Rapid report

No evidence for substantial aerobic methane emission by terrestrial plants: a ^{13}C-labelling approach

Author for correspondence:
Tom A. Dueck
Tel: +31 317475918
Fax: +31 317423110
Email: tom.dueck@wur.nl

Received: *19 March 2007*
Accepted: *30 March 2007*

Tom A. Dueck,[1] Ries de Visser,[2] Hendrik Poorter,[3] Stefan Persijn,[4] Antonie Gorissen,[2] Willem de Visser,[1] Ad Schapendonk,[5] Jan Verhagen,[1] Jan Snel,[1] Frans J. M. Harren,[4] Anthony K. Y. Ngai,[4] Francel Verstappen,[1] Harro Bouwmeester,[1] Laurentius A. C. J. Voesenek[3] and Adrie van der Werf[1]

[1]Plant Research International, PO Box 16, 6700 AA, Wageningen, the Netherlands; [2]IsoLife, PO Box 349, 6700 AH, Wageningen, the Netherlands; [3]Plant Ecophysiology, Utrecht University, PO Box 800.84, 3508 TB, Utrecht, the Netherlands; [4]Molecular & Laser Physics, Radboud University, Toernooiveld 1, 6525 ED Nijmegen, the Netherlands; [5]Plant Dynamics, Englaan 8, 6703 EW, Wageningen, the Netherlands

Key words: ^{13}C-labelling, climate change, methane emission.

Summary

- The results of a single publication stating that terrestrial plants emit methane has sparked a discussion in several scientific journals, but an independent test has not yet been performed.
- Here it is shown, with the use of the stable isotope ^{13}C and a laser-based measuring technique, that there is no evidence for substantial aerobic methane emission by terrestrial plants, maximally 0.3% (0.4 ng g^{-1} h^{-1}) of the previously published values.
- Data presented here indicate that the contribution of terrestrial plants to global methane emission is very small at best.
- Therefore, a revision of carbon sequestration accounting practices based on the earlier reported contribution of methane from terrestrial vegetation is redundant.

New Phytologist (2007) **doi**: 10.1111/j.1469-8137.2007.02103.x

Introduction

Methane is a very potent greenhouse gas and originates both from anthropogenic and natural sources (Bousquet *et al.*, 2006). Recent findings suggest that terrestrial plants may also emit methane under aerobic conditions by an as yet unknown physiological process (Keppler *et al.*, 2006), and in this way may substantially contribute to the annual global methane budget (Bousquet *et al.*, 2006). Scaling up from individual plants to global vegetation resulted in estimated values for methane emission by terrestrial plants varying between 10 and 260 Tg yr^{-1} (Houweling *et al.*, 2006; Keppler *et al.*, 2006; Kirschbaum *et al.*, 2006). These values are impressive and can have large repercussions for the mitigation of climate change. The high emission rates might account for the plumes of methane observed above tropical forests (Frankenberg *et al.*, 2005). These high methane emissions might also provide a link between the annual decline in growth rate of atmospheric methane, on the one hand, and deforestation during the last decade on the other (Dlugokencky *et al.*, 1998; Bousquet

et al., 2006; Keppler *et al.*, 2006). Keppler *et al.* (2006) sparked a discussion among both the scientific community and the general public (Parsons *et al.*, 2006; Lelieveld, 2006; Lowe, 2006; Schiermeier, 2006) and their data are being used in global methane modelling (Bousquet *et al.*, 2006; Houweling *et al.*, 2006), However, these discussions are based on short-term experiments in one single laboratory which were criticized for the experimental setup (Kirschbaum *et al.*, 2006). Therefore, our aim was to re-examine their findings in an independent study by testing, in both the short and longer terms, whether plants are, in fact, able to emit methane.

Materials and Methods

Plant growth

Six plant species were used – *Ocimum basilicum* L. (basil), *Triticum aestivum* L. (wheat), *Zea mays* L. (maize), *Salvia officinalis* L. (sage), *Lycopersicon esculentum* Miller (tomato), and *Oenothera biennis* L. (common evening primrose) – the first three of which were also used by Keppler *et al.* (2006). Since methane emission appeared to be species-dependent, wheat and maize were included because they showed the highest methane emission rates in the Keppler *et al.* (2006) study. The plants were grown together in the ESPAS (Experimental Soil Plant Atmosphere System) facility (Gorissen *et al.*, 1996), a unique hermetically sealed plant growth chamber with a volume of 3500 l, specifically designed for atmospheric isotope labelling. Plants were grown hydroponically (i.e. soil-free) to exclude any methane production derived from anaerobic soil pockets.

The environmental and atmospheric conditions were fully controlled in the ESPAS. Plants were labelled (IsoLife BV, Wageningen, the Netherlands) from seed on hydroponics for a period of 9 wk in ^{13}C-CO_2 (99 atom % ^{13}C, 1% ^{12}C using CO_2 from cylinders in which no ^{13}C-methane could be detected; Isotec, Inc., Miamisburg, OH, USA) instead of the natural atmospheric ^{13}C-CO_2 concentration (1.1% ^{13}C, 98.9 atom % ^{12}C). Plants were grown at a light intensity of 500 μmol m^{-2} s^{-1} during a 16 h day, a day : night temperature of 23 : 18°C and RH of 75 : 80%. Under these light conditions, most herbaceous plant species grow at their maximum rate (Poorter & Van der Werf, 1998). CO_2 concentrations in the growth chamber were 550 ppm on average during the light period.

Measurements of methane emission

In the first experiment, two to four plants of basil, sage, wheat and maize were transferred to continuous-flow gas exchange cuvettes (Poorter & Welschen, 1993) 7 and 8 wk after sowing for methane measurements. In this system, shoots were sealed off from the roots, that is, any methane measured would have been derived from the shoots only. All measurements were performed in comparison to measurements of control cuvettes without plants, at background ^{13}C- and ^{12}C-methane concentrations of 22 and 2100 ppb, respectively. The plants were allowed to attain steady-state conditions for at least 2 h, after which measurements were performed. In order to increase the sensitivity of the methane measurements, the flow was set to a relatively low value of 60–120 l h^{-1} for plants with a shoot dry weight (DW) of 5–14 g (leaf areas varying from 800 to 1700 cm^2). Consequently, ^{13}C-CO_2 concentration in the cuvettes was *c.* 300 ppm (with *c.* 900 ppm CO_2 entering the cuvette), except for the larger maize plants, which were measured at *c.* 200 ppm ^{13}C-CO_2 and RH was above 90%. Plants were measured at a light intensity of 300 or 600 μmol m^{-2} s^{-1}, and with a corresponding air temperature in the cuvette of 25 or 35°C, respectively.

In the second experiment, the ESPAS growth chamber was briefly vented after 9 wk with ambient air to remove any possible accumulated methane. The incoming ^{12}C-CO_2 in the ambient air was captured in soda lime and replaced by 99 atom % ^{13}C-CO_2, after which air samples were taken at 2 d intervals during a 6 d period for methane analysis. Contrary to measurements in the first experiment, roots were not sealed off from the shoots. Any methane measured in the ESPAS growth chamber would have originated from both shoots and roots.

The ^{13}C enrichment of plant material was determined using GC-MS analysis (after derivatization) of leaf extracts. The average atom % ^{13}C was deduced from the mass spectral peak intensities of ^{12}C and ^{13}C containing fragments of fructose.

Measurement of methane

Gas samples (2–4 l) from the continuous-flow gas cuvettes and the ESPAS facility were collected in Tedlar and aluminium-coated Teflon bags. Before measurements, transpiration water was removed with a $CaCl_2$ scrubber in order to prevent dilution effects. The concentration of ^{13}C-methane in the samples was determined using photo-acoustic spectroscopy in combination with a continuous-wave, optical parametric oscillator (OPO). The OPO combines high power (> 1 watt), a broad tuning range (2.75–3.83 μm) and a narrow line width (4.5 MHz over 1 s) (Van Herpen *et al.*, 2002). A high sensitivity for trace gas detection is achieved when operating in the mid-infrared wavelength region where molecules have their strongest vibrational absorption bands. The precision of the laser-based system is shown in Fig. S1 (Supplementary Material). At the natural background concentration (22 ppb ^{13}C-methane), a detection limit of 3 ppb is realized (Van Herpen *et al.*, 2002; Ngai *et al.*, 2006). The gas from the sampling bags was sucked through the detection cell at a flow rate of 1–2 l h^{-1}. ^{13}C-methane was detected using one of its strong absorption features centred at 3240.08 nm. Figure 1 shows calculated and measured ^{12}C-methane and ^{13}C-methane absorption spectra in air. To subtract interferences from ^{12}C-methane, the OPO was scanned over a wider wavelength range of 0.5 nm. The complete detection system was calibrated using bags with a known concentration of ^{13}C-methane (Isotec, Inc, Miamisburg, OH, USA).

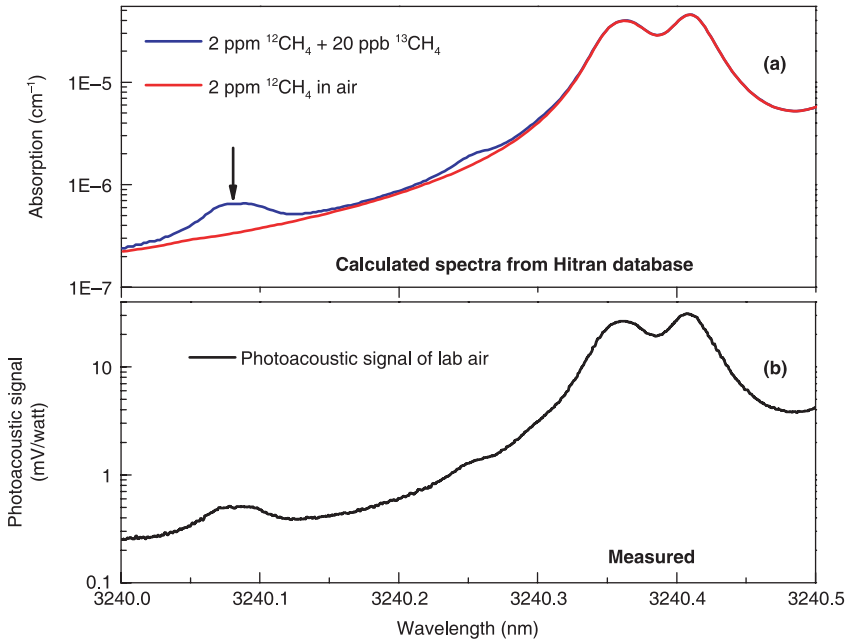

Fig. 1 Methane spectra. (a) Spectra calculated from the Hitran database (http://cfa-www.harvard.edu/Hitran/). The arrow indicates the ^{13}C-methane peak used for the measurements. (b) Measured photoacoustic spectrum of laboratory air.

Calculation of methane emission rates

In Expt 1, the methane emission rate (MER) was calculated by multiplying the flow rate of incoming air into the cuvette by the difference in methane concentration between cuvettes with and without a plant. No correction for dilution of the airstream by transpiration was made, as air samples were dried before the measurements. In Expt 2, MER was calculated as follows:

$$\text{MER (nl g}^{-1}\text{ h}^{-1}) = (\text{AM}t_2 - \text{AM}t_1)/(((\text{PDW}t_2 + \text{PDW}t_1)/2) \times (t_2 - t_1)) \quad \text{Eqn 1}$$

(AM, amount of ^{13}C-methane in the ESPAS (nl); PDW, total plant DW in the ESPAS (g); t, time (h)).

Photosynthesis

Photosynthesis was measured using a Li-Cor 6400-40 with an incorporated light source with a programmable light intensity. Stomatal conductance and intercellular CO_2 concentration were calculated based on vapour pressure deficits and transpiration rates of the leaves, which were measured along with CO_2 assimilation rates.

Results

Expt 1

The $^{13}CO_2$ plants grown in the hermetically sealed ESPAS chamber did not differ visually in their morphology from $^{12}CO_2$-grown plants. They also showed normal rates of photosynthesis and photosynthesis-related parameters under these conditions (Table 1). Almost 99% of the carbon (98.4–99.0%) found in these plants was in the form of ^{13}C (data not shown). Thus, we can expect that nearly 99% of the methane emitted by these plants is in the form of ^{13}C-methane. After 7 and 8 wk of growth in the ESPAS growth chamber, four of the plant species were transferred to continuous-flow gas exchange cuvettes (Poorter & Welschen, 1993) and analysed for ^{13}C-methane emission from shoots under various environmental conditions. In general, the methane concentrations in the continuous-flow gas cuvettes with plants were not significantly higher than those of control cuvettes without plants (Table 2), the difference between the two being close to or below the detection limit. Based on this difference, we calculated emission rates for the four species ranging from –10 to 42 ng g^{-1} h^{-1}, with an overall mean of 21 ng g^{-1} h^{-1} (Table 3). These emission rates were not

Table 1 Net photosynthesis per unit leaf area, stomatal conductance and intercellular CO_2 concentration (C_i) of leaves at a photosynthetic quantum flux density of 600 μmol m^{-2} s^{-1} for four plant species grown in ^{13}C-CO_2

Species	Photosynthesis (μmol m^{-2} s^{-1})	Stomatal conductance (mol m^{-2} s^{-1})	C_i (ppm)
Ocimum basilicum (basil)	10.1 ± 0.8	0.15 ± 0.01	242 ± 2
Salvia officinalis (sage)	12.8 ± 0.8	0.39 ± 0.01	292 ± 3
Triticum aestivum (wheat)	9.6 ± 0.8	0.28 ± 0.09	290 ± 15
Zea mays (maize)	8.3 ± 0.0	0.07 ± 0.01	165 ± 18

Values are means ± SE (n = 2 – 3).

Table 2 Mean methane difference in concentrations between measuring and control cuvettes (with 22 ppb background ^{13}C-methane) from four plant species under steady-state conditions

Species	LL (ppb)	HH (ppb)	Mean (ppb)
Ocimum basilicum (basil)	2.0 ± 0.9	0.1 ± 0.5	1.2 ± 0.7
Salvia officinalis (sage)	1.0 ± 1.8	3.1 ± 1.6	1.8 ± 1.2
Triticum aestivum (wheat)	1.1 ± 1.1	−0.5 ± 2.0	0.6 ± 1.0
Zea mays (maize)	1.8 ± 1.0	1.0 ± 1.5	1.5 ± 0.8

LL, low light, low temperature; HH, high light, high temperature. Values are means ± SE.
Measurements were performed under conditions of low light and low temperature (LL, 300 μmol m^{-2} s^{-1}, 25°C), and high light and high temperature (HH, 600 μmol m^{-2} s^{-1}, 35°C). ^{13}C-methane concentrations in the control cuvettes varied from 17 to 24 ppb, with a mean of 19.3 ppb. Mean value per species are given (n = 2–4). Differences between treatments and species were tested with a weighted analysis of variance at $\alpha = 0.05$.

Table 3 Emission rates of ^{13}C-methane from four plant species under relatively low light and low temperature (LL), and relatively high light and high temperature (HH)

Species	LL (ng g^{-1} h^{-1})	HH (ng g^{-1} h^{-1})	Mean (ng g^{-1} h^{-1})
Ocimum basilicum (basil)	42 ± 42	−1 ± 6	25 ± 24
Salvia officinalis (sage)	16 ± 23	20 ± 17	17 ± 12
Triticum aestivum (wheat)	26 ± 33	−10 ± 12	14 ± 36
Zea mays (maize)	39 ± 54	7 ± 12	28 ± 36
All species	31 ± 17	4 ± 7	21 ± 11

Values are means ± SE.
LL, 300 μmol m^{-2} s^{-1}, 25°C; HH, 600 μmol m^{-2} s^{-1}, 35°C. Emission rates are based on the concentration values in Table 2 (n = 2–4). Differences between treatments and species were tested with a weighted analysis of variance at $\alpha = 0.05$.

statistically significant from zero. Our emission rate with the continuous-flow system was six to 18 times lower than the average methane emission rates given by Keppler *et al.* (2006), that is, 119 and 374 ng g^{-1} h^{-1}, compared with our measurement of 21 ng g^{-1} h^{-1}. Increasing the light intensity and temperature did not increase the emission of methane (Table 3). An additional test was performed to check for leakage, oxidation or adsorption, by adding a known amount of labelled methane to the flow-through system. A recovery of *c.* 98% was achieved. Therefore, we can preclude the loss of methane in the experimental system.

Expt 2

Even with our approach to boost sensitivity by measuring ^{13}C-methane, we came close to the detection limit of our laser-based technique. In order to substantiate our findings with additional evidence, we performed a second, longer-term experiment with a much greater plant biomass. We grew a large number of plants from six species in the ESPAS facility. It was briefly flushed with ambient air after 9 wk to remove any possible accumulated methane. Air samples were taken at 2 d intervals during a 6 d period for ^{13}C-methane analysis. Contrary to the first experiment, in which only shoots were measured, any methane measured here would have been derived from shoots and/or roots. During this 6 d period, the total plant biomass in the ESPAS growth chamber increased from 289 to 374 g DW. Based on the measured average methane emission of 21 ng g^{-1} h^{-1} in the first experiment and the plant biomass present in the growth chamber, we expected to measure 495 ppb ^{13}C-methane at the end of this period, a value well above our detection limit. In reality, we found an increase over time of less than 1 ppb ^{13}C-methane (Table 4), which is only 0.1 and 0.3%, respectively, of the emissions that would have been expected on the basis of the rates under 'sunlight' and 'no sun' conditions reported by Keppler *et al.* (2006) (Fig. 2b). This implies an emission rate of between –0.9 and 0.4 ng g^{-1} h^{-1}, which is not statistically different from zero (Fig. 2a). Recovery checks in the ESPAS growth chamber without plants following injection of ^{13}C-methane showed that only 0.3% of the 400 ppb methane spike was lost daily during a 6 d period. This rules out the possibility of substantial loss through leakage, oxidation or adsorption.

Discussion

One of the consequences of using a flow-through gas exchange system is that methane measurements must be highly sensitive to measure changes in methane concentrations against a background concentration of *c.* 2000 ppb methane. Therefore, we made use of plants uniformly labelled with ^{13}C, an optical parametric oscillator for methane detection, and used low air flow rates relative to the amount of biomass present. Uniform ^{13}C-labelling of lower plant species such as algae has already been performed for decades (Berthold *et al.*, 1995). Even though fractionation of ^{13}C occurs to some extent in a range of enzymatic reactions and metabolic pathways (Farquhar *et al.*, 1989; O'Leary *et al.*, 1992; Paré *et al.*, 1998), the strength of the fractionation is generally far less than 1%. No indication of significant 'metabolic shifts' exists in uniformly ^{13}C-labelled plants (Kurilich *et al.*, 2003; Kikuchi *et al.*, 2004; Novotny *et al.*, 2005; Kelliher *et al.*, 2006). We are therefore confident that our ^{13}C-grown plants behaved similarly to ^{12}C-grown plants.

In their experiment, Keppler *et al.* (2006) made use of relatively small closed cuvettes, which results in a continuous decline in CO_2 and an increase in relative humidity, air temperature and especially leaf temperature, as well as an accumulation of methane. Under these conditions the emission rate for detached leaves of basil, wheat and maize were similar to those measured in this study. However, they observed

Table 4 Calculated and measured concentrations of methane in the plant growth chamber based on average emission rates for 'sunlight' plants and 'no-sun' plants reported by Keppler *et al*. (2006), based on our measurements in continuous flow gas exchange chambers on individual ^{13}C-plants and actual measurements on a mixture of plant species in the ESPAS growth chamber

	Methane concentration in growth chamber (ppb)				
	Calculated			Measured	
Time (d)	'Sunlight' plants (374 ng g^{-1} h^{-1})	'No sun' plants (119 ng g^{-1} h^{-1})	Continuous-flow gas exchange cuvettes (21 ± 11 ng g^{-1} h^{-1})	ESPAS	Biomass (g)
0	23	23	23 ± 5	23 ± 5	289
2.2	2248	649	170 ± 89	22 ± 5	317
3.8	4265	1217	303 ± 159	24 ± 5	346
5.9	7185	2038	495 ± 260	23 ± 4	374

Means ± SE are given (n = 24 for individual plants in continuous-flow gas exchange chambers; n = 6 for growth chamber). Biomass was calculated on the basis of the daily amount of ^{13}C-CO_2 injected in the ESPAS and the conversion efficiency from ^{13}C-CO_2 to biomass. ^{13}C-methane measured in the ESPAS includes the natural background (22 ppb ^{13}C-methane).

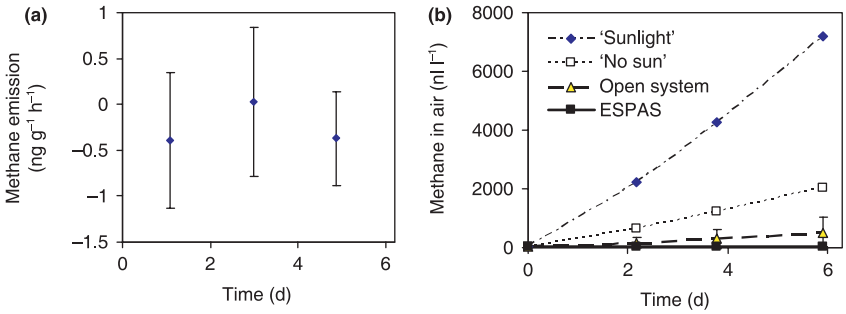

Fig. 2 Long-term steady-state methane emissions by vegetation. (a) Measured ^{13}C-methane emissions (mean ± SE) by a mixture of ^{13}C-enriched plants in the ESPAS (Experimental Soil Plant Atmosphere System) growth chamber under controlled steady-state conditions. Plant biomass increased from 289 (day 0) to 374 (day 6) g dry weight during the experiment (n = 3), and the emissions are given at the median of the time for accumulated emission. (b) Measured (solid line) and predicted (dashed lines) accumulation of methane by ^{13}C-enriched plants in the ESPAS growth chamber. Measured methane concentrations (solid line, closed squares), and methane concentrations predicted from our continuous-flow experiment (Table 3; 21 ng g^{-1} h^{-1}, dashed line, open triangles), or from Keppler *et al*. (2006: 'sunlight', 374 ng g^{-1} h^{-1}, dot-dashed line, closed diamond; 'no sun', 119 ng g^{-1} h^{-1}, dotted line, open squares).

much higher methane emission values for intact plants, that is, 119 and 374 ng g^{-1} h^{-1} for 'no sun' and 'sunlight' plants, respectively. By contrast, we used open systems in the form of continuous-flow gas exchange cuvettes in order to realize steady-state conditions with respect to light, temperature, ambient CO_2 concentrations and relative humidity. All measurements were performed at a constant natural methane background concentration of *c*. 2000 ppb. Thus, performed under physiologically relevant and controlled conditions, we were not able to measure substantial methane emissions (Fig. 2, Table 3).

In the longer-term experiment with a large plant biomass to increase the potential emission of methane, no increase above the background concentration of 22 ppb ^{13}C-methane was measured. With the large plant biomass and the emission rates indicated by Keppler *et al*. (2006) for 'sunlight' and 'no-sun' plants, or even the rate we found in the continuous-flow cuvettes, the methane concentration would have been greatly increased in the ESPAS growth chamber. But this was not the case, which can only mean that the 375 g of plant biomass in the growth chamber did not emit any methane at all.

To date, the Keppler *et al*. (2006) study had yielded the only experimental data on methane emission from plants, and concluded that plants are indeed able to emit substantial amounts of methane. Is there, then, an explanation for the large difference between our results and those of Keppler *et al*. (2006)? One possible explanation may lie in the flushing procedure before measurements. Keppler *et al*. (2006) flushed their cuvettes with methane-free air to remove ambient methane. However, if plants still contain ambient methane concentrations in intercellular air spaces and air spaces in the soil system (*c*. 2000 ppb), as well as in lipid membranes and water, this may have diffused to the surrounding air during their measurements following a concentration gradient. The rate of this

diffusion process would have been influenced by temperature. Keppler *et al.* (2006) provided no details on plant growth conditions before the experiment, and the description of their experiments and methodology is rather poor. For example, no information was given on the light intensities of the 'no sun' and 'sunlight' conditions. Furthermore, no mention was made of possible stress conditions in their static air cuvettes. Therefore this explanation is a suggestion, at best, and we cannot be sure if this might fully explain the discrepancy.

Up until now, no other data on methane emissions of plants have been published. However, our results are indirectly confirmed by a recent study (Ferretti *et al.*, 2007), in which methane emissions were modelled using stable isotope data from ice cores. Their best estimate was 80% lower than that of Keppler *et al.* (2006), and their confidence interval for methane emission even included zero emission.

Conclusions

In this paper we measured both short- and longer-term emissions of methane from various plant species. The experimental design entailed measurements under physiologically relevant and controlled conditions. We did not find any evidence of a substantial emission of methane by terrestrial plants under aerobic conditions.

Acknowledgements

This project was supported with a grant from the Dutch Programme on Non-CO_2 Reduction (ROB), a joint initiative of the Ministries of Housing, Spatial Planning and the Environment, of Agriculture, Nature and Food Quality and of Economic Affairs. The programme is coordinated by SenterNovem. Stan Smeulders is acknowledged for his role in the inception of this project.

References

Berthold HK, Jahoor F, Klein PD, Reeds PJ. 1995. Estimates of the effect of fasting on whole-body protein degradation in women vary with the amino acid used as tracer: studies of multiple amino acid kinetics using uniformly labeled ^{13}C-algal protein to label plasma and hepatic amino acids. *Journal of Nutrition* **125**: 2516–2527.

Bousquet P, Ciasis P, Miller JB, Dlugokencky EJ, Hauglustaine DA, Prigent C, Van der Werf GR, Peylin P, Brunke E-G, Carouge C, Langenfelds RL, Lathiére J, Papa F, Ramonet M, Schmidt M, Steele LP, Tyler SC, White J. 2006. Contribution of anthropogenic and natural sources to atmospheric methane variability. *Nature* **443**: 439–443.

Dlugokencky EJ, Masarie KA, Lang PM, Tans PP. 1998. Continuing decline in the growth rate of the atmospheric methane burden. *Nature* **393**: 447–450.

Farquhar GD, Ehleringer JR, Hubick KT. 1989. Carbon isotope discrimination and photosynthesis. *Annual Review of Plant Physiology* **40**: 503–537.

Ferretti DF, Miller JB, White JWC, Lassey KR, Lowe DC, Etheridge DM. 2007. Stable isotopes provide revised global limits of aerobic methane emissions from plants. *Atmospheric Chemistry and Physics* 7: 237–241.

Frankenberg C, Meirink JF, Van Weele M, Platt U, Wagner T. 2005. Assessing methane emissions from global space-borne observations. *Science* **308**: 1010–1014.

Gorissen A, Kuikman PJ, Van Ginkel JH, Van de Beek H, Jansen AG. 1996. An advanced phytotron for measuring carbon dynamics in a whole plant-soil system. *Plant and Soil* **179**: 81–87.

Houweling S, Röckmann T, Aben I, Keppler F, Krol M, Meirink JF, Dlugokencky EJ, Frankenberg C. 2006. Atmospheric constraints on global emissions of methane from plants. *Geophysical Research Letters* **33**: L15821.

Kelliher BP, Simpson MJ, Simpson AJ. 2006. Assessing the fate and transformation of plant residues in the terrestrial environment using HR-MAS NMR spectroscopy. *Geochimica et Cosmochimica Acta* **70**: 4080–4094.

Keppler F, Hamilton JTG, Braß M, Röckmann T. 2006. Methane emissions from terrestrial plants under aerobic conditions. *Nature* **439**: 187–191.

Kikuchi J, Shinozaki K, Hirayama T. 2004. Stable isotope labeling of *Arabidopsis thaliana* for an NMR-based metabolomics approach. *Plant Cell Physiology* **45**: 1099–1104.

Kirschbaum MUF, Bruhn D, Etheridge DM, Evans JR, Farquhar GD, Giffford RM, Paul KI, Winters AJ. 2006. Comment of the quantitative significance of aerobic methane release by plants. *Functional Plant Biology* **33**: 521–530.

Kurilich AC, Britz SJ, Clevidence BA, Novotny JA. 2003. Isotopic labeling and lc-apci-ms quantification for investigating absorption of carotenoids and vitamin k1 from kale. *Journal of Agriculture and Food Chemistry* **51**: 48774889.

Lelieveld J. 2006. Climate change: a nasty surprise from the greenhouse. *Nature* **443**: 405–406.

Lowe DC. 2006. A green source of surprise. *Nature* **439**: 148–149.

Ngai AKY, Persijn ST, Von Basum G, Harren FJM. 2006. Automaticallly tunable continuous wave optical parametric oscillator for high resolution spectroscopy and sensitive trace gas detection. *Applied Physics* **B85**: 173–180.

Novotny JA, Kurilich AC, Britz SJ, Clevidence BA. 2005. Appearance of labeled beta-carotene, lutein, retinol, and phylloquinone in plasma after consumption of isotopically-labeled kale. *Journal of Lipid Research* **46**: 1896–1903.

O'Leary MH, Madhavan S, Paneth P. 1992. Physical and chemical basis of carbon isotope fractionation in plants. *Plant, Cell & Environment* **15**: 1099–1104.

Paré PW, Alborn HT, Tumlinson JH. 1998. Concerted biosynthesis of an insect elicitor of plant volatiles. *Proceedings of the National Academy of Sciences, USA* **95**: 13971–13975.

Parsons R, Newton P, Clark H, Kelliher FM. 2006. Scaling methane emissions from vegetation. *Trends in Ecology and Evolution* **21**: 423–424.

Poorter H, Van der Werf A. 1998. Is inherent variation in RGR determined by LAR at low irradiance and by NAR at high irradiance? A review of herbaceous species. In: Lambers H, Poorter H, van Vuren M, eds. *Inherent variation in growth rate. Physiological mechanisms and ecological consequences.* Leiden, the Netherlands: Backhuys Publishers, 309–336.

Poorter H, Welschen RAM. 1993. Variation in RGR underlying carbon economy. In: Hendry GAF, Grime JP, eds. *Methods in comparative plant ecology – a laboratory manual.* London, UK: Chapman & Hall, 107–110.

Schiermeier Q. 2006. The methane mystery. *Nature* **442**: 730–731.

Van Herpen MMJW, Li S, Bisson SE, Harren FJM. 2002. Photoacoustic trace gas detection of ethane using a continuously tunable, cw optical parametric oscillator based on periodically poled lithium niobate. *Applied Physics Letters* **81**: 1157–1159.

Supplementary Material

The following supplementary material is available for this article online:

Fig. S1 Calibration curve for a series of methane concentrations. Horizontal bars indicate variations in the standard, and vertical bars indicate the standard error of the measurement signal ($n = 3$).

This material is available as part of the online article from: http://www.blackwell-synergy.com/doi/abs/10.1111/j.1469-8137.2007.02103.x
(This link will take you to the article abstract).

Please note: Blackwell Publishing are not responsible for the content or functionality of any supplementary materials supplied by the authors. Any queries (other than missing material) should be directed to the journal at *New Phytologist* Central Office.

