmoA* (methanotroph) mRNA expression with methane flux

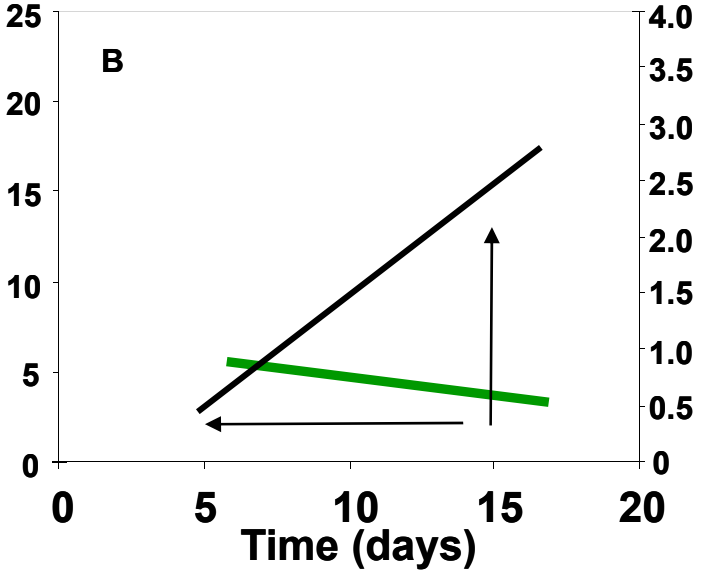
CH_4 emitting heather site EC6 ↑



- **Linear relationship of *mcrA* gene transcript abundance ratios with CH_4- flux; increasing ratios with increasing flux.**
- **No/weak linear relationship of *pmoA* gene with CH_4- flux.**
- **No linear relationship of *mcrA: pmoA* ratio with CH_4- flux.**

Methanogen response over time at constant temp.: physiological rates and *mcrA* transcript :gene ratios





AEM 2009, 75:6679–6687