

## Emission of methane from plants

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It has been proposed that plants are capable of producing methane by a novel and unidentified biochemical pathway. Emission of methane with an apparently biological origin was recorded from both whole plants and detached leaves. This was the first report of methanogenesis in an aerobic setting, and was estimated to account for 10–45 per cent of the global methane source. Here, we show that plants do not contain a known biochemical pathway to synthesize methane. However, under high UV stress conditions, there may be spontaneous breakdown of plant material, which releases methane. In addition, plants take up and transpire water containing dissolved methane, leading to the observation that methane is released. Together with a new analysis of global methane levels from satellite retrievals, we conclude that plants are not a major source of the global methane production.

**Keywords:** methane; methanogenesis; plant; satellite

### 1. INTRODUCTION

Early in 2006, [Keppler \*et al.\* \(2006\)](#) reported measurements that implied that plants produced methane in aerobic conditions, and that the fluxes were large (a global total of 62–236 Tg (million tonnes) yr<sup>-1</sup>, or approx. 10–45% of the total global methane source; [Keppler \*et al.\* 2006](#)). This report was supported by elegant analytical measurements of mixing ratios (concentrations) and carbon isotopes in the methane emitted. If correct, this implies either that the classical methanogenic pathway operates under aerobic conditions and has gone undetected in plants, or that there exists an unknown, yet extremely important, biochemical pathway. A novel pathway has recently been found in some bacteria, where methane is a by-product of methylphosphonate decomposition ([Kittridge & Roberts 1969](#); [Karl \*et al.\* 2008](#)).

Natural sources of atmospheric methane remain poorly quantified, so the hypothesis that plants produce methane prompted a reappraisal of the global methane budget. One subsequent model allocated a maximum of 85 Tg methane yr<sup>-1</sup> to plants ([Houweling \*et al.\* 2006](#)), although other studies ([Ferretti \*et al.\* 2006](#)) have since shown that the plant source must be <46 Tg yr<sup>-1</sup>. The debate is important because accurate estimates of the global methane budget are needed as a basis for the efforts to ameliorate fluxes of this potent greenhouse gas, which may contribute significantly to global warming.

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We have conducted further experiments on plants grown in controlled conditions and have re-analysed the previously published data. We show that plants do not contain a biochemical mechanism for methanogenesis, and cannot produce methane as an end-product or by-product of their metabolism. Instead, when plants transpire, any methane that is already dissolved in the water derived from the soil will be released into the atmosphere. In addition, under high stress conditions, such as high UV radiation, methane is released as a part of the cellular breakdown process.

### 2. MATERIAL AND METHODS

#### (a) Collection of air samples from *Chlamydomonas* and *Arabidopsis*

*Chlamydomonas reinhardtii* was grown in 500 ml of tris-acetate-phosphate (TAP) medium (made with distilled water) in sterile conditions at room temperature (approx. 20°C) on a 12 hours light/8 hours dark cycle.

*Arabidopsis thaliana* seeds were surface-sterilized to kill any surface microbes and several hundred were planted in 2 l vessels under sterile conditions onto 0.5 l Murashige–Skoog (MS) medium plus 0.8 per cent agar media, buffered with 0.05 per cent morpholino-ethanesulphonic acid (pH 5.7). Plants were grown at 20°C for 2 weeks. Biologically sterile ambient London air (filtered through a 0.22 µm filter) from a tank containing air of known methane mixing ratio was supplied to the flask containing *Chlamydomonas* or *Arabidopsis* at 5 ml min<sup>-1</sup> and collected periodically for monitoring. Samples of air (3 l) were removed from each

Table 1. Measurement of methane emissions from plants grown under controlled conditions. (For *Chlamydomonas* and *Arabidopsis*, input refers to the methane concentration of air supplied at  $5 \text{ ml min}^{-1}$ . For rice and maize, input refers to the air inside the closed chamber when the experiment was started. Samples were removed for analysis (output). Values for the mean and standard deviation of methane mixing ratio and  $\delta^{13}\text{C}$  in triplicate analyses of each air sample are shown.)

plant	input $\text{CH}_4$ (ppb)	output $\text{CH}_4$ (ppb)	input air $\delta^{13}\text{C}$ (‰)	output air $\delta^{13}\text{C}$ (‰)	mass of leaves at start (g)	mass of water lost (g)
<i>Chlamydomonas</i>	$1901 \pm 4$	$1904 \pm 9$	n.a.	n.a.	n.a.	n.a.
<i>Arabidopsis</i>	$1893 \pm 5$	$1887 \pm 5$	n.a.	n.a.	n.a.	n.a.
rice, 25 hours	$1914 \pm 7$	$1916 \pm 9$	$-47.3 \pm 0.0$	$-47.1 \pm 0.1$	n.a.	n.a.
maize, 24 hours	$2021 \pm 5$	$2027 \pm 15$	$-48.5 \pm 0.1$	$-48.5 \pm 0.0$	156.3	2.4
maize, 48 hours	$1984 \pm 6$	$1977 \pm 2$	$-48.0 \pm 0.1$	$-48.0 \pm 0.1$	153.9	5.1

flask for analysis. Controls were carried out, using flasks of media only (i.e. not containing *Arabidopsis*).

#### (b) Collection of air samples from *Zea mays* and *Oryza sativa*

*Zea mays* (maize) and *Oryza sativa* (rice, japonica cultivar) plants were grown from seed in inert support (vermiculite), soaked in MS medium. This was to prevent the build-up of decayed organic matter in the soil, and thus prevent the colonization by methanogenic bacteria, and to control the supply of gas present in the plants' transpiration stream. Plants were grown under cool-white fluorescent light ( $180 \mu\text{mol m}^{-2} \text{ s}^{-1}$  photosynthetically active radiation) using 16 hour photoperiods. Plants were watered once weekly with fresh MS medium, or topped up with distilled water at other times, with both the MS medium or water having been previously saturated with identical air of known composition. Initially, 156 g of *Z. mays* leaves were harvested and placed in a 15 l glass chamber. Air samples were removed after 24 and 48 hours for analysis. In another experiment, four pots, each containing multiple *O. Sativa* plants, were placed inside a 15 l glass chamber. Air samples were removed after 25 hours for analysis. The results are shown in table 1.

#### (c) Collection of air samples from *Ocimum*

Distilled water was saturated with  $^{13}\text{C}$ -enriched methane ( $\delta^{13}\text{C}$  value given in table 2) by bubbling methane from a high pressure cylinder (Air Products, 3.5 Grade) through the water for 10 min. A basil (*Ocimum basilicum*) plant, growing in a pot in soil, was starved of water for 4 days. 100 ml of water saturated with methane (as above,  $\delta^{13}\text{C} = -39\text{‰}$ ) was placed in the saucer under the basil plant pot on each of the fourth and fifth days. On day 6, all the shoots and leaves were removed from the plant and placed in a 2 l closed vessel. On day 7, a 1 l sample of air was pumped out of the chamber into a Tedlar bag, and methane concentrations and isotopic composition were analysed. The results are presented in table 2.

#### (d) Collection of air samples from *Apium graveolens*

The base of two celery (*Apium graveolens*) stalks of known mass was placed in methane-saturated ( $\delta^{13}\text{C} = -39.0 \pm 0.1\text{‰}$ ) coloured distilled water. Water was allowed to flow up the *A. graveolens* stems as measured by the red dye. The base of the stems (that had been in contact with the water) was cut off, and the new mass measured. The remainder of the stems were placed in a 20 l glass chamber containing laboratory air with a known methane concentration. After 16 hours, an air sample was removed for analysis, and the apparatus was dismantled, and the mass of the celery stem recorded again.

#### (e) Measurement of methane concentration and stable isotopic composition

Air samples were analysed in triplicate. Methane concentrations were analysed using a HP 5890 gas chromatograph with standard precision of  $\pm 3$  ppb on background ambient air ( $1\sigma$ ). Methane isotopic composition was analysed by continuous flow-isotope ratio mass spectrometry using a GV Instruments IsoPrime mass spectrometer and modified Trace Gas preparation system. This technique has a precision of  $\pm 0.05\text{‰}$  ( $1\sigma$  in 10 consecutive analyses of a standard).

### 3. NO GENETIC OR BIOCHEMICAL BASIS FOR METHANE PRODUCTION

#### (a) Classical methanogenesis

Methanogenesis, the classical process of microbial methane production, is carried out by archaea in strictly anaerobic conditions. The biochemical pathway of anaerobic methanogenesis has never been found outside the archaea. Although it is sometimes possible for methanogenic archaea living in the dead anaerobic xylem in the centre of trees to produce methane (Zeikus 1977), plant methane production cannot be explained by the presence of such methanogens, as methane emissions were seen from all types of plants, as well as detached leaves (Keppeler *et al.* 2006).

During classical methanogenesis, methane is formed from  $\text{CO}_2$  or acetate via a series of biochemical reactions. The last two reactions are catalysed by the enzymes methyl tetrahydromethanopterin:methyl transferase and methyl-coenzyme M reductase (Ellis *et al.* 2006). In order to determine whether plants might be able to carry out methanogenesis by the same pathway as archaea, we carried out an NCBI BLAST (bioinformatics) search of all genomes and partial sequences of Viridiplantae (including the complete genomes of *A. thaliana* and *Oryza sativa*; see below). We were unable to identify any genes that could be plant homologues of archaeal methyl tetrahydromethanopterin:methyl transferase or methyl-coenzyme M reductase, even at very low stringency (high 'e' value). This implies that the classical methanogenesis pathway is not present in plants. These genes were not found when the search was widened to cover all eukaryotic gene sequences, including those of eukaryotic algae. Furthermore, the production of coenzyme M-associated enzymes requires vitamin B12, which plants cannot synthesize (Croft *et al.* 2005). There is therefore no evidence for a classical methanogenesis biochemical pathway in plants, or a distantly related methanogenesis pathway that has the same origin. We conclude that if plants do indeed release methane, it must be synthesized

by a novel biochemical pathway, as suggested by *Keppler et al.* (2006) or produced by an abiological chemical or photochemical process.

### (b) Methylphosphonate decomposition

It has recently been shown that methane is a by-product of methylphosphonate decomposition (*Kittridge & Roberts* 1969; *Karl et al.* 2008), which is carried out by many bacteria in an aerobic setting (*Huang et al.* 2005). These include important cyanobacteria such as *Trichodesmium* (*Dyhrman et al.* 2006) under low-phosphate conditions. A BLAST search of fully sequenced cyanobacterial genomes confirmed the presence of genes from the *phn* operon encoding proteins from the phosphonate degradative pathway in *Trichodesmium erythraeum* IMS101 (found in tropical oceans) and *Nostoc* sp. PCC 7120 (previously named *Anabaena* sp. PCC 7120, and found in terrestrial, nutrient-poor environments; *Dyhrman et al.* 2006). The genome searches also identified the pathway in *Synechococcus* sp. JA-3-3Ab, a cyanobacterium isolated from Yellowstone hot springs, which grows between 58 and 65°C, but it was not found in other *Synechococcus* species. Although fragments from several *phn* genes were identified in other cyanobacteria, no fully sequenced species contained the full set of genes necessary to decompose methylphosphonate and thus release methane. Significant levels of lateral gene transfer have been reported in cyanobacteria, including cases of genes acquired from other phyla (*Zhaxybayeva et al.* 2006). It thus seems most likely that a few species of cyanobacteria have acquired the methylphosphonate decomposition pathway by lateral gene transfer from other bacteria, but that it is not widespread. No *phn* genes were found in the fully sequenced plant genomes, indicating that plants are unable to release methane by this process.

### (c) No methane emission from whole plants

Owing to the large numbers of plant species, a small number have been chosen as model species. These organisms have fully sequenced genomes, and their biochemical and genetic pathways have been extensively studied. A wide range of plants have been shown to emit methane, including monocotyledonous and dicotyledonous plants (the two angiosperm groups), and conifers. Given this distribution, and the fact that it is unlikely the pathway evolved multiple times in land plants, we chose the model plant *Arabidopsis thaliana* (thale cress) as a representative plant. In order to determine whether the aerobic production of methane arose within the plants, or is more widespread within the Archaeplastida, we also examined the green alga *Chlamydomonas reinhardtii*, which shares a common ancestor, and thus many biochemical pathways, with the plants (*figure 1*; *Adl et al.* 2005). Should both *Chlamydomonas* and plants produce methane, then aerobic production of methane in plants must be very ancient, predating the emergence of green land plants 450 Ma ago (*Willis & McElwain* 2002).

*Arabidopsis thaliana* and *C. reinhardtii* were grown under aseptic conditions to avoid possible contamination by methanogenic archaea and to control the nutrient and water supply to the plants (§2a). For experiments conducted in methane-free air (*Keppler et al.* 2006), release of gas from water is inevitable as a result of

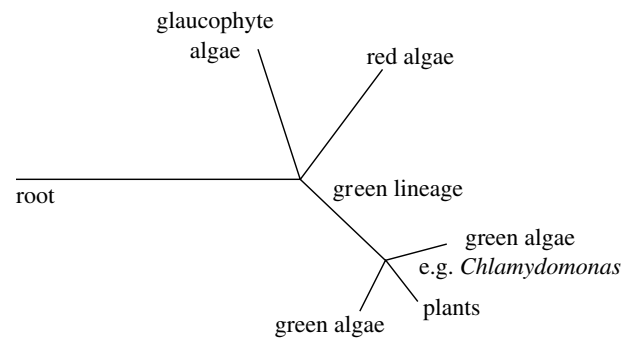


Figure 1. Evolution of the Archaeplastida supergroup, showing the relationship between green plants and algae.

methane equilibrating along the concentration gradient. We carried out our experiments in air containing ambient levels of methane to reduce this effect.

In both cases, the output air was found to contain the same mixing ratio of methane (*table 1*), indicating either that neither organism had produced or used methane, or that net production, had it occurred, exactly equalled consumption. When higher flow rates of air were applied, uptake of methane was seen. However, this was due to the methane dissolving into the medium in which the plants were growing (as flasks containing medium only also showed uptake of the same amounts of methane). The lower limit for detection using our set-up was 2.5 ng (4 ppb) methane per litre. It would thus appear that neither *Arabidopsis* nor *Chlamydomonas* was emitting significant quantities of methane under ambient lighting in laboratory-controlled conditions.

We also examined other plant species grown for a longer time under non-sterile conditions. Maize (*Z. mays*, a C<sub>4</sub> plant) and rice (*O. sativa*, a C<sub>3</sub> plant; §2b) were grown in the laboratory on vermiculite, an inert mineral support, in order to avoid the presence of decaying organic matter (§2). Measurements were taken from different amounts of plant material and plants at different stages of growth. Neither appeared to produce methane. The role of rice plants in the transport of methane from methane-rich anoxic paddy fields to the atmosphere has been known for many years (*Nouchi et al.* 1990). These experiments confirm that rice plants serve as a conduit for transporting methane, rather than producing the methane themselves.

A recent paper by *Dueck et al.* (2007) has also shown that there was no evidence for methane emission from plants. This study examined four plant species grown hydroponically in a controlled chamber supplied with <sup>13</sup>C-labelled CO<sub>2</sub>. Methane emissions from plants ranged from an uptake of 10 ng g<sup>-1</sup> h<sup>-1</sup> to emissions of 42 ng g<sup>-1</sup> h<sup>-1</sup>, values which *Dueck et al.* (2007) showed statistically were not significantly different from zero. Taken together with our results presented above, and the fact that the enzymes necessary for classical methanogenesis are not present in plants, it seems clear that plants are not capable of producing methane when grown under controlled conditions in the laboratory under artificial lights.

## 4. STATISTICAL ANALYSIS OF METHANE EMISSION

Key evidence for the production of methane in plants was the observed difference in δ<sup>13</sup>C values of methane emitted

from C3 and C4 plants (Keppler *et al.* 2006). We re-analysed data reported by Keppler *et al.* (2006, table S1) by the use of an ANOVA (R Development Core Team 2007) to examine methane emission from plant material under various conditions. It is not clear whether the data in their table S1 come from separate plants for each condition, or whether the same plant was subjected to each treatment. We therefore carried out two analyses. One assumed that a different plant was subjected to each condition (an ANOVA with four between-subject factors: country (Northern Ireland/Germany), light conditions (light/dark), temperature (30/40°C) and type of plant (C3/C4)). The other analysis assumed that one plant was subjected to each and all of the treatments (a mixed-model ANOVA with two levels, Northern Ireland/Germany and C3/C4, and two within-subject factors, light (light/dark) and temperature (30/40°C)). This was chosen to avoid any pseudo-replication and thus inflation of the apparent sample size. Some of the data were given as a mean of two observations, while others were given as a mean of 3–5, with a standard deviation. The mean value was used in our analyses.

An ANOVA was therefore performed on four separate between-subject factors using R software: type of plant (20 C3 plants and 3 C4 plants), light conditions (15 plants in sunlight and 22 plants in the dark), temperature (22 at 30°C and 21 at 40°C) and country (3 from Northern Ireland and 16 from Germany).

We assessed the  $\delta^{13}\text{C}$  of methane emitted from 20 C3 plants (range:  $-68.4$  to  $-48.6\text{‰}$ ) and 3 C4 plants (range:  $-52.9$  to  $-47.5\text{‰}$ ) from Keppler *et al.* (2006, table S1). If we assume that separate plants were used for each experiment, then a significant difference exists between the  $\delta^{13}\text{C}$  values of methane emitted from C3 and C4 plants ( $F=29.6$ , d.f. = 1, 53,  $p=1.44\times 10^{-6}$ ). This observation holds when we assume that the same plants are used, under a mixed-model ANOVA ( $F=14.49$ , d.f. = 1, 50,  $p=0.00039$ ). This conclusion remains if the data are re-analysed using the non-parametric Kruskal–Wallis test, confirming the Keppler *et al.* (2006) observation. This is in contrast to our experimental data presented above, which did not show the production of methane from either C3 (rice) or C4 (maize) plants grown under identical, controlled conditions.

The plants used by Keppler *et al.* (2006) originated in Northern Ireland and Germany. There is a statistically significant difference between the  $\delta^{13}\text{C}$  values of methane emitted from plants grown in Northern Ireland and those grown in Germany ( $F=5.4$ , d.f. = 1, 53,  $p=0.024$ ) when analysed assuming different plants for each treatment. This difference is lost if a mixed-model ANOVA (assuming the same plant) is used ( $F=0.0612$ , d.f. = 1, 50,  $p=0.81$ ). Light ( $F=0.079$ , d.f. = 1, 50,  $p=0.80$ ) and temperature ( $F=0.000038$ , d.f. = 1, 50,  $p=1$ ) do not appear to influence the  $\delta^{13}\text{C}$  values.

The rates of methane emission from detached leaves were also analysed using an ANOVA. The results of the analysis indicated that the country (Germany/Northern Ireland) where the experiment was conducted influenced the rate of methane release ( $F=12.79$ , d.f. = 1, 53,  $p=0.00075$ ), as did the location of the plant (indoors or outside), when whole plants were analysed ( $F=10.57$ , d.f. = 1, 12,  $p=0.0069$ ).

It should be noted that the amount of methane measured in these experiments was extremely small ( $1\text{--}20\text{ ng g}^{-1}\text{ dw h}^{-1}$ ), and highly variable, suggesting that care should be taken when upscaling to provide an estimate of the global methane emissions from plants. In addition, at these low levels of methane production, the variability in the  $\delta^{13}\text{C}$  measurement is large. For example, the same species of plant grown in two different countries yielded notably different  $\delta^{13}\text{C}$  values (e.g. ash:  $-58.2\text{‰}$  in Northern Ireland and  $-54.9\text{‰}$  in Germany). If the same plant-specific biological process is producing the methane, then these  $\delta^{13}\text{C}\text{--CH}_4$  values ought to be very similar. While some variability would be expected, we would suggest caution in using these figures as proof of methane production.

## 5. SATELLITES PROVIDE LITTLE EVIDENCE FOR METHANE EMISSION

A major source of support for the hypothesis that plants produce and emit methane was based upon satellite data suggesting that elevated methane mixing ratios exist in tropical regions above evergreen forests. The UV/visible/near-infrared spectrometer SCanning Imaging Absorption spectroMeter for Atmospheric CHartography (SCIAMACHY; Frankenberg *et al.* 2005) on-board the European Space Agency's satellite ENVISAT records the intensity of solar radiation, reflected from the Earth's surface or the atmosphere. Frankenberg *et al.* (2005) retrieved column densities of methane from these measurements and converted the data into column-averaged mixing ratio, using  $\text{CO}_2$  concentrations as a standard. Their comparison with methane mixing ratios simulated using a global chemistry-transport model (taking into account then-current emissions inventories) indicated that there may be an additional unidentified source of methane in the tropics, accounting for approximately 30 Tg of methane emissions over the period of investigation (August–November 2003). This finding was interpreted by Keppler *et al.* (2006) as methane emission from plants in tropical rainforests.

Subsequent studies have improved the procedure for obtaining methane mixing ratios from the satellite data (Bergamaschi *et al.* 2007) and have also considered a new inventory for methane emissions from wetlands (Bergamaschi *et al.* 2007). These developments have led to a significantly improved agreement between SCIAMACHY data and model simulations. However, the satellite-derived results still indicate a tendency for enhanced methane mixing ratios in the Andes and the tropical regions of South America and Africa. Clouds are a potential source of bias in the satellite retrievals, which may explain some of the discrepancy. Although an attempt is made to minimize the errors introduced by partial cloud cover over the SCIAMACHY swath (which has a ground pixel size of  $30\times 60\text{ km}$ ) by using a filter based on an effective cloud top height, it is evident from the work of Frankenberg *et al.* (2006; see their figs 4 and 5) that the results are sensitive to the choice of cloud filter. This is especially true in the regions of the tropics where deep penetrative convection and high altitude cirrus clouds occur, potentially introducing a positive bias. There are also considerable uncertainties in the spatial distribution of wetland emissions (Bergamaschi *et al.* 2007). Although

it is likely that there is methane emission from wetlands, especially due to transpiration (§6), caution is needed in attributing significance to the remaining discrepancy between the observations and simulations of methane mixing ratios based upon satellite data.

## 6. EVIDENCE FOR METHANE EMISSION

### (a) *Transpiration of water*

Keppler *et al.* (2006) clearly showed that both whole plants and detached leaves emitted methane when placed in methane-free air, as did Whiticar & Ednie (2007). Given that it is not biochemically possible for plants to create methane by known pathways, how is it that these plants are seen to emit methane?

We suggest that the most obvious explanation for the emission of methane from plants involves transpiration. Plants take up a large amount of water each day through the roots and transport it via the xylem to the leaves. A small amount of water (generally less than 1%) is used for growth and photosynthesis, and the remainder is transpired (released by evaporation) through stomata in the leaf. If the water that is taken up by the roots of a plant contains dissolved methane, and if the methane is not used in the plant, then methane gas must be released by transpiration. As the methane in soil is produced from methanogenic archaea, it will have a  $\delta^{13}\text{C}$  value reflecting a biological origin.

It is widely accepted that various aquatic plants, such as rice, that are rooted in anoxic sediment, release methane. Various trees (e.g. alder) are also known to absorb methane from soil water or soil gas and transport it to the atmosphere (Rusch & Rennenberg 1998). Rice plants emit significantly more methane than can be explained by passive transpiration alone, as methane is also absorbed directly from soil water, gasified, transported up the plant stem and released from micropores in the leaf sheaths (Nouchi *et al.* 1990). Other aquatic plants, such as the common cattail, *Typha latifolia* (Yavitt & Knapp 1995), emit methane via pressurized ventilation of their roots. Another process involves gaseous transport in plants in which compounds such as ethylene (a plant hormone,  $\text{C}_2\text{H}_4$ ) and carbon dioxide ( $\text{CO}_2$ ) accumulate in the roots, and diffuse upwards in the plant stem in gaseous form (Colmer 2003). Plants, including land plants (i.e. non-aquatic flora), contain gas-filled spaces, allowing gas distribution throughout the plant (Raven 1996). The methane molecule is similar in size to these other gases and it seems likely that methane could be transported via the same mechanism.

The transport of water containing methane through the plant can explain why plants grown in different locations (§4) emitted methane with different  $\delta^{13}\text{C}$  values, as the water will contain methane from different sources. Mineral soils containing high levels of oxygen (9–19%) have been demonstrated recently to contain significant quantities of biogenic methane (up to 3% v/v) in pore spaces (Teh *et al.* 2005). Thus, the production of  $\text{CH}_4$  from degradation of organic matter in soils is not confined solely to the fully anoxic subsurface of wetlands and peatlands, and may occur widely in anoxic micro-sites within damp finely textured soils. When plants are grown on the same soil for a number of years, the  $\delta^{13}\text{C}$  value of the soil organic matter reflects the type of plant grown

(C3 or C4; Staddon 2004). Methanogens in the soil will therefore have a carbon source with a different  $\delta^{13}\text{C}$  value, and thus potentially produce methane with  $\delta^{13}\text{C}$  values that differ by 10–15‰. The uptake of the methane by the plant via soil water will give the appearance that C3 and C4 plants produce methane with different  $\delta^{13}\text{C}$  values, as seen in the original experiments. This could explain why our results, using plants grown on inert vermiculite with the same water rather than that on established soil, showed no difference in  $\delta^{13}\text{C}\text{-CH}_4$  values between C3 and C4 plants.

### (b) *Methane dissolved in the soil water*

For transpiration to be the primary source of methane emission, there must be a supply of methane to soil water. Even if the soil is well aerated, almost all soil water contains dissolved methane (Billings *et al.* 2000). This methane is of biological origin, coming from methanogenic archaea that occur throughout the soil in micro-aerobic environments and decaying organic matter (Zeikus & Ward 1974). Methane emitted from these organisms will dissolve into soil water. Although some will be consumed by methanotrophs, low levels will remain and will be taken up into the plants. Measurements of methane levels from soil water from a spruce ecosystem (Black Forest, Germany), ranged from 0.008 to 151.3  $\mu\text{M}$  (Fiedler *et al.* 2005). This is much greater than atmospheric methane, as a dissolved concentration of 0.003  $\mu\text{M}$  corresponds approximately to equilibrium with ambient levels of atmospheric methane (approx. 1700 ppb). Plants living on dry land will therefore be expected to emit low levels of methane, while those in swamps should be expected to emit high levels of methane, in both cases reflecting the amount of methane dissolved in the water, which they transpire. Clearly, the amount of methane dissolved in soil water will vary widely across different ecosystems, and at different times of the year. For example, plants growing in regions of the world with periodic long-term flooding (e.g. owing to monsoon rains) will transpire water with hugely varying methane content over the course of a year.

The emission of methane from detached leaves or whole plants through transpiration explains a number of the observations of Keppler *et al.* (2006). For example, methane emissions were at least one order of magnitude greater from whole plants than from detached leaves. This would be expected, as detached leaves would not be replenished with water, and their stomata would close in response to drought stress. Similarly, Keppler *et al.* observed that emissions increased when plants were exposed to direct sunlight, which is expected according to our proposal, as higher light levels cause full opening of stomata and an increase in transpiration rates (Shimazaki *et al.* 2007). The mechanism we propose is also consistent with the observed correlation between methane emission rates above rice paddy fields and both the number of leaves and the leaf area index of the crop, and thus with the quantity of surface area evapo-transpiring, rather than that with the amount of crop biomass (Gogoi *et al.* 2005).

### (c) *Experimental methane emission*

This hypothesis was tested by experiments on *Ocimum basilicum* (basil). *Ocimum basilicum* plants were dehydrated and rehydrated with methane-enriched water (from a

Table 2. Measurement of methane emissions by transpiration. (Basil (*Ocimum*) plants were rehydrated with methane-enriched water and placed in a closed vessel containing air of known methane concentration (input). Following overnight incubation, air samples were removed (output). Values for the mean and standard deviation of methane mixing ratio and  $\delta^{13}\text{C}$  in triplicate analyses of each air sample are shown.)

plant	input $\text{CH}_4$ (ppb)	output $\text{CH}_4$ (ppb)	input air $\delta^{13}\text{C}$ (‰)	output air $\delta^{13}\text{C}$ (‰)	methane $\delta^{13}\text{C}$ (‰) in water
basil	2026 $\pm$ 3	2242 $\pm$ 7	-47.3 $\pm$ 0.1	-45.5 $\pm$ 0.0	-39.0 $\pm$ 0.1

non-biological source), as described in §2. The methane concentration in the flask containing the basil shoots had risen by 216 ppb (table 2). The extra methane must have been derived from the gas in the water supplied to the plants because of the increased (i.e. less negative)  $\delta^{13}\text{C}$  value. When plants were supplied with distilled water unsaturated with respect to methane, no rise in methane concentration was observed. The methane must therefore have entered the plant through the roots, and was retained in the cut shoots and leaves.

In order to measure the amount of methane that was transpired, we examined *Apium graveolens* (celery) stalks with leaves (§2c). After overnight incubation, there was an increase in methane levels of 31 ppb in the vessel containing celery stems. The decrease in mass of 0.57 g from the celery stems is due to the amount of water released by transpiration. When compared with the measured rise in methane, this would indicate that 0.45 mg l<sup>-1</sup> methane was released from the transpired water. This is well within the theoretical maximum of 26 mg l<sup>-1</sup> (the saturation point of methane in water). Therefore, significant amounts of methane could be transpired through plants.

## 7. STRESS-MEDIATED BREAKDOWN OF PLANT MATERIAL RELEASES METHANE

Recently, several papers and abstracts have been published that show that detached leaves emit methane under stress conditions. The amount of methane released in each case is an order of magnitude smaller than those observed by Keppler *et al.* but rose with increasing light levels (Whiticar & Ednie 2007), significant UV (Bruhn *et al.* 2007; Rockmann *et al.* 2007; Vigano *et al.* 2007), heating (Vigano *et al.* 2007) and gamma irradiation (Whiticar & Ednie 2007). In some cases, this can be accounted for by evaporation of methane that was found in water in the leaf (which would otherwise have been transpired, if the plant were still alive) but in other cases there must be a new source. No known biochemical pathway exists in plants to produce this methane. As all the treatments that induce methane release are stress conditions, we propose that methane is a photo-, high UV-, heat- and irradiation-mediated breakdown by-product of plant cell material. During high stress conditions, a variety of chemical changes occur to plant cells, causing morphological changes and damaging compounds within the cell (Kovacs & Keresztes 2002). These include the release of free radicals (Hideg & Vass 1996), which could induce the breakdown of cellular material and the release of methane.

These are plant stress conditions and are not found under normal growth. For example, plants are not normally exposed to the high temperatures used in the

various experiments (up to 70°C). Thus, the release of methane from a breakdown product is unlikely to happen under normal growth conditions. Because of this, we believe that the predominant source of methane for plants growing under normal conditions is more likely to be transpiration, which explains the observations in Keppler *et al.* (2006), Dueck *et al.* (2007) and Wang *et al.* (2008).

It has been proposed that one source of methane is the breakdown of pectin (Keppler *et al.* 2006, 2008). Despite this, analysis of the results from Keppler *et al.* (2006) revealed that per unit mass, plants release approximately 100 times more methane than pure pectin per unit mass (in methane-free air) at room temperature. Plants contain approximately 1 per cent pectin (Sterling & Kalb 1959), which suggests that the amount of methane released by plants is approximately 10 000 times more than pure pectin. This indicates that, regardless of whether the breakdown of pectin releases methane during ambient conditions, it cannot account for the source of methane in plants.

However, methane emission due to stress-induced breakdown of plant material does have important consequences. If, for example, there is exposure to radiation, high UV (due to an ozone hole) or heat (from a forest fire), it is likely that the plants will be damaged and thus release methane. This also has consequences for the methane budget of the early Earth; before there was a significant atmosphere, it seems reasonable to assume that cyanobacteria were damaged by high UV levels, inducing the release of methane from methylated polymers. This should be accounted for in the estimates of the composition of the early atmosphere.

## 8. CONCLUSIONS

There has been considerable recent controversy over whether plants produce methane. Some experimental results have shown that plants do emit methane (Keppler *et al.* 2006), although others have shown that they do not (Dueck *et al.* 2007). It would initially appear that these results are incompatible with each other. We believe that both sets of results are correct, and can be explained by the complex nature of plant physiology. In contrast to the previously published statements, we show that plants do not have the necessary biochemical pathways to synthesize methane, and therefore reports of aerobic production of methane are incorrect. Instead, emission of methane under normal growth conditions is due to the transport of water containing dissolved methane from the soil to the atmosphere. Under certain stress conditions, methane may be generated by the breakdown of plant material. The emission of methane by plants is therefore a passive consequence of other physiological processes. Thus

Kepler *et al.* (2006) primarily measured transpired methane; when this methane is removed (Dueck *et al.* 2007), no methane emission is observed.

Identifying all sources of methane is important in setting global methane inventories. The role of plants in moving methane about is not accounted for. In addition, transpiration could help to explain the satellite-measured methane enhancement over the tropical forest regions of South America and Africa. Transpiration produces most of the water vapour at lower levels in rainforests (Moreira *et al.* 1997), accounting for some of the published ground-based methane measurements (Braga do Carmo *et al.* 2006; Miller *et al.* 2007). Although these measurements ( $4\text{--}38\text{ Tg yr}^{-1}$  in the global methane budget) are considerably less than the enhancement suggested earlier ( $236\text{ Tg yr}^{-1}$ ; Kepler *et al.* 2006), they indicate the importance of plants in the global cycling of methane.

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