LETTERS

Methane emissions from terrestrial plants under aerobic conditions

Frank Keppler[1], John T. G. Hamilton[2], Marc Braß[1,3] & Thomas Röckmann[1,3]

Methane is an important greenhouse gas and its atmospheric concentration has almost tripled since pre-industrial times[1,2]. It plays a central role in atmospheric oxidation chemistry and affects stratospheric ozone and water vapour levels. Most of the methane from natural sources in Earth's atmosphere is thought to originate from biological processes in anoxic environments[2]. Here we demonstrate using stable carbon isotopes that methane is readily formed *in situ* in terrestrial plants under oxic conditions by a hitherto unrecognized process. Significant methane emissions from both intact plants and detached leaves were observed during incubation experiments in the laboratory and in the field. If our measurements are typical for short-lived biomass and scaled on a global basis, we estimate a methane source strength of 62–236 Tg yr^{-1} for living plants and 1–7 Tg yr^{-1} for plant litter (1 Tg = 10^{12} g). We suggest that this newly identified source may have important implications for the global methane budget and may call for a reconsideration of the role of natural methane sources in past climate change.

Methane (CH_4) is the most abundant organic trace gas in the atmosphere (mixing ratio ~1.8 p.p.m.) and is important to both tropospheric and stratospheric chemistry. Therefore, the atmospheric CH_4 budget has been intensively studied over the past two decades using flux measurements on sources[3], global observation networks[4] and global atmospheric models[5,6]. In addition, stable carbon isotope ratios ($^{13}C/^{12}C$) have been applied to investigate sources and sinks of atmospheric CH_4 (refs 7, 8). Although uncertainties in the estimates of individual source strengths are large (50–100 Tg), it is generally thought that all major sources, including wetlands, animals, rice cultivation, biomass burning and fossil fuel production, have been identified and sum up to a global source strength of ~600 Tg yr^{-1} (refs 1, 2). However, significantly elevated CH_4 mixing ratios were recently observed in tropical regions above evergreen forests[9] indicating an additional tropical source of 30–40 Tg over the time period of the investigation (August–November), which could not be explained within the currently accepted global budget of CH_4.

Following our observations of non-enzymic production of methyl halides from senescent plants and leaf litter[10,11], we investigated the possibility of methane formation by plant material. A large set of laboratory experiments using freshly collected and dried plant material—including tree and grass leaves from C_3 and C_4 plant categories—were conducted, in which CH_4 release rates and stable carbon isotope composition ($\delta^{13}C$ values) of emissions were measured under controlled conditions (see Methods). Whereas CH_4 emissions were difficult to quantify for samples incubated in ambient air owing to the high atmospheric background levels of CH_4, production was clearly evident when samples were incubated in CH_4-free air. Emission rates typically ranged from 0.2 to 3 ng per g (dry weight) h^{-1} at 30 °C (see Supplementary Table S1). Release of CH_4 was very temperature sensitive—concentrations approximately doubled with every 10 °C increase over the range 30–70 °C (Fig. 1a), suggesting a non-enzymic rather than an enzyme-mediated process. $\delta^{13}C$ of the emitted CH_4 ranged from −51.8‰ to −68.4‰ (mean = −58.2‰, $n = 61$) and −46.9‰ to −53.1 (mean = −49.5‰, $n = 13$) for C_3 and C_4 plants, respectively. The mean value determined for C_3 plant emissions is comparable with the average $\delta^{13}C$ value for CH_4 emitted from wetlands and rice paddies (approximately −60‰; ref. 8) and thus would be generally regarded as an indication for biological production by anaerobic bacteria. Even though this possibility was remote since most of our experiments were performed under aerobic conditions, we measured CH_4 production by leaf tissue sterilized with γ-radiation (Fig. 1b, c). No significant difference, either in emission rates or $\delta^{13}C$ values of the emissions, was noted between sterilized and non-sterilized samples, thus further excluding microbial activity as the CH_4 source and clearly indicating the existence of a hitherto unknown pathway for CH_4 production in leaf tissue.

Having established CH_4 production by detached leaf tissue, we investigated the possibility of CH_4 formation by intact plants, using incubation chambers in the laboratory and in the field (see Methods). CH_4 formation was observed for all plant species investigated, with release rates ranging from 12 to 370 ng per g (dry weight) h^{-1}, thus one to two orders of magnitude higher than the emissions from detached leaf material. Furthermore, emission rates were found to increase dramatically, by a factor of 3–5 (up to 870 ng per g (dry weight) h^{-1}), when chambers were exposed to natural sunlight, an effect also observed with detached leaf tissue (see Supplementary Information). As can be seen from Fig. 2, CH_4 concentrations increased continuously when plants were incubated in chambers at ambient temperatures. We conducted most laboratory chamber experiments in a CH_4-free air atmosphere where, in addition to concentration measurements, reliable $\delta^{13}C$ measurements were also recorded when CH_4 concentrations in the chamber were above 40 p.p.b. (Fig. 2b–d). $\delta^{13}C$ values were in the range of −48‰ to −59.5‰ (mean = −52‰, $n = 29$) and −45 to −47‰ (mean = −46.5‰, $n = 11$) for C_3 and C_4 plants, respectively. Our experiments were performed in a well circulated atmosphere containing ~20% oxygen, so it was unlikely that the observed CH_4 production could have been mediated by anaerobic acetate fermentation or CO_2 reduction, since obligate anaerobes metabolize only under anoxic conditions at redox levels $E_h < -200$ mV.

Nevertheless, we conducted a series of experiments that have enabled us to unequivocally demonstrate *in situ* formation of CH_4 in plants. First, we could not detect any differences in either the emission rates or the $\delta^{13}C$ values for CH_4 produced by plants of the same species that were grown hydroponically or on soil. Second, since

[1]Max-Planck-Institut für Kernphysik, Saupfercheckweg 1, 69117 Heidelberg, Germany. [2]Department of Agriculture and Rural Development for Northern Ireland, Agriculture, Food and Environmental Science Division, Newforge Lane, Belfast BT9 5PX, UK. [3]Institute for Marine and Atmospheric Research Utrecht, Utrecht University, Princetonplein 5, 3508 TA, Utrecht, The Netherlands.

© 2006 Nature Publishing Group

the methyl group of acetate is considered to be the principal substrate from which CH_4 is readily formed by microbial activity in anaerobic soils, 2-^{13}C labelled acetate was added to the soil of some plants and the CH_4 released monitored for ^{13}C enrichment. Using this approach, any contribution to CH_4 production via the acetate fermentation pathway would be clearly identifiable by a massive increase in its ^{13}C content. No significant incorporation of ^{13}C was observed, eliminating microbial formation via the anoxic pathway. Finally, the difference between the δ^{13}C values for CH_4 emissions from C_3 and C_4 plants is similar to the difference between the δ^{13}C values for the biomass of the two plant categories (Supplementary Fig. S1). All these findings lead us to conclude that the observed CH_4 emissions during these studies must have originated from a thus far unknown process in the plant itself, and that this process is clearly distinct from the widely accepted process requiring anoxic conditions.

The observed ^{13}C depletion of the CH_4 emitted relative to bulk biomass indicates that the process must either have an associated large kinetic isotope effect or the source substrate must be isotopically depleted. It was recently demonstrated that the major plant C_1 (one-carbon unit) pool, which includes methoxyl groups from pectin and lignin, has a unique carbon isotope signature exceptionally depleted in ^{13}C (ref. 10). Since this pool has been shown to be responsible for the emissions of C_1 compounds such as CH_3Cl and CH_3OH from senescent leaves and leaf litter[11,12], its involvement in the *in situ* formation of CH_4 by plants can also be envisaged. Indeed, in experiments with purified apple pectin we also found formation of CH_4; emission rates, δ^{13}C values and response to temperature and sunlight were similar to those observed with detached leaves (see Supplementary Table S1 and Supplementary Fig. S2). These results indicate that the structural plant component pectin plays a prominent role in the *in situ* formation of CH_4 in plants. However, since no chemical mechanism for CH_4 production in plants is known, an explanation for its unprecedented release must await much more detailed investigations.

To assess the global environmental impact of this newly established CH_4 source, we assume that the range of measured emission rates are globally representative for short-lived biomass. We also make the assumption that these emissions can be scaled relative to the annual net primary production (NPP), where we distinguish between the various types of biome[13], differences in the length of vegetation period and average daily sunshine hours (Table 1). We are aware that this simple approach neglects the complexity of terrestrial ecosystems, in particular that the influence of solar radiation will be more variable than that indicated. Hence, our calculations should be considered as a first estimate. CH_4 released by living vegetation is calculated to be in the range 62–236 Tg yr^{-1} (average 149 Tg yr^{-1}) with the main contribution, 46–169 Tg yr^{-1} (average 107 Tg yr^{-1}), assigned to tropical forests and grasslands (Table 1). Between one and two orders of magnitudes lower, in the range 0.5–6.6 Tg yr^{-1}, is our estimate for annual global production of CH_4 by plant litter.

The detection of an additional source of this magnitude, some 10–30% of the present annual source strength, would necessitate reconsideration of the global CH_4 budget. Even though in some budget estimates it is possible to accommodate an additional source of some 50 Tg in order to match the well-established sink strength[2], it appears that a reduction in other source terms would be necessary to keep the budget balanced. We suggest that production due to the source we have identified here may overlap with production assigned

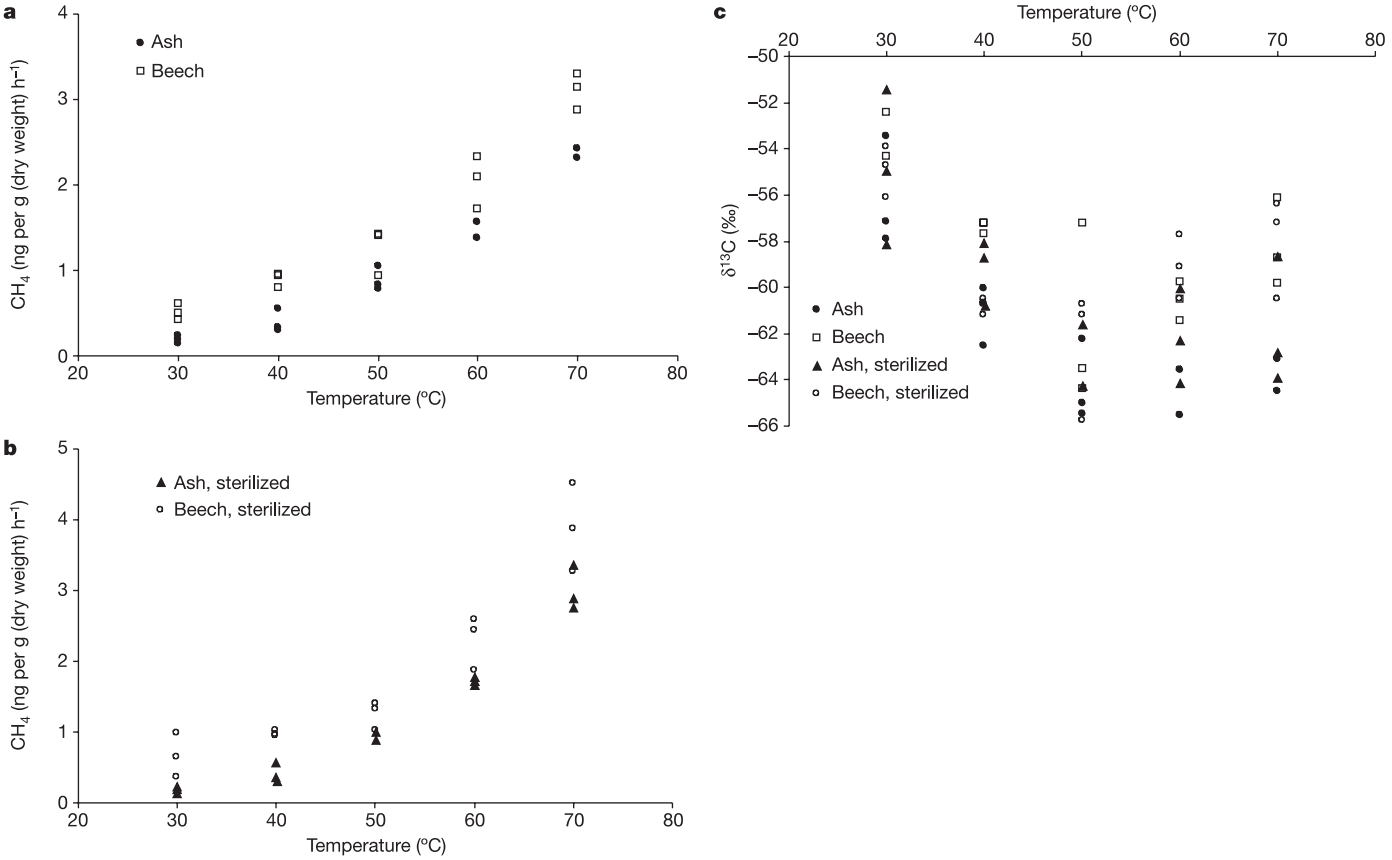

Figure 1 | Release rates and isotopic signatures of CH_4 formed by leaf tissue incubated in the dark. **a**, CH_4 release rates of air dried ash (*Fraxinus excelsior*) and beech leaves (*Fagus sylvatica*) in the temperature range 30–70 °C. **b**, **c**, CH_4 release rates (**b**) and $\delta^{13}CH_4$ values (**c**) for ash and beech leaves with and without sterilization using γ-radiation. Data from tropical plant species are shown in Supplementary Fig. S3.

© 2006 Nature Publishing Group

to other sources such as wetlands or rice cultivation. For example, recent work with different rice cultivars showed a strong positive correlation of the crop growth parameters leaf number and leaf area index with total CH_4 flux[14]. In other studies with rice plants, it was shown that a 1% reduction in the solar radiation resulted in a ~2% reduction in CH_4 emissions[15]. A straightforward explanation for part of these emissions would be *in situ* formation in the plants themselves and, as we would anticipate, a concomitant enhancement of emissions with increased leaf biomass and solar radiation.

Direct CH_4 emissions from plants may also provide simple explanations for several findings reported in the literature that up to now were not understood. For example, the source is of the correct magnitude and also at the correct location to explain the observed elevated CH_4 levels over tropical evergreen forests[9], a finding that could not be accounted for within the previously accepted global budget. Furthermore, since severe anthropogenic deforestation has considerably reduced tropical biomass over the past decades (a −12.3% net change in tropical forest area between 1990 and 2000; ref. 16), a corresponding reduction in tropical plant CH_4 emissions would have resulted in a decrease of 6–20 Tg yr^{-1} over this time period. This is similar in magnitude to the present global source–sink imbalance[2], and thus reduced biomass has probably contributed to the recent decrease in the atmospheric growth rate of CH_4 concentration[4,17].

We also suggest that in pre-industrial times, that is, without anthropogenic emissions, the relative contribution of CH_4 to the atmosphere by direct plant emissions may have been even larger than today. This could have far reaching implications for the interpretation of atmospheric CH_4 levels and climate signals in the past. For example, variations in total global biomass over glacial cycles[18] must contribute to reported differences in atmospheric CH_4 mixing ratios between glacial and interglacial periods[19]. Recent measurements of $\delta^{13}C$ values of atmospheric CH_4 from ice cores covering the past 2,000 years (ref. 20) provide additional observational support for a prominent role of a plant source in the pre-industrial atmosphere. Unexpectedly enriched $\delta^{13}C$ values of around −47‰ were shown to persist over the time period 0 to 1200 AD. This cannot be reconciled with a pre-industrial methane budget dominated by isotopically depleted wetland emissions ($\delta^{13}C \approx -60$‰; ref. 8), as this would lead to atmospheric $\delta^{13}C$ values in the region of −54 to −49‰ (refs 21, 22). Since direct plant emissions are enriched in ^{13}C compared to wetland emissions (from our measurements we derive a $\delta^{13}C$ value of about −50‰ based on a 60:40 ratio of C_3 and C_4 plants), the isotope mass balance for the pre-industrial atmosphere

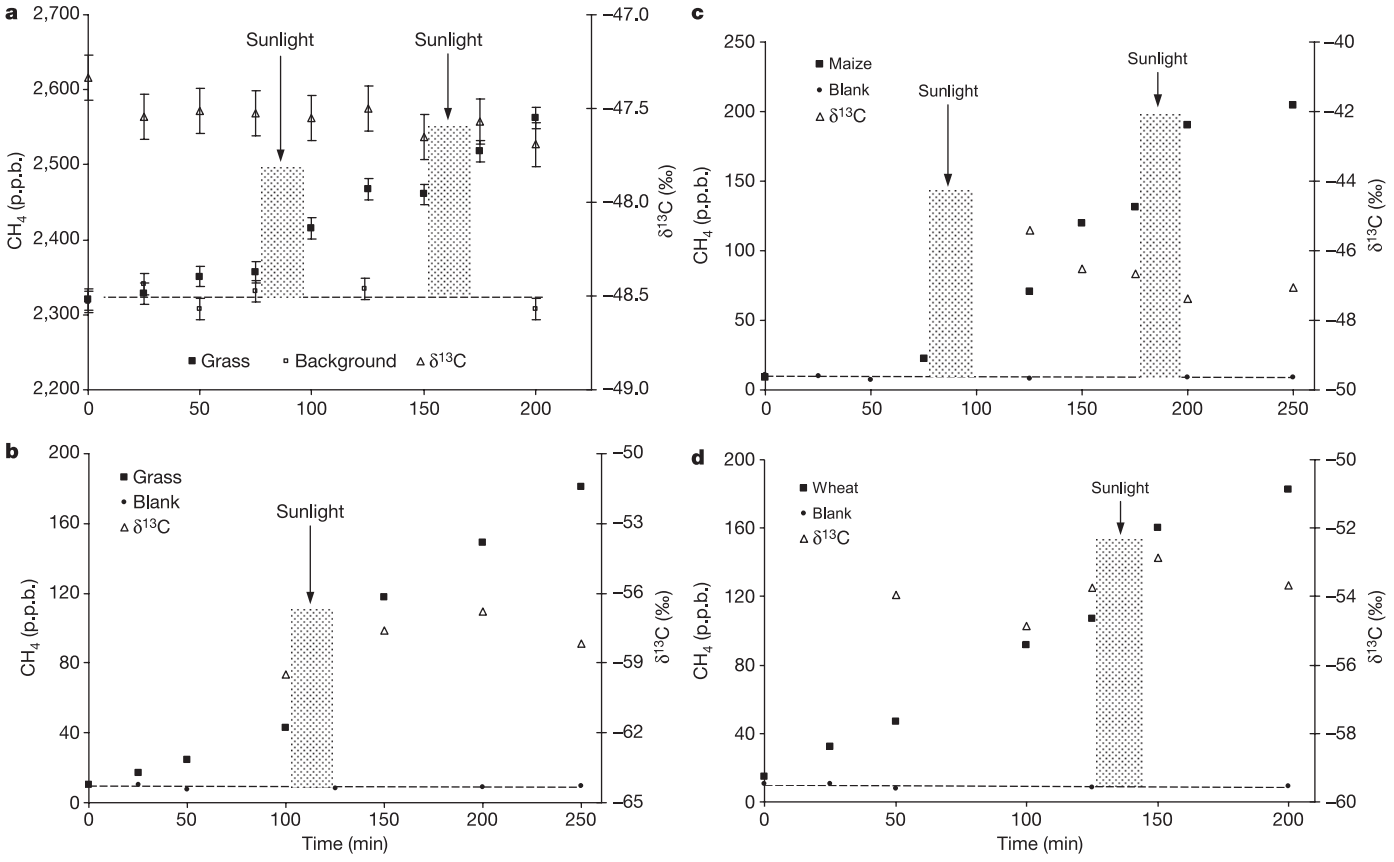

Figure 2 | Mixing ratios and $\delta^{13}C$ values of CH_4 formed by intact plants. **a**, Increase of CH_4 mixing ratio during incubation of sweet vernal grass (*Anthoxanthum odoratum L.*) in ambient air. CH_4 mixing ratios in the chamber increased from 2,320 to 2,357 p.p.b. within 75 min, yielding emission rates in the range 36–126 ng per g (dry weight) h^{-1} for the individual time steps. Exposure to sunlight (indicated by dotted areas) significantly increased CH_4 release to ~ 320 ng per g (dry weight) h^{-1}. After sunlight exposure, release rates decreased again. A comparison between plants with and without exposure to sunlight is shown in Supplementary Fig. S4. Dashed line shows the mean CH_4 concentration without plants. $\delta^{13}C$ values of CH_4 from incubation experiments ranged from −47.3‰ to −47.7‰. Error bars shown reflect the uncertainty (−/+ 1σ) of the measurements of the analytical system. **b**, Profile of CH_4 mixing ratio and associated $\delta^{13}C$ values during incubation of sweet vernal grass (*Anthoxanthum odoratum L.*, C_3 plant) in a chamber purged with CH_4-free air before experiment. CH_4 increased from 10 to 43 p.p.b. within the first 100 min, corresponding to emission rates in the range of 48 to 63 ng per g (dry weight) h^{-1}. Sunlight exposure increased CH_4 release to ~256 ng per g (dry weight) h^{-1}. After sunlight exposure, release rates decreased to ~110 ng per g (dry weight) h^{-1}. $\delta^{13}C$ values of emitted CH_4 ranged from −56.8‰ to −59.5‰. **c**, **d**, Profiles of CH_4 concentrations and associated $\delta^{13}C$ values during incubation of maize (*Zea mays*; C_4 plant) (**c**) and wheat (*Triticum aestivum L.*; C_3 plant) (**d**) in chambers purged with CH_4-free air before experiment. The $\delta^{13}C$ of CH_4 from maize was in the range −45.5‰ to −47.4‰, while that from wheat was more depleted in ^{13}C, from −52.7‰ to −54.9‰.

© 2006 Nature Publishing Group

Table 1 | Estimated annual global emissions of CH_4 by living plants and leaf litter

Vegetation type/biome	Season length* (d)	NPP† (Pg C yr^{-1})	Sunshine hours‡ (h d^{-1})	Annual CH_4 production§ low/mean/high‖ (Tg yr^{-1})
Living biomass				
Tropical forests	365	21.9	8	33.2/78.2/123
Temperate forests	250	8.1	6	7.1/17.7/ 28.4
Boreal forests	150	2.6	4	1.1/3/4.1
Mediterranean shrublands	200	1.4	8	1.2/2.7/4.3
Tropical savannas and grasslands	200	14.9	8	12.4/29.2/45.9
Temperate grasslands	150	5.6	6	2.9/7.4/11.8
Deserts	100	3.5	10	1.7/3.8/5.9
Crops	200	4.1	8	2.9/7.2/11.5
Total		62.1		62.3/149/236
	Period# (d)	**NPP† (Pg C yr^{-1})**	**Sunshine hours (h d^{-1})**	
Leaf litter¶				
Tropical forests	365	21.9	8	0.23/1.53/ 3.2
Temperate forests	90	8.1	6	0.02/0.12/0.25
Boreal forests	60	2.6	4	0.01/0.02/0.05
Mediterranean shrublands	180	1.4	8	0.01/0.05/0.1
Tropical savannas and grasslands	365	14.9	8	0.16/ 1/2.1
Temperate grasslands	90	5.6	6	0.01/0.08/0.18
Deserts	365	3.5	10	0.04/0.28/0.56
Crops	90	4.1	8	0.01/0.07/0.15
Total		62.1		0.49/3.2/6.6

*Estimated.
†Data from ref. 13.
‡Estimated hours of sunshine per day during vegetation period.
§For calculation, see Methods.
‖Low (high) estimates are derived as mean of the CH_4 emission rates − (+) 1σ, value for the mean is similar to the median.
¶Emissions measured by detached leaf tissue (fresh and dried) are considered to reflect those from leaf litter.
#Estimated period of plant decay with similar ambient temperatures as in our experiments.

can be closed if plant emissions are included as an important natural CH_4 source. Consequently, the role of natural CH_4 sources in past climate change, particularly when biospheric productivity changed dramatically, may have to be reconsidered.

Finally, it has been suggested that the so-called 'CO_2 fertilization effect' could lead to a substantial increase in NPP over the next 100 years (ref. 23), which should have an effect on CH_4 emissions from plants. Thus for the future it is essential that we fully understand the relationship between climate change and plant CH_4 emissions.

METHODS

Incubation experiments with detached leaves. Fresh leaves (1–6 g; detached from the intact plants) and dried leaves (1–5 g, air dried at 25 °C for 48 h) were placed in glass vials (44 ml) and sealed with caps containing PTFE-lined silicon septa. Vials were purged with CH_4-free air for one hour before the start of the experiment, and controls (blanks) were measured after purging. After incubation in the dark for 16 h at 30 °C and 40 °C, CH_4 formed in the vial was analysed with continuous-flow isotope ratio mass spectrometry (CF-IRMS). Leaf dry matter was determined by drying at 105 °C for 24 h. Experiments with solar radiation were performed by placing glass vials in direct sunlight for a 1 h period between 10:00 h and 15:00 h in Heidelberg, Germany.

Chamber experiments with intact plants. Plexiglas chambers (volume 18 l, diameter $d = 29$ cm) were used for static incubation experiments. Plants were placed in sealed chambers and purged with CH_4-free air until CH_4 background levels were below 10 p.p.b. Chamber temperature, pressure, CO_2 concentration and humidity and external atmospheric pressure were all monitored online during the experimental period. In all experiments, CO_2 concentration never dropped below 300–250 p.p.m. Concentrations and $\delta^{13}C$ values of CH_4 were measured every 25 min by transferring 40 ml of chamber headspace gas into the analytical system. Small electric fans were used to circulate the chamber air. Dry matter content was determined at the end of the experiment. Experiments with solar radiation were performed by placing chambers in direct sunlight for a 20 min period between 10:00 h and 15:00 h in Heidelberg, Germany.

Methane measurements. Headspace gas samples were transferred from the sample vial or the incubation chamber to an evacuated 40 cm^3 sample loop. CH_4 was trapped on Hayesep D, separated by gas chromatography from interfering compounds and transferred via an open split to the isotope ratio mass spectrometer (ThermoFinnigan Delta^{plus} XL). Concentration and $\delta^{13}C$ values were determined using an air standard with known concentration and isotopic composition as internal reference, and a measurement of the inlet pressure in the sample loop. The reference gas was also used to establish a robust linearity correction, since sample CH_4 concentrations were very variable. $\delta^{13}C$ values are reported relative to Vienna-PDB and defined by the equation $\delta^{13}C = (R_{sample}/R_{V\text{-}PDB} - 1) \times 1{,}000‰$, with $R = {}^{13}C/{}^{12}C$.

Calculation of annual CH_4 production. As a first estimate, average daily CH_4 emission rates are calculated as $ER_{day} = (ER_{sun} \times h_{sun}) + (ER_{nosun} \times h_{nosun})$, where ER_{sun} and ER_{nosun} are the measured emission rates of CH_4 with/without direct sunshine (10^{-9} per g (dry weight) biomass h^{-1}), and h_{sun} and h_{nosun} are the estimated daily hours with/without sunshine (h d^{-1}), respectively.

Mean (low/high) values of ER_{sun} for intact plants and detached leaves were 374 (198/598) and 8.7 (1.6/15.8) ng per g (dry weight) h^{-1}, respectively. Mean (low/high) values of ER_{nosun} for living plants and detached leaves were 119 (30.7/207) and 1.6 (0.1/4.4) ng per g (dry weight) h^{-1}, respectively. Low (high) estimates are derived as mean of the CH_4 emission rates − (+)1σ. Solar radiation experiments were carried out in direct sunshine in springtime (March–May 2005) in Heidelberg, Germany (~49.4° N); living plants: 8 different plant species, $n = 33$; detached leaves: 14 different plant species, $n = 23$ (see Supplementary Information). 'No sun' experiments were carried out in the laboratory without direct solar radiation; living plants: 9 different plant species, $n = 46$; detached leaves: 19 different plant species, $n = 61$ (see Supplementary Information).

Annual production of CH_4 per biome type is calculated as $P(CH_4)_{annual} = NPP \times 2 \times SL \times ER_{day}$, where $P(CH_4)_{annual}$ is the annual production (g yr^{-1}), NPP is the net primary production of the biome type (10^{15} g C yr^{-1}) and SL is season length (d); the factor 2 is needed to convert NPP, which is usually expressed as carbon equivalent, to plant biomass, assuming that plant biomass is 50% carbon.

Received 14 July; accepted 3 November 2005.

1. Lelieveld, J., Crutzen, P. J. & Dentener, F. J. Changing concentration, lifetimes and climate forcing of atmospheric methane. *Tellus B* **50**, 128–150 (1998).
2. Houghton, J. T. *et al.* (eds) *Climate Change 2001: The Scientific Basis* (Cambridge Univ. Press, Cambridge, 2001).
3. Bartlett, K. B. & Harriss, R. C. Review and assessment of methane emissions from wetlands. *Chemosphere* **26**, 261–320 (1993).
4. Dlugokencky, E. J., Masarie, K. A., Lang, P. M. & Tans, P. P. Continuing decline in the growth rate of the atmospheric methane burden. *Nature* **393**, 447–450 (1998).
5. Walter, B. P., Heimann, M. & Matthews, E. Modeling modern methane emissions from natural wetlands 2. Interannual variations 1982–1993. *J. Geophys. Res.* **106**, 34207–34219 (2001).

© 2006 Nature Publishing Group

6. Houweling, S., Dentener, F., Lelieveld, J., Walter, B. & Dlugokencky, E. The modeling of tropospheric methane: How well can point measurements be reproduced by a global model? *J. Geophys. Res.* **105,** 8981–9002 (2000).
7. Lowe, D. C. *et al.* Concentration and ^{13}C records of atmospheric methane in New Zealand and Antarctica—Evidence for changes in methane sources. *J. Geophys. Res.* **99,** 16913–16925 (1994).
8. Quay, P. *et al.* The isotopic composition of atmospheric methane. *Glob. Biogeochem. Cycles* **13,** 445–461 (1999).
9. Frankenberg, C., Meirink, J. F., van Weele, M., Platt, U. & Wagner, T. Assessing methane emissions from global space-borne observations. *Science* **308,** 1010–1014 (2005).
10. Keppler, F., Kalin, R. M., Harper, D. B., McRoberts, W. C. & Hamilton, J. T. G. Dramatic ^{13}C depletion in the plant methoxyl pool and its biogeochemical implications. *Biogeosciences* **1,** 123–131 (2004).
11. Hamilton, J. T. G., McRoberts, W. C., Keppler, F., Kalin, R. M. & Harper, D. B. Chloride methylation by plant pectin: An efficient environmentally significant process. *Science* **301,** 206–209 (2003).
12. Keppler, F., Harper, D. B., Röckmann, T., Moore, R. M. & Hamilton, J. T. G. New insight into the atmospheric chloromethane budget gained using stable carbon isotope ratios. *Atmos. Chem. Phys.* **5,** 2403–2411 (2005).
13. Saugier, B., Roy, J. & Mooney, H. A. in *Global Terrestrial Productivity* (eds Roy, J., Saugier, B. & Mooney, H. A.) 541–555 (Academic, San Diego, 2001).
14. Gogoi, N., Baruah, K. K., Gogoi, B. & Gupta, P. K. Methane emission characteristics and its relations with plant and soil parameters under irrigated rice ecosystem of northeast India. *Chemosphere* **59,** 1677–1684 (2005).
15. Sass, R. L. & Cicerone, R. J. Photosynthate allocations in rice plants: Food production or atmospheric methane? *Proc. Natl Acad. Sci. USA* **99,** 11993–11995 (2002).
16. FAO, *Global Forest Resources Assessment 2000—Main Report* (FAO Forestry Paper No. 140, Food and Agriculture Organization of the United Nations, Rome, 2001).
17. Dlugokencky, E. J. *et al.* Atmospheric methane levels off: Temporary pause or a new steady-state? *Geophys. Res. Lett.* **30** doi:10.1029/2003GL018126 (2003).
18. Mayle, F. E., Beerling, D. J., Gosling, W. D. & Bush, M. B. Responses of Amazonian ecosystems to climatic and atmospheric carbon dioxide changes since the last glacial maximum. *Phil. Trans. R. Soc. Biol. Sci.* **359,** 499–514 (2004).
19. Petit, J. R. *et al.* Climate and atmospheric history of the past 420,000 years from the Vostok ice core, Antarctica. *Nature* **399,** 429–436 (1999).
20. Ferretti, D. *et al.* Unexpected changes to the global methane budget over the past 2000 years. *Science* **309,** 1714–1717 (2005).
21. Lassey, K. R., Lowe, D. C. & Manning, M. R. The trend in atmospheric methane $\delta^{13}C$ implications for isotopic constraints on the global methane budget. *Glob. Biogeochem. Cycles* **14,** 41–49 (2000).
22. Houweling, S., Dentener, F. & Lelieveld, J. Simulation of preindustrial atmospheric methane to constrain the global source strength of natural wetlands. *J. Geophys. Res.* **105,** 17243–17255 (2000).
23. Cramer, W. *et al.* Global response of terrestrial ecosystem structure and function to CO_2 and climate change: results from six dynamic global vegetation models. *Glob. Change Biol.* **7,** 357–373 (2001).

Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

Acknowledgements We thank R. Conrad, J. Kesselmeier and D. Harper for comments on the manuscript; B. Knape, P. Franz, R. Shaheen, F. Kleinbongardt, V. Mallinger, R. Runck and C. McRoberts for technical assistance; the Botanical Garden of the University of Heidelberg for providing plant species from tropical regions; and the European Commission for a Marie Curie-Research Training Grant (F.K.). The ISOSTRAT project in Heidelberg was funded by the BMBF within the AFO2000 project.

Author Information Reprints and permissions information is available at npg.nature.com/reprintsandpermissions. The authors declare no competing financial interests. Correspondence and requests for materials should be addressed to F.K. (frank.keppler@mpi-hd.mpg.de).

© 2006 Nature Publishing Group