

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

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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

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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 CH₄-free air
- Headspace was exchanged with synthetic air containing 20% of oxygen and 385 ppm ¹³CO₂ (99 at% ¹³C)
- Flasks were kept in glove box filled with pure N₂ 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

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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-I 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 μ mol m⁻² s⁻¹ photosynthetic photon flux density (PPFD)

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mrcA primers used for PCR

Forward primer: GGATTCACACARTAYGCWACAGC

Databases: thousands of mcrA sequences but only "few" are full-length

Alignment: to
see conserved
regions and
design primers

ber 0 501		1100
AJ584650MeThanosphae T AA TAACTITA AA TTATT T A CA CACCATTATAC ACCAA TAT TTA TTCATACAT TCT T	T T CATTCACACAATAT CTACA	CATACACA AT AAATATTA AT ACTTCATTTACTAT TAAA ACT
NC 007681MeThanospha T as TaboTTTE as TTATT T & Ca Caccattata: accas tat. TTA TTCATACAT TCT. T	T T ATTCACA BATAT CTACA	З сатасаса ат завтатта ат асттсатттастат тава аст.
NC 000909MeThanofald are taboatt a trait T ca ca cracettrat accas fit TTA as cratat for a	A TA ATTTACA A TAT . TCA	ACATACACA AT ACATCTTA AT ATTTT TTTATTAT AAT A
NC 009637MeThanofoff T as astratta as to ta cast T C C TTATAC ACCESSTCT CTC T CATACAT TCT T	T TI APPEARACACATAC CTACE	ТСАТАСАСС АТ АТАТСТТА АТ АСТТСТСАТАСТАС. АТТА АТТ
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NE 005791MeThAnoEoCC T BACBATCATTA BA TA TA CARA T CT CTTATAT BCCA BTCT CTC T CATACAT TCT T	T TI ATTCACTCAATAC CTACC	ЭТСАТАСАСС АС АТАТСТТА АТ АСТТСТСАТАСТАС САСТС АСТ
NC 009634MeThanolocc T & CA TCATTA BA TA TA CA CT T CT CATTATAT ACCRATATAT CTT T CATACAT TCT T	C T1 TTTCACACABATAC CTACA	CATACACC AC ATATCTT, AC ATTTCTCATACTAT CACTT ACT
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AV386125MeThAnobalTe TO T TA CTOTA AT TT TA CATCE T CAAT TTATAC ACCADATCE OTO ATCATACAT TOT T	T TI ATTCACACTATAT CTACC	сатасасс всавсаттстт вс асттсасствствт, тава ват
D0677519MeThannhACTE as Table TO AT TT TT CARC T CART TTATE ACCA ATCT CTC ATCATACAT TCC T	T TE ATTCACACA TAC CTACA	CTTACACC ATAAT TTCTT AC ACTTCT TTACTAC TABA BAT
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NE 008942hypoTheTiEA BASA CATCOCT BASAT TO CA CA A COST CTOTTO ACCA ATCT CTO ATCCTACAT TOC C	T T TTTCACCCA TAC CAACT	GATACAC ATABCATCOTT AT ATTTCACCTACTAC AAT ACT
NC 0089427 hosomalpr aaaa carcer aaaar re ca ca a coar crerre acca arer ere areratar ree c	T T TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT	CATACAC ATAACATCCTT AT ACTTT TATACA C AAT ACT
NC 007796MeThanoshir and carcart a TT TC T CA TT TAT CTCTTC acca aret of TTCTTCACA ATCT TTCTTC TC	T T ATTACCTA TAT CARCO	сатасасс всавсатосто ат а ттовостастат тат аст
NE 009051MeThanoEull and CETCETT & TO TO C CO ABCOT CTCTED BOOK BTOT CTC CTCCTECHT TOC C	C T CTTCACCCA TAC CAACC	сстасасс всавсатесто вс в ттелестветве тат вст
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NC 009464UnCulTuredm as TOTE OTC & SCOTE CATCE OT CAT CT TAC ACCA ATCT OTC CA CTACAT TOC C	T T CTTCACCCA TAT CAACC	CATACACT AC ACATECTE AT ACTTCT CTACTAC CTAC
AV327049unCulTuredAr CC CT CACACTC BA TT TT C BCC CAC AT CTCTAC BCCA ATAT CTT ATCATACAT TCA T	T T ATTCAC CA TAC CAACA	CATACAC AAC AT T CT AT ACTTCAC TACTAC ATAC ACT
AV714839unCulTuredAr CTT TACACAGTC & TC TT CTT C CAC &T CTCTAC ACCA STAT CTT ATCATACAT TCA T	T T ATTCAC CA TAC C ACA	CATACACCARC AT T CT AT ACTTCAC TACTAC ATAC A
NC 008553MeThAnosAeT ACA TETCACT ABC T TO CT CA CAC AT CTCTAC ATCA ATCT CT CA CTACAT TOC A	C T ATTCACACA TAC CCACA	C TACACCARC AT TCCT. AT ACTTCTCCTACTAT. C TT ACT
AY260439MeThAnosArCi AAA TC CTCTC AA TC TA T CA CT TAT CTCTAT ACCA ATCT CTC ATCCTACAT TCC T	T T TTCACACA TAT CAR A	CATACACE AT ACATECTT ACAACAACACCTACTAC AC TT ACT
AY260438MeThAnosArCi AAA TO CTOTO BATO TA TOTA OT TAT CTOTAT ACCA ATCT OTO ATCOTACAT TOO T	T.T. TTCACACA TAT CAA A	CATACACC AT ACATCOTT ACAACAACACCTACTAC AC TT ACT
NC 003901MeThAnosArC ANA ATT CTCTT BA TA TO T CA CT TAT CTCTAC ACCA ATCT CTC ATCATACAT TCC T	T TI ATTCACACE TET CAN A	а сатасаса ас асатесте асаасаасасстастат ас III аст
NC 003552MeThAnosArC AAA ATT CCCTT BA TA TO C CA CT TAT CTCTAC ACCA ATCT CTC ATCCTACAT TCC T	T T TTCACCCA TAT CAACT	CATACACE AT ACATECTE ACAACAACACETACTAT AC TT ACT
NC 007355MeThAnosArC ARA ATT CTCTT BA TO TO T CA OT TAT CTCTAC ACCA ATCT CTC ATCCTACAT TCC T	T T ATTCACACA TAT CAACA	CATACACC AT ATATCCTC ACAACAACACCTACTAC AC TT ACT
NC 007955MeThAnoCoCC ACAC TA CTCTA A TA TA TA CA AT TAT CTCTAC ATCA ATCT CTC ATCATACAT TCT T	A T TTTCACACAATAC CAACC	CATACACCARCARCATCCTC AT ACARCCT TACTACARC TT ACT
NC 003551MeThAnopyru CAA TC C CT A T ATC CC CC A C AT CTCTAC ACCA ATCT CTA ATCCTACAT TCA A	T T TTTCAC CA TRC C AC	T TACACT ATAACATCCT AC ACTAC T TACTAC TCTC A T
AY714816unCulTuredar an ant antra a carc ca tt ct ctat tctac acca tt t ttc anc ta at tct a	T T & ATTCAC CA TRC CATCT	ACATACAC - ACAACATTCT - A - ACTTCT - CTACAA CT - T - A - A
AV714825unCulTuredar an ant antra a care ca tt et ctat tetas acca tt t tto and tacat tet a	T T L ATTCAC CA TAC CATCT	ACATACAC - ACAACATTCT - A - ACTTCT - CTACAA CT - T - A - A
AY714837unCulTuredAr AA AAT AATTA A A CATC CA TT CT CTAT TCTAC ACCA TT T TTC AAC TACAT TCT C	TTLEATCAC CA TAC CATCT	ACCTACAC - ATAATATCCT - A - ACTTCT - CTACAA CT - T - A - A
AY714852unCulTuredar an ant antra a a care ca tt et ctat tere acca tt t tte ane tagat tet a	T T L ATTCAC CA TAT C A T	ACATACACH ACAACATCTTA AH ACTTCT CTACAAH AT CAA A
BX649197unCulTuredAr AA CT A TTA A A CATC CA TT CT CTAT TCTAT ACCA TT T TTC AAC TACAT TC A	T T L ATTCAC CA TAT C A T	ACATACAC SEACAACATCCTA - A SEACTTCT - CTACAA - SEAT - T - A - A
AY327048unCulTuredAr AA AAC AATTA A A CATC C IT CA CACT ITTAC ACCA TT T TC AAC TACAT TC IT	TT ATTCAC CA TAT C A T	ACATACACH ACAACATCCT - AC ACTTCT CTACAA - AT T AAA
AY714819unCulTuredar AA AAC AATTA A A CATC C IT CA C CT ITTAC ACCA TT T ITC AAC TACAT TCA IT	TT ATTCAC CA TAT C A T	ACATACACH ACAACATCCT - AC ACTTCT - CTACAA - C AT - T - AAA
AY714830unCulTuredar AA AAT AATTA A A CATC CA TT CC CAAT TCTAT ACCA TT T TTC AAC TACAT TC T	T T & ATTCAC CA TAT C A C	ACATACACH ACAACATCCT - AC ACTTCT - CTACAA - C AT T A A
AY714870unCulTuredar AA AAT AATTA A A CATC CA TT CC CAAT TCTAT ACCA TT T TTC AAC TACAT TC T	TTL ATTCAC CA TAT C A T	ACATACACH ACAACATCCT HAF ACTTCT CTACAA HEAT TA A

Reverse primer: TCATBGCRTAGTTHGGRTAGT

sel=0	1470				1624
AJ584650MeThAnosphAe	CTCACTCTCT TAA AA T AT AA CACTTATTC	AC AATTAA A TCCT ACTACCCTAAC	PAC CART & IC TA TCACCAACCI	I AATAT CA TATT CTCAA CACCACAC CA CAA A	A AC CATTL CACTTAACCCAT
NC 007681MeThAnosphA	CTCACTCTCT TAA AA T AT AA CACTTATTC	AC AATTAA A TOOT IACTACCOTAACI	PACE CAATE A LC. TA COACCAACCI	I AATAT CA TATT CTCAA CACCACAC CA CAA A	A AC CATTL CACTTAACCCAT
NC 000909MeThAnoCAld	CTCACTATCAATCA AA T AT AA STTTATT C	AT AAT TA A ACCA ATTATECARACI	FAC CART A IC TT ACATCA CCI	A A TAT CTI AATT CCCAA CTCCCCAT CHICAA GO	A AT CATTTT CTTAAACCCAA
NC 009637MeThAnoCoCC	CTCACTO CAATCA AAAC AC AA CT CACCTO	TT AATTAA A ACCT HICTHCCCTRACI	FAC CRAT R IC TA TCACCA 7	A AATAC CA TATT CACAATCT CTCACTCC CAA A	A AC CATTT CAAT AACCCAT
ABFP01000001MeThAnoC	CTCACTC CAATCA AAAC AC AA CT CACCTC	TT AATTAA A ACCT ACTACCETRACI	PAC CRAT & IC TA TCACCA 7	A AATAC CA TATT CACAATCT CTCACTCC CAA A	A AC CATTL CAAT AACCCAC
NC_009135MeThAnoCoCC	CTCACTC CAATCA AAAC AC AA CT CACCTC	TT AATTAA A ACCT INCTRECEPARCI	PAC CART & IC TA TCACCA 7	A AATAC CA TATT CACAATCT CCCACTCC CAA A	A AT CATTL CACTLA CCCAT
NC_005791MeThAnoCoCC	CTCACTC CAATCA AAAC AC AA CT CACCTC	TT AATTAA A ACCT ACTACCCTAACI	PACECEATER ICETAL TCACCALES 7	A AATAC CA TATT CACAA CT CACACTCC CAA A	A AC CATTT CACTTA CCCAT
NC_009634MeThAnoCoCC	CTCACTC CAATCA AAAC AC AATCT CACCTC	TT AATTAA GUO CCT ACCTACCCTAAC	PACICART A <mark>LC TA TCACCA I 7</mark>	A AATAC CA TATT CACAA CT CTCACTCA CAA A	A AT CATTT CAAT A T CAT
ABHB01000001MeThAnoC	CTCCTTA CAATAA AAAC AC AA CTTCACCAT	T AATTAA A TCCT ACTACCCTAACI	PAT CAAT A IC TA TCACCAA T	A AATAC CT. TATT CACA TCT CACACTCA CTA A	A AC CATTC CAACAAAC CAT
NC_009635MeThAnoCoCC	CTCATTATCTATTA AAAT AC AATCCTCACCAT	T AATTAA A TCCA ACTATCCARAC	FAT CAAT A IT TT TCACCAA 7	A AATAT CA TATCACCCAA CA CACACTCT CAA A	A AT CTTTT CTAT AATCCAT
NC_000916MeThAnoTher	CTCCCTCTCAATCAL A C ACLALLTCTCATAC	AC AACTCC T ACCT ERCTRECCHARCI	PACE COMPER IC TELE TCACCA CCI	A A TAC CA I TATA CTCA I CACCACACI CT CAA GU	C AT CCTTCT TACAAACCCAC
U09990	CTCCCTCTCAATCA - A - C - AC - A - F - TCTCATAC	AC AACTCO T ACCT INCTATECAAAC	PACECCATER IC THE TCACCA CCI	A A TAC CAL TATA CTCA CACCACAC CT CAA GO	C AT CCTTCT TACAAACCCAC
NC_009515MeThAnobrev	C TATTCTCCATCA AAAT AT AA TTTACCAC	TC AAAT A A ACCA ACTACCCTAAC	FAC CART A <mark>IC TA TCACCAA I</mark>	A ATACICTI TATCICTCAA CTCCTCACICA CTCITI	A AT CTT TCTTTCAACCCAT
AV386125MeThAnobACTe	С ТАТТСТСААТТА А А АТ АА АСТАССАС	TA AACTTA A A CA ACTACCCCAAC	TAT		
DQ677519MeThAnobACTe	C TATTT CAATAA GOLA AT AA CATTACCTA	CA AATTAA A CCCT HACTACCCTAACI	TACCCCATC		
U10036	CFTCTTCTCAATAACGUGCFACLAUGUACTUCCAC	T CA CTTC TO ACCT HETHTECHARES	PACE COATER IC TO STOACCASSO	A BATACICCI FTATATCACA FCACCACAC ICT ICCCI TH	A AC CCTTC T TTCAACCCAC
NC_008942hypoTheTiCA	CTCACTCTCCATCA ACCORCIACION TATC	CATTCCT ACCURCTACCC RACI	PAT CAAT A <mark>IC TC TCACCA</mark>	A RATAC CA CRATT CA CCTCT CCCACTTC CCCCC	C AC CAT CAC CTCTCCCCCC
NC_008942ribosomAlpr	CTCACTCTCCATCA ACCORCEACE ASSECT STATCS	C A TTCC T ACC ACTACCE AACT	PAT CAAT A <mark>IC TC TCACCA 7</mark>	A RATAC CA CCATCO A CA CA CCCACTTCO CC CO	C AC CAT AC CTCTCCCCCC
NC_007796MeThAnospir	CTCACTCTCT TCA ACCT ACTACT TT TATC	T AATACC T TCCA ACTACCC AAC	PACE CAATER <mark>ICET</mark> EEE TCACCA I	A AATAC CA CAATT TT CATCTTCCCACTACACCA AAI	ACCATESTITCT TCT TCCCCAC
NC_009051MeThAnoCull	CTCCCTCTCCATESTCTCCSACCSTSSTCTSATS	C AACT C T TCCC ACTACCC AACI	FACECERTER IC TO EACACCA SEC	I A TAC CO CCATO TO STOTTCCCACTAC COCCO	C AT C TTCT CTACA CCC C
NC_009712CAndidATusM	CTOTATOTCO TCC SCCC SACOA SSOCT SCCT S	C AACTCC T CCC ACTACCC ARC	FACECRATER <mark>IC-TCE-ACACCA-E-</mark> J	A TAC CA CCATT CATCO CC CCCACTACE AC CCI	AFFACFCAT FFTACT TCCCCFC
NC_009464UnCulTuredm	C.T.TTCTCCT.CA. TCC.AC.A. C.C.CAATC	AT AACT A A CCC LACTACCC AAC	FAC CRAT R IC TA CCACCA I	I CTACACC TATE CC C CA CCCACTAC CC T	C AT C T TC CAA CCC C
AY327049unCulTuredAr	C-TAT-CTC-TACCA-G-T-AC-AGG-AT-CT-CC	TC AACTCC T C CA ACTACCC AACI	PAC CART A IC TA TCACCA C	. A TAC CA TATCACACA TCT CACACTCA CAACCI	AC AT CATACT CT CAACCCAC
AY714839unCulTuredAr	C TAT CTC TACCA C C AC A CAT CT CC	TC AACTCA A C CA ACTACCC RACT	PAC C AT A LC TA TCACCA C	. A TAC CA ATTCACT T CA C CAC CA T CACI	AT AT CATACT CT CAACCCAC
NC_008553MeThAnosAeT	C TETTETE TACCA TEL ACIA CAACCO C	TC A CT A GEOCOCC ACTATOCIAACI	FACECE AT A COTCOCCACCA STO	I A TAT CA CATCTCAA C CT CTCAC CA CCC C	C AC CCTTC CAT CAACCE C
AY260439MeThAnosArCi	C TTCT TCCTACCA C AACAA		**********		
AY260438MeThAnosArU1	C TTCT TCCTACCA C C				
NC_003901MeThAnosArC	C TTCT TCCTACCA C AC AA TCTCCCA	AC AACTCC T TCCA IACTACCCCAAC	TAC CRAT A IC TC TCACCA	ATAC CA TATC CTCA CA CCCACTCC AC T	A AC CATTCACC TCAACCCAC
NC_003552MeThAnosArC	C TTCT TCCTACCA - C AC AA TCTCCCA	AC AACTCC T TCCA IA TA A AA	TAC CAAT A IC TC TCACCA	ATAC CA TATC CTCA CA CCCACTCA CC C	C AC CATTCACC TCAACCC C
NC_007355MeThAnosArc	T. TTCT. TCCTACCAC. AC. AA., TCTCCCA	AC AACTCC T TCCA IA TA CARA	IR ARE ALL TT TCACCA	A ATAC CA TATC CTCA CA CTCACTCC CC T	C AC CATTTACC TCAACCCAC
NL_00/955MeInAnololl	C TATTCTCCTTCCA TCC AC AA TCT CCAC	IT ARCTAC T TUCK IN THE STAR	EAST CRAFT & L. TA TUACUA TU	TTAC CC CARTCACITCA CA CACACICA ACIT	A AT CAT CA TTARCCCAC
NC_003551MeThAnopyru	CTC.CT.A.C.T.AA.C.AC.AACT.CC.C	TC A CT C T TCC IACTACCO ARC	LAC C. AL A C. T TCACCT	A TACICI TATCITICA COLCACICACICI CAL	C AC C TTCT T T CACCCI
AT/14816unculturedar	TTC TATTCATACCA A C AT A A AAT CCAT	TU A AT A A UT INCLATOU ARC	IAC U AI A II TU CUATUA A I	A C TAU C TUTA TA U A CAUACT T C AATU	AC AT CAT STT UT TUACUAT
AT/1482SunculturedAr	TIC TATICATACCA A C AT A C ART CCAT	ICARIAA CI.IRTALCAR	TAC. C. AL. A II. T. C. CAICA A	A CUTAC CULTURA TA CULUA CACACI I CUARICI	ACCAL CALCULUT CACCAL
AT/1483/unculturedar	TTC TATTCATACCA A C AT A A AAT CCAT	TC A AT A A C TA A TATA RR	LAU X AX A II TU CUATUA A I	A CATACI CUITCTA TA CUITA CACACTITIC AATCI	IC AT CAT SOT CTOTCACCOT
AT/14852untulTuredAr	TTC TRITCATACCA A C AT A C AAT CCAT		IR C AT A C TC CATCA A	COTRECE CONTENTS OF CONCERNMENT OF THE CONCERNMENT.	C AT CAT OF CT TCACCAT
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AT32/048unculluredAr	CICCINITCATACCA A C AC A AAT CCAT	IC A HI CA CT HEIROC ARC	INC. C. H. R. C. T. ACACCA A.	CONTRACT TOTOTT CALLAR COURCE CONTRACTOR	
AT714619unculTuredAr	THE THE CHIRCH A C AC A C ART COAT	TO A ALL OT COT LACTATICE ARC		CATRONIC TO TOTOTT CA CA COCACT CCC AATC	
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δ^{13} C of CH₄



Relationship between δ^{13} C-CH₄ and δ^{13} C of bulk plant material





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Range of aerobic CH₄ from living and detached plant material



ng CH ₄ g ⁻¹ DW h ⁻¹	References
ND	Kirschbaum & Walcroft, 2008; Nisbet et al., 2009, two species;
0.03	Vigano <i>et al.,</i> 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 <i>et al.</i> , 2008, nine emitting species (35 non-emitting species)
-10-42	Dueck <i>et al.</i> , 2007, six species
(not significantly different from 0)	
Up to 32	Vigano <i>et al.,</i> 2008, for a fully ¹³ C- labelled wheat leaf of Dueck <i>et al.</i> , 2007, without UV light
32–49	Beerling <i>et al.</i> , 2008, two species
(not significantly different from 0)	
12–370	Keppler <i>et al.</i> , 2006, five species

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Summary



- We have observed release of ¹³C-labelled CH₄ from poplar significantly different from zero under low (UV-free) light conditions after ¹³CO₂ labelling
- The ¹³C-label was detectable in CH₄ released from the plants already several minutes after start of ¹³CO₂ labelling
- However, poplar methane emission rates are at the lower end of the reported CH₄ 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 CH₄ under aerobic conditions

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The "perfect" aerobic methane experiment?



Goal:

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

- Experiments at ambient gas (CH₄, O₂, CO₂) 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)

Nicolas Brüggemann & Jörg-Peter Schnitzler | IMK-IFU | Garmisch 1st workshop on aerobic methane formation, 26/27 Feb 2009, Mainz KIT – a Cooperation between Karlsruhe Research Center and University of Karlsruhe

