Aerobic methane formation in Grey poplar plants grown under sterile conditions

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Introduction

Objections to the experimental design of Keppler *et al*. (2006), criticizing the use of static chambers and methane-free air: e.g.,

Kirschbaum *et al*. (2006), *Functional Plant Biology* **33**: 521–530
Dueck *et al*. (2007), *New Phytologist* **175**: 29–35

No observation of aerobic methane emission from plants: e.g.,

Dueck *et al*. (2007), *New Phytologist* **175**: 29–35
Beerling *et al.* (2008), *Global Change Biology* **14**: 1821–1826
Kirschbaum & Walcroft, *Biogeosciences* **5**: 1551–1558

Observation of aerobic methane emission from plants: e.g.,

Vigano *et al.* (2008), *Biogeosciences* **5**: 937–947
Wang *et al.* (2008), *Environmental Science & Technology* **42**: 62–68

Mechanisms of aerobic methane formation: e.g.,

Keppler *et al.* (2008), *New Phytologist* **178**: 808–814
McLeod *et al.* (2008), *New Phytologist* **180**: 124–132
Messenger *et al.* (2009), *Plant, Cell & Environment* **32**: 1–9

Open research questions

- **Missing proof for the absence of methanogenic microorganisms potentially contributing to aerobic methane emission from plants**
- **Convincing evidence that aerobic methane originates in living plant material**

Our experimental design

- Plant species: Grey poplar (*Populus* x *canescens*, syn. *Populus tremula* x *P. alba*), derived from cell cultures under sterile conditions
- Plants on sterile medium in gas-tight flasks in CH_4-free air
- Headspace was exchanged with synthetic air containing 20% of oxygen and 385 ppm $^{13}CO_2$ (99 at% ^{13}C)
- Flasks were kept in glove box filled with pure N_2 for 33 days under a 16/8 h light/dark regime
- GC-IRMS analysis of methane in the headspace
- Molecular biological analysis of plant material and medium for the methyl coenzyme M reductase alpha subunit (*mcrA*) gene
- EA-IRMS of bulk plant material after end of the experiment

Plant material

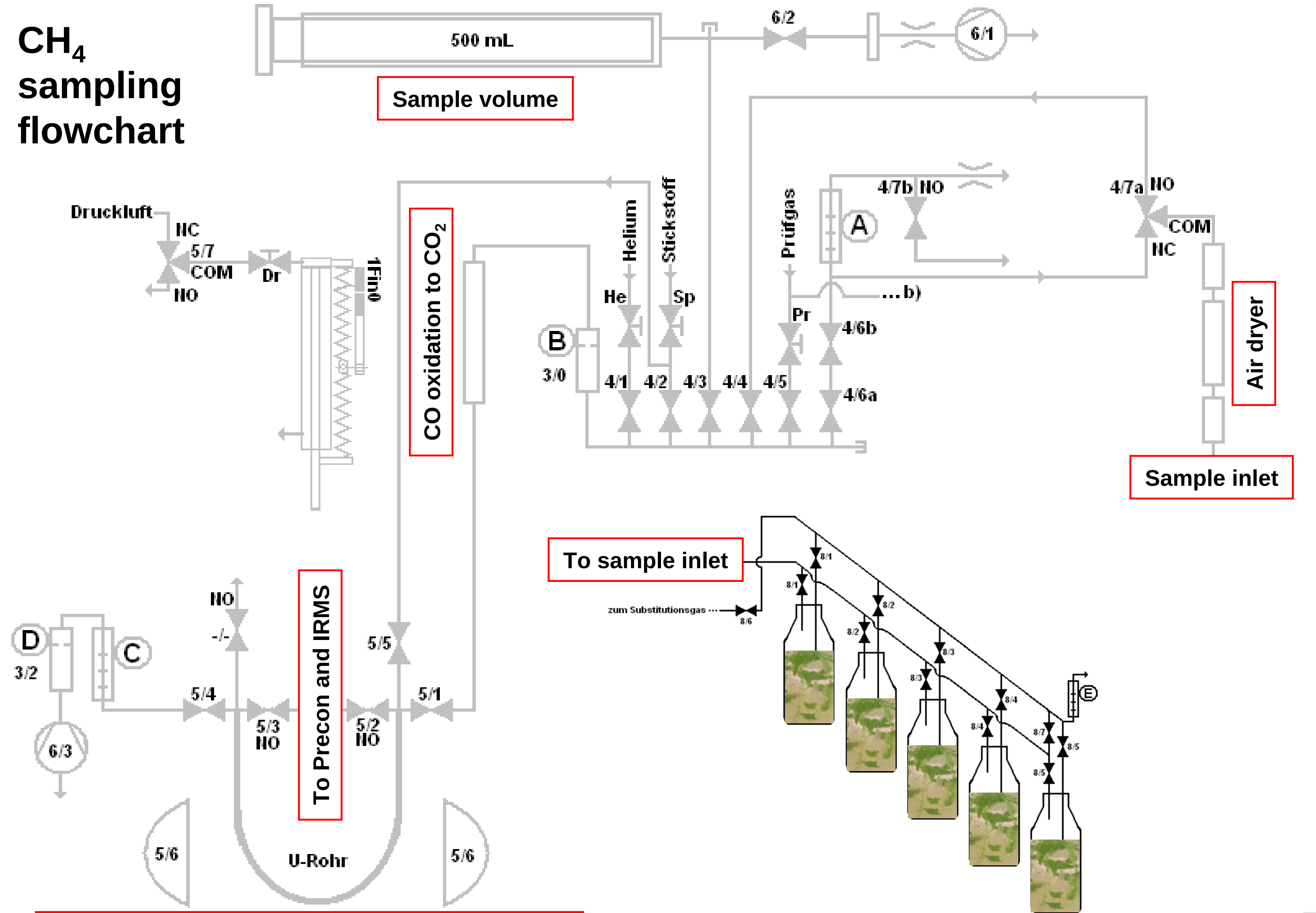
Wild type *Populus* × *canescens* (Aiton) Sm. (syn. *Populus tremula* × *P. alba*) lines, amplified by micro-propagation

7-8 plantlets were transferred under sterile conditions to 1-l sterile glass flasks, containing sterilized quartz sand and MS medium

The flasks were sealed with screw caps and sterilized valves; the inlet ports were additionally equipped with sterile filters (0.22 µm pore size)

The poplar plants were grown under standard conditions of 27°C : 24°C (day : night) and a light period of 16 h with approx. 100 µmol m^{-2} s^{-1} photosynthetic photon flux density (PPFD)

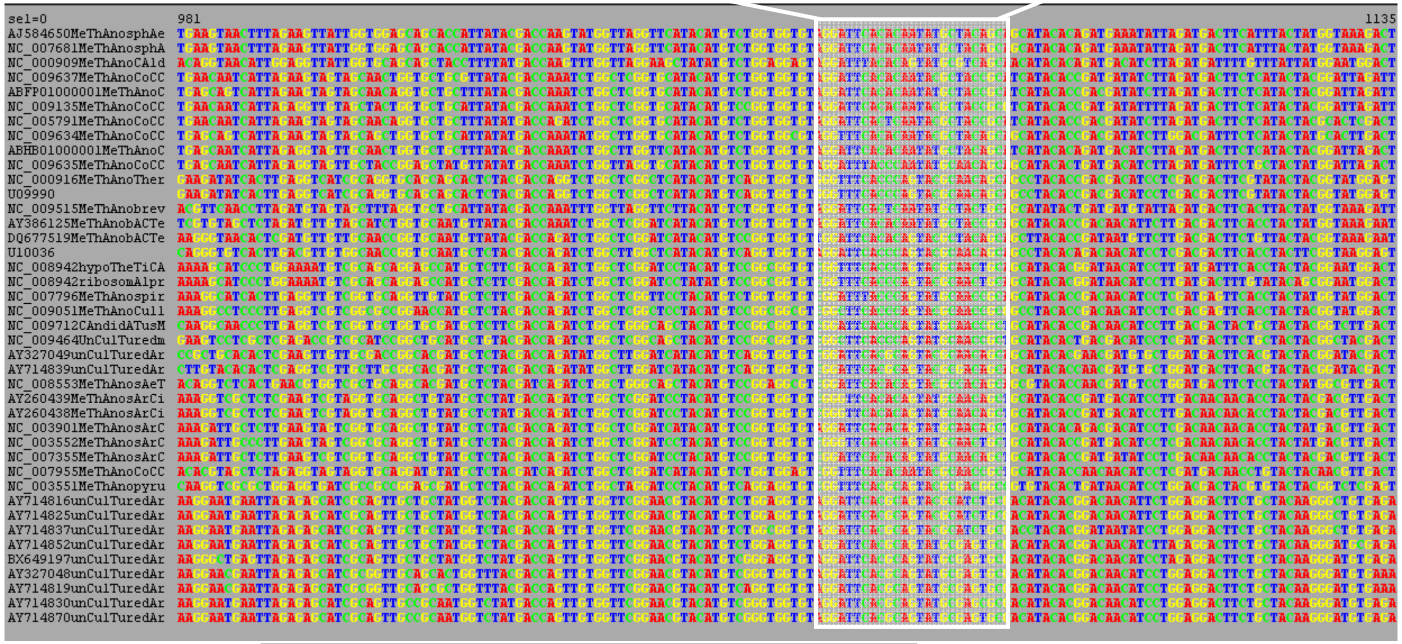
CH_4 sampling flowchart



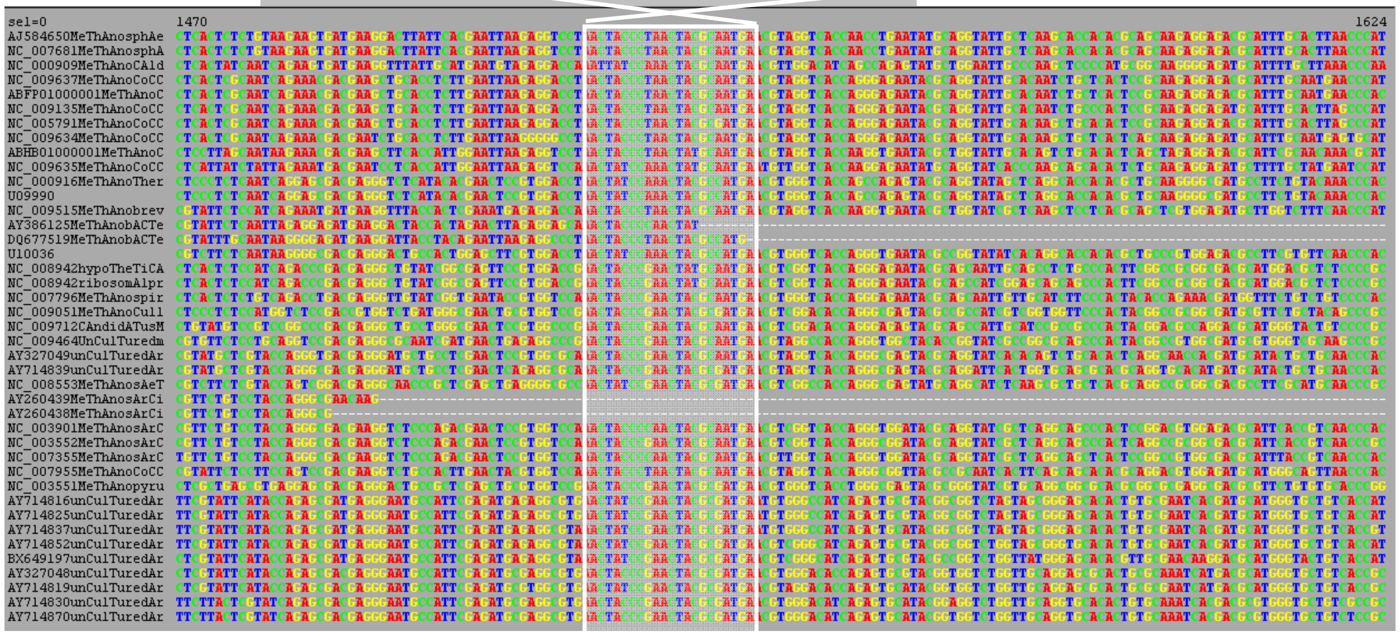
Condensation of sample air with freezing of CO_2

mrcA primers used for PCR

Forward primer: GGATTCACACARTAYGCWACAGC

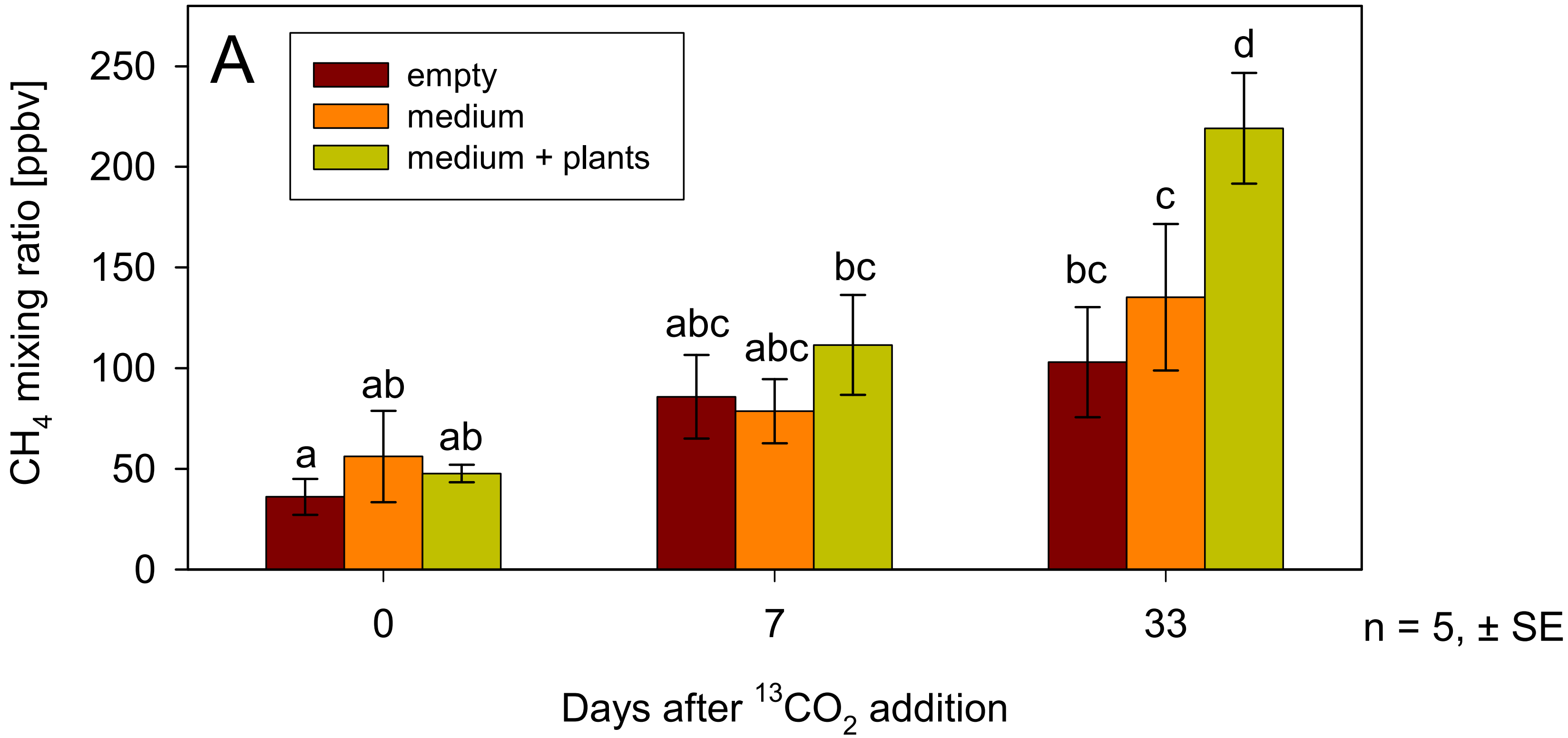


Reverse primer: TCATBGCRTAGTTHGGRTAGT



- Databases: thousands of mcrA sequences but only „few“ are full-length
- Alignment: to see conserved regions and design primers

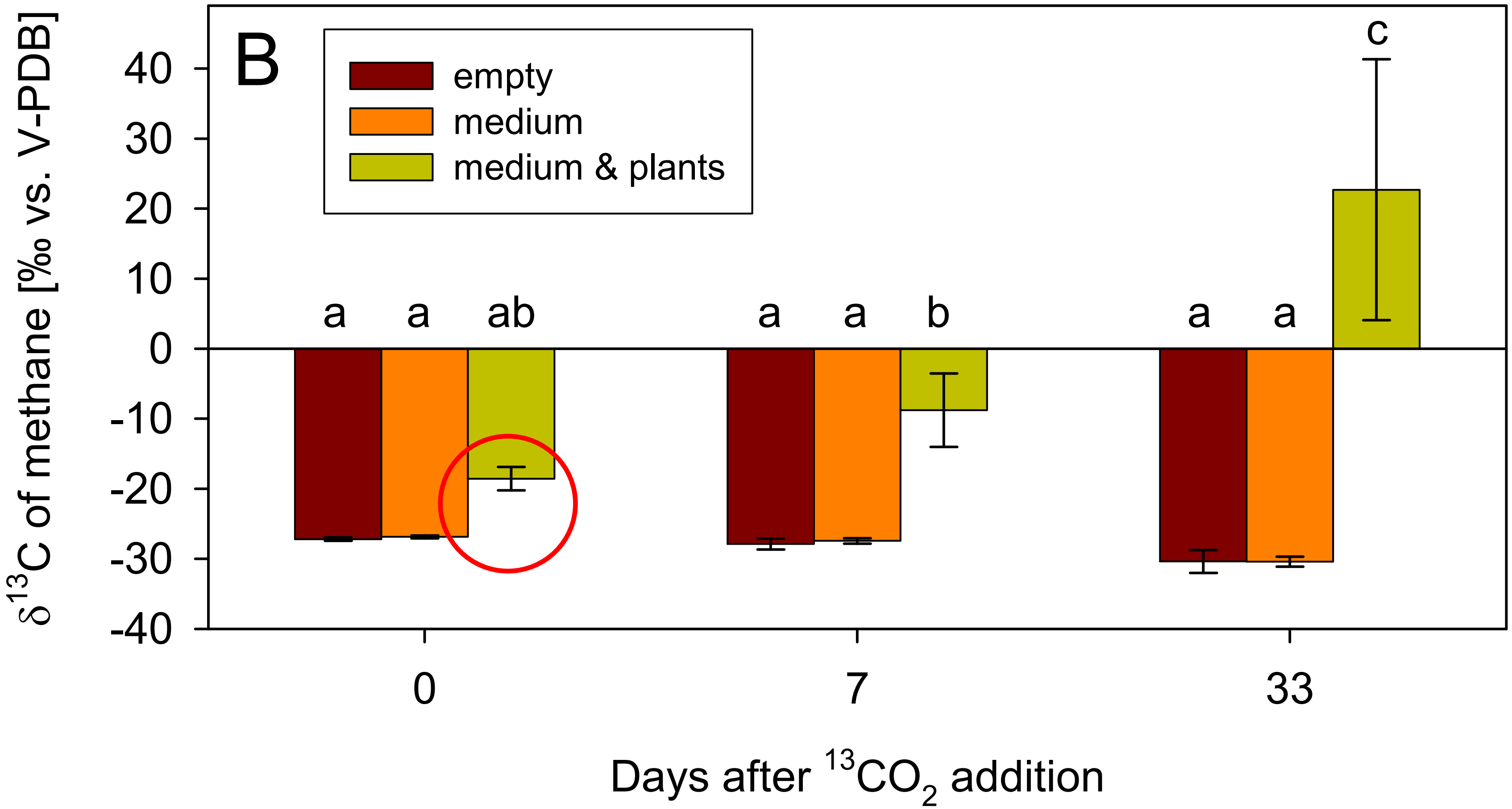
CH_4 formation



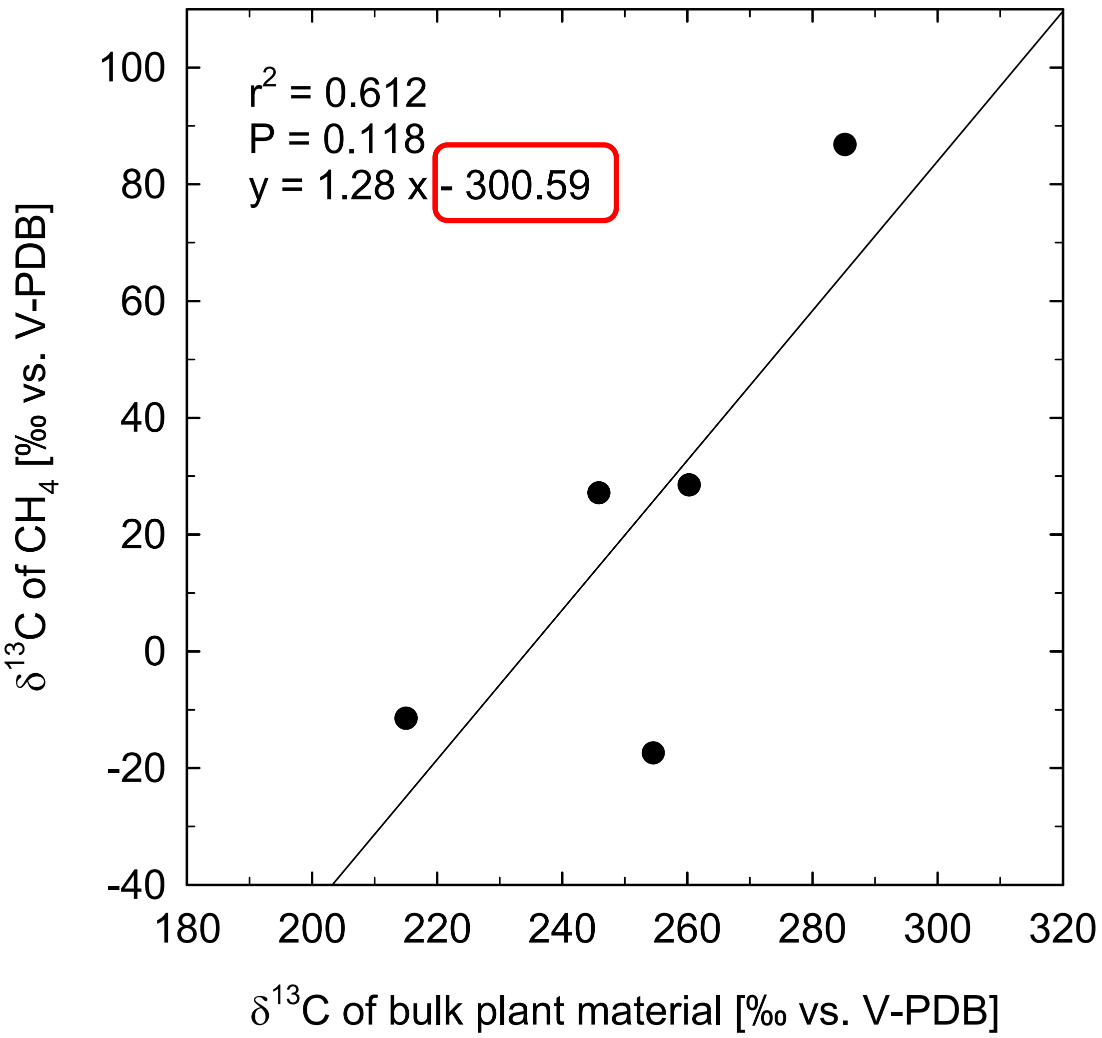
CH_4 release rate from plants (mean, ± s.e.m.)

0-7 days: 0.70 ± 0.37 ng g^{-1} dry weight h^{-1}
7-33 days: 0.16 ± 0.11 ng g^{-1} dry weight h^{-1}
0-33 days: 0.24 ± 0.06 ng g^{-1} dry weight h^{-1}

$\delta^{13}C$ of CH_4

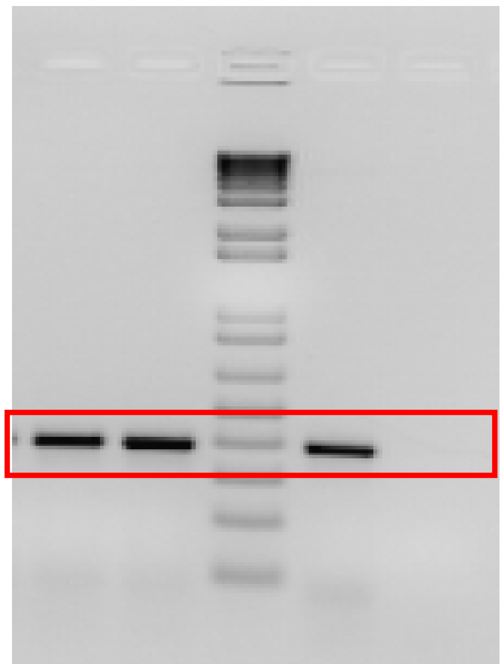


Relationship between $\delta^{13}C-CH_4$ and $\delta^{13}C$ of bulk plant material



Electrophoretic analysis of PCR products of medium & plants

M1 M2 L + -

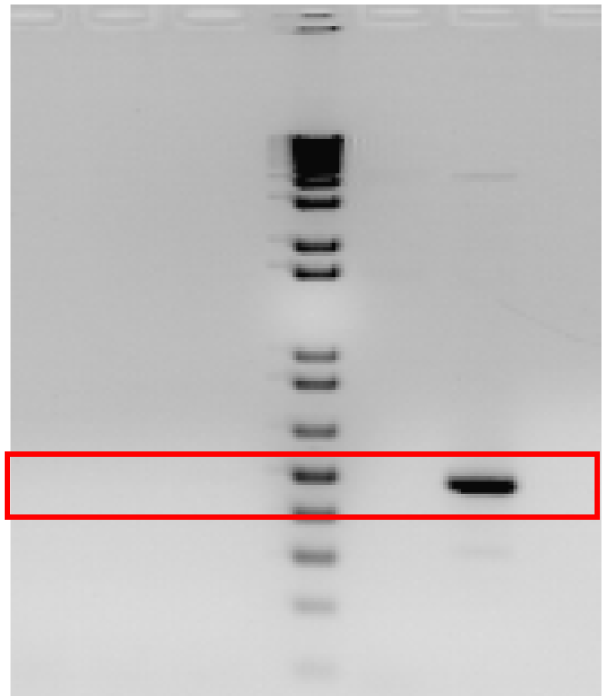


PcPSY

P1 P2 L + -

PcTUB

M1 P1 L - +



mcrA

PcPSY = oligonucleitid primer specific for the <u>*Populus × canescens* phytoene synthase</u> gene

PcTUB = oligonucleitid primer specific for the <u>*Populus × canescens* β-tubulin</u> gene

mcrA = oligonucleitid primer specific for the <u>methyl coenzyme M reductase</u> alpha subunit

Range of aerobic CH_4 from living and detached plant material

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ng CH_4 g^{-1} DW h^{-1}	References
ND	Kirschbaum & Walcroft, 2008; Nisbet et al., 2009, two species;
0.03	Vigano *et al.,* 2008, for a fully ^{13}C-labelled wheat leaf of Dueck et al., 2007, without UV light
0.16–0.7	**Our work**
0.5–13.5	Wang *et al.*, 2008, nine emitting species (35 non-emitting species)
–10–42 (not significantly different from 0)	Dueck *et al.*, 2007, six species
Up to 32	Vigano *et al.,* 2008, for a fully ^{13}C-labelled wheat leaf of Dueck *et al.*, 2007, without UV light
32–49 (not significantly different from 0)	Beerling *et al.*, 2008, two species
12–370	Keppler *et al.*, 2006, five species

Summary

- We have observed release of ^{13}C-labelled CH_4 from poplar significantly different from zero under low (UV-free) light conditions after $^{13}CO_2$ labelling
- The ^{13}C-label was detectable in CH_4 released from the plants already several minutes after start of $^{13}CO_2$ labelling
- However, poplar methane emission rates are at the lower end of the reported CH_4 emission rates from living or detached plant material
- Our work is the first molecular biological proof for the absence of methanogenic microorganisms in plants emitting CH_4 under aerobic conditions

The “perfect” aerobic methane experiment?

Goal:
Elucidation of CH4 mechanism(s) with simultaneous determination of realistic emission rates

- Experiments at ambient gas (CH_4, O_2, CO_2) concentration levels
- Stable isotope labelling essential to differentiate between plant and atmospheric methane
- Analysis of plant-internal reactive oxygen species (ROS)
- Molecular biological verification of the absence of methanogenes
- Application of defined stress situations initiating ROS formation
- …(open for discussion)