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No detectable aerobic methane efflux from plant material, nor from adsorption/desorption processes

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Abstract. In early 2006, Keppler et al. (Nature, 439:187–191) reported a novel finding that plant leaves, and even simple organic materials, can release methane under aerobic conditions. We investigated here whether the reported methane release might simply arise from methane desorption from sample surfaces after prior exposure to higher methane concentrations. We exposed standard cellulose filter papers (i.e. organic material with a high surface area) to atmospheric methane concentration and then transferred them to a low-methane atmosphere. Our results suggest that any desorption flux was extremely small ($-0.0001\pm0.0019\,ngCH_4\,kgDW^{-1}\,s^{-1}$) and would play no quantitatively significant role in modifying any measured methane fluxes.

We also incubated fresh detached leaves of several species and intact *Zea mays* seedlings under aerobic and low-light conditions. After correcting for a small measured methane influx into empty chambers, measured rates of methane emission by plant materials were zero or, at most, very small, ranging from $-0.25\pm1.1\,ngCH_4\,kgDW^{-1}\,s^{-1}$ for *Zea mays* seedlings to $0.10\pm0.08\,ngCH_4\,kgDW^{-1}\,s^{-1}$ for a mixture of freshly detached grasses. These rates were much smaller than the rates originally reported by Keppler et al. (2006).

1 Introduction

Methane is an important greenhouse gas, contributing about 20% to the current radiative forcing of the enhanced greenhouse effect (Ramaswamy et al., 2001). It has been intensively studied and it had been thought that all of its sources and sinks had been identified. Hence, it came as a surprise when Keppler et al. (2006) reported a new finding that methane could be released under aerobic conditions by living plants and even dead plant tissues.

This was corroborated in field measurements of tropical C_4 grasses by Sanhueza and Donoso (2006) who observed greater net methane efflux from intact grass swards than after the same swards had been cut. The implication of this observation was that additional methane was being released by the grass foliage. Contrary to those findings, Dueck et al. (2007) and Beerling et al. (2008) conducted detailed laboratory-based investigations of possible aerobic methane release. They used different experimental approaches to overcome some of the measurement challenges of measuring minute methane fluxes and did not observe any significant methane emissions in their systems.

Wang et al. (2008) measured aerobic methane released from a variety of plants from inner Mongolia. They reported that under dark conditions, about 80% of their test plants did not produce any measurable amount of methane. Of the 20% that did produce methane, Wang et al. (2008) showed that methane release for some of those was simply due to apparent storage of soil-derived methane in stem tissues.

For one species, the xerophytic shrub *Artemisia frigida*[1], however, Wang et al. (2008) excluded a range of possible artefacts and still found that leaves produced methane under aerobic conditions. It is particularly interesting that one of the studied species clearly produced methane while most of the others did not. If unaccounted artefacts had played a role in the work of Wang et al. (2008), one might have expected aerobic methane release to have been reported for either all of their samples or for none. On the other hand, this work contrasts with the observations of Keppler et al. (2006) who

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[1]The species had been described as *Achillea frigida* by Wang et al. (2008). However, it was subsequently confirmed that the species was actually *Artemisia frigida* (Z.-P. Wang, personal communication)

observed methane release from all plant materials they studied.

In other recent studies, Vigano et al. (2008) and McLeod et al. (2008) showed that aerobic methane release was linearly related to exposure to UV radiation. They showed that a variety of plant materials, and plant constituents such as pectin and lignin, released substantial quantities of methane under exposure to UV radiation or at high temperatures above 80°C. Highest emissions were recorded at UV levels that exceeded levels normally found under natural conditions, but the studies showed that aerobic methane release was, indeed, possible under these conditions.

Other indirect evidence for the existence of aerobic methane release came from field studies by Crutzen et al. (2006), do Carmo et al. (2006) and satellite observations reported by Frankenberg et al. (2005, 2006), although the latest re-analysis of the satellite data point to somewhat lower apparent methane emissions from tropical regions (Frankenberg et al., 2008). Together, these studies presented findings that were consistent with the presence of aerobic methane emissions, but other possible sources or adjustments in the source/sink balance could not be excluded.

At present, the question is still open whether the significant apparent methane emissions were actually just artefacts of the measurement conditions. Kirschbaum et al. (2006) listed a range of possible artefacts that could have led to spurious observed rates, and Kirschbaum et al. (2007) subsequently tried to quantify some of these in greater detail. They concluded that methane absorption (in the liquid and lipid phase inside living leaves) and the amount of methane held within intercellular air spaces, would be unlikely to cause quantitatively important artefacts, but that methane adsorption/desorption was of more serious concern (Kirschbaum et al., 2007).

Methane readily adsorbs onto the surfaces of organic materials that have been activated to increase their internal surface area. Cell walls of plants consist of a complex network of a highly porous polysaccharide matrix with large numbers of small pores (Cheng and Huang, 2004; Celzard and Fierro, 2005). Pore sizes are typically around 5 nm (e.g. Carpita et al., 1979; Carpita and Gibeaut, 1993) which approach the molecular diameter of methane to allow a strong interaction between organic materials and methane molecules. This is largely responsible for the high adsorption capacities of organic matter (Biloe et al., 2002; Lozano-Castello et al., 2002).

Many organic materials, such as activated charcoal formed from coconut shells, can strongly adsorb methane, and at high pressure and moderate temperatures, some are able to adsorb more methane than their own weight (Wegrzyn and Gurevich, 1996). Because of that, methane adsorption has even been considered as a means of storing methane in natural-gas powered road vehicles (e.g. Wegrzyn and Gurevich, 1996).

As adsorption potential decreases with increasing temperature (e.g. Garcia-Perez et al., 2007), adsorption/desorption could explain not only observed base rates but also the strong temperature response of aerobic methane release that had been reported by Keppler et al. (2006). As an increase in temperature reduces the methane adsorption capacity of leaves, methane will desorb even if the external methane concentration does not change (Harrison et al., 2000; Shao et al., 2004; Thammakhet et al., 2005).

Adsorption and desorption are also relatively slow processes, in particular to and from organic materials (Pignatello and Xing, 1996). Some studies have suggested that methane adsorbed to organic materials can be released into methane-free air at steady rates for periods of days to weeks (Zhang and Krooss, 2001; Cheng and Huang, 2004). Unfortunately, few data are available for low-pressure methane adsorption capacities of non-activated compounds, and extrapolation from available adsorption isotherms to a low pressure range is limited due to the very strong pressure-dependence of adsorption at low pressures (Shao et al., 2004; Walton et al., 2005; Garcia-Perez et al., 2007; Saha et al., 2007).

Kirschbaum et al. (2007) estimated an adsorption potential of about 40 000 $ngCH_4\ kgDW^{-1}$based on the adsorption characteristics of plant cell walls of coconut charcoal when they assumed a simple linear dependence of adsorption on methane partial pressure. With reported flux rates of less than $1\ ngCH_4\ kgDW^{-1}\ s^{-1}$ for dead plant materials (Keppler et al., 2006), Kirschbaum et al. (2007) postulated that methane desorption could potentially play an important role in contributing to observed apparent fluxes.

As adsorption/desorption is a simple physical process that occurs everywhere, one has to expect that it would modify any apparent emission fluxes. The key questions are 1) whether the amounts potentially adsorbed or desorbed are large enough to be quantitatively significant; and 2) whether any fluxes occur at a rate and for a duration over which they can interfere with experimental measurements. If desorption is very fast it might be completed during any experimental equilibration period; if it is very slow, any desorption could occur at only very small rates.

We conducted a simple experiment to expose a standard organic material to a normal atmospheric methane concentration and then to a sub-atmospheric concentration to observe any methane efflux due to methane desorption. We used standard cellulose filter paper for the experiment in order to facilitate the repeatability of the experiment. Samples were pre-incubated in an atmospheric methane concentration because this is of most practical relevance for experiments studying the release of methane under aerobic conditions.

Following the adsorption experiment with filter papers, we introduced various detached, but living, plant materials and intact growing *Zea mays* seedlings into the chambers to determine whether we could observe any detectable aerobic methane efflux from these plant materials.

2 Materials and methods

We used six cylindrical plexiglass chambers with an internal diameter of 140 mm and a volume of 5.7 l. The chambers were sealed at either end with rubber O-rings and placed on a laboratory bench where they received light from fluorescent lamps at about 5 μmol quanta m^{-2} s^{-1}.

Two chambers were left empty (to provide blank tests) and four chambers were filled with 100 Whatman No. 1 filter papers (diameter 12.5 cm). The filter papers were stacked on a stainless steel rod (2 mm diameter) placed in the middle of the chambers. A gap between each filter paper ensured that air could diffuse to both sides of each individual filter paper. The total weight of each stack of filter papers was approximately 112.2 g.

The chambers were left open and exposed to the ambient atmosphere for approximately two weeks. The chambers were then closed and flushed with methane-free air for 10 min at 1,500 ml min^{-1} via sampling ports at either end of the chamber. Because of some mixing between gas inside the chamber and the flushing gas, some methane remained inside the chamber even after flushing for 10 min. After flushing, the sampling ports were sealed, and the methane concentration was recorded over the following six days.

To measure the methane concentration inside the chambers, 5 ml of gas was withdrawn from the sample chamber using a syringe with a hypodermic needle inserted through a rubber septum in the sampling port. Sample methane concentrations were measured with a gas chromatograph (Varian CP-3800, Varian Inc., USA) using a flame-ionization detector. The instrument was calibrated against certified gas standards. After analysis, 5 ml of methane-free air was injected back into the chambers to replace the sampled gas. A correction for this dilution was made in the subsequent calculation of methane concentrations.

For measuring the methane efflux from living plant material, six different plant materials were collected from plants growing near our laboratory or from a local plant nursery. We used leaves of Five Finger (*Pseudopanax arboreus* (Murray) Philipson) and Broadleaf (*Griselinia littoralis* Raoul) as two examples of large and fleshy leaves that were thought to be least likely to show adverse physiological responses to detachment from the parent plant. Foliage from a mixture of local grasses was used as an example of plant material with likely higher growth and gas exchange rates than the tree leaves. Recently formed and expanded dandelion flowers (*Taraxacum officinale* F. H. Wigg) were used as an example of plant material with high specific growth rate as it has been suggested that aerobic methane release could be related to cell wall synthesis (Keppler et al., 2006).

We also used Yarrow (*Achillea millefolium* L.), a locally available relative of *Achillea frigida*, that had been reported to have been used by Wang et al. (2008). Once we learnt that the material had been mislabelled and the species used by Wang et al. (2008) was actually *Artemisia frigida* Willd, we obtained a locally available relative of that species, Wormwood (*Artemisia absinthium* L.).

Excised plant material was placed in plastic bags, immediately taken to the laboratory and placed inside our sample chambers. Plant materials did not noticeably dry out during the incubation, and it is likely that the plant materials remained physiologically active during measurements.

We also used intact seedlings of corn (*Zea mays* L.; var. "Early Chief") growing in small pots containing vermiculite. Seedlings were 18 days old at the start of the experiment. Pots were last watered two days before the start of the experiment to avoid any possibility of anaerobic conditions developing in the rooting medium. The development of anaerobic conditions is unlikely in a vermiculite medium in any case, but partial drying was used as an extra precaution. The partial drying was not enough to have caused any water stress for plants as evidenced by water exudation on the leaves inside our sample chambers.

In that experiment, we used empty chambers and chambers with vermiculite-filled pots as controls. However, there were no apparent differences between empty chambers and those with pots without plants so that no results from the empty pots are shown below.

All plant material was exposed to normal atmospheric methane concentration while growing outside, as well as inside the chambers until the start of the incubation when chambers were flushed with methane-free air for 10 min at 1500 ml min^{-1}. Mean ambient methane concentration in the laboratory from repeated measurements over a number of days was 1818 ppb. The methane concentration varied between days, but measurements on the same day were repeatable within $\pm$15 ppb.

As there was no gas flow through the sample chambers after they had been sealed, relative humidity inside the chambers probably built up to close to 100%, after which further water loss from the plant materials was prevented. The CO_2 concentration inside sample chambers was not monitored. At the end of the experiment, all plant material was dried in an oven at 80°C for 48 h and weighed. Data were statistically analysed by using a linear mixed effect model, with confidence intervals based on a t-distribution, fitted using the lme function in the nlme library of R (R Development Core Team 2008). Confidence intervals for parameters were based on a t-distribution.

3 Results

Figure 1 shows the change in methane concentration as a function of time after the methane concentration surrounding the test samples had been changed from atmospheric to a lower methane concentration. Concentrations did not fall below about 100 ppb because the chambers were flushed with methane-free air for only 10 min before measurements. Flushing for 10 min constituted a compromise

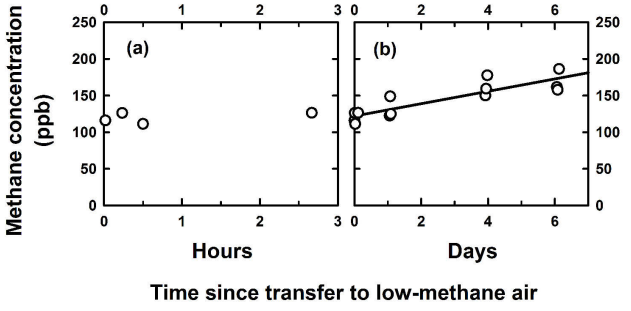

Fig. 1. Observed methane concentrations in one of the sample chambers with a stack of filter papers, shown as a function of time after incubating samples in air with a low methane concentration. Up to time zero, samples had been pre-incubated at atmospheric methane concentrations of about 1800 ppb. The line in (b) is a linear regression line shown here only for visualisation of the trend.

between flushing for a long period that would have reduced the initial concentration further and starting measurements as early as possible in order to observe any early methane desorption during the first few minutes after reducing the concentration of the surrounding air.

There was no consistent trend in methane concentrations over the first few hours after the initial flushing with methane-free air (Fig. 1a). However, methane concentrations increased linearly over the full six days of incubation. With the absence of measurable trends over the first few hours of incubation, we then normalised all data relative to the methane concentrations observed after the end of flushing with methane-free air on day 0 and combined data from all chambers (Fig. 2).

On each measurement occasion, individual data points scattered around mean values by 20–40 ppb. Despite the scatter on individual days, the trend was also clear with an apparent flux into sample chambers of 9.5 ± 4.2 ppb d^{-1} (Mean ±95% confidence interval; Fig. 2b), which was almost identical to the apparent flux into empty chambers of 9.9 ± 5.9 ppb d^{-1} (Fig. 2a).

We then subtracted the rate of methane increase in empty chambers from the change in methane concentration in chambers with filter papers to derive an apparent flux due to the presence of filter papers. This was expressed as a flux per unit dry weight in Fig. 2c. These derived data showed no apparent trend (-0.0001 ± 0.0019 $ngCH_4$ $kgDW^{-1}$ s^{-1}) over the six days of measurement (Fig. 2c), indicating that any desorptive flux was not discernable within the resolution of the instrument and the experimental set-up.

We then incubated six different types of plant material in our chambers. Detached leaf samples were left in the chambers for only one to three days to minimise physiological changes after removal from the parent plant. Detailed results for the incubation of *Achillea millefolium* are shown as an example in Fig. 3.

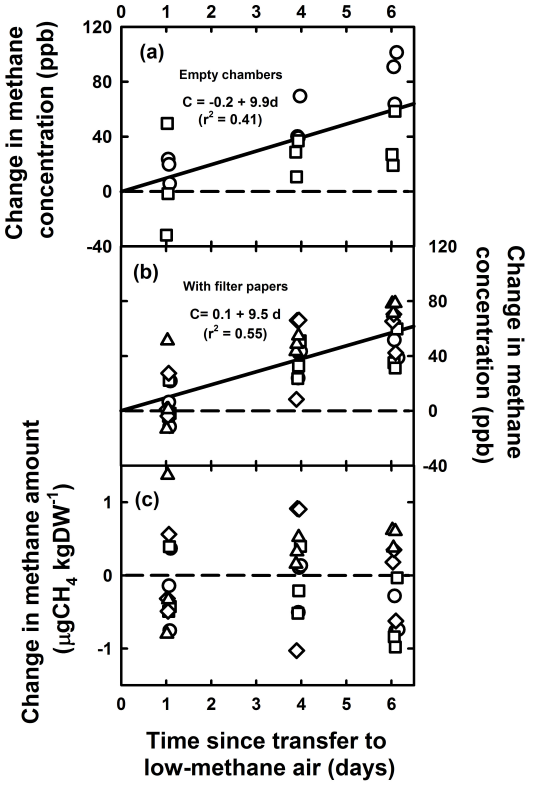

Fig. 2. Methane concentration as a function of time after incubating samples in methane-free air for empty chambers **(a)**, chambers with filter papers **(b)**, and derived apparent methane change in chambers with filter papers after subtracting the flux into empty chambers **(c)**. Different symbols refer to different sample chambers. Lines in (a) and (b) are linear regressions, with relevant coefficients shown in the figure. No regression line was fitted to data in (c) as the trend in the data did not differ significantly from 0.

Table 1. Apparent methane fluxes from living detached leaves and intact plant materials observed under low-light conditions. Data shown are means ±95% confidence intervals.

Scientific name	Common name	Rate ($ngCH_4$ $kgDW^{-1}$ s^{-1})	
		Fresh detached	Intact plants
Z. mays	Corn		-0.25 ± 1.1
P.arboreus	Five finger	0.007 ± 0.022	
G. littoralis	Broadleaf	0.026 ± 0.021	
–	Mixed grasses	0.102 ± 0.082	
T. officinale	Dandelion flowers	0.043 ± 0.38	
Achillea millefolium	Yarrow	0.049 ± 0.049	
Artemisia absinthium	Wormwood	0.068 ± 0.077	

In this experiment, methane concentrations increased in the sample chambers (Fig. 3a), and at a slightly faster rate than in empty chambers, resulting in an apparent flux of $5\,\mu gCH_4$ $kgDW^{-1}$ d^{-1} (Fig. 3b) or 0.049 ± 0.049 $ngCH_4$ $kgDW^{-1}$ s^{-1}. Derived flux rates for other plant materials are shown in Table 1.

Table 2. Comparison of methane fluxes reported by Keppler et al. (2006) and those calculated from plant materials and filter papers in the present experiment. All numbers are given in $ngCH_4\,kgDW^{-1}\,s^{-1}$. Detached plant material measured by Keppler et al. (2006) consist of a mixture of fresh and dried plant material, whereas our detached plant materials were living and probably more comparable to intact plants measured by Keppler et al. (2006).

Intact plant materials, dark	32±13	Keppler et al. (2006)
Detached plant materials, 30°C, dark	0.33±0.25	
Pectin, 30°C, dark	0.036	
Filter papers, 20°C, low light	−0.0001±0.0019	This experiment
Plant materials, 20°C, low light	−0.25±1.1 to 0.10±0.08	

The rates shown in Table 1 are much smaller than the rates reported by Keppler et al. (2006) of $32\pm13\,ngCH_4\,kgDW^{-1}\,s^{-1}$ for emissions from intact plant materials (Table 2). Rates reported here are, at most, comparable to the rates for pure pectin reported by Keppler et al. (2006).

4 Discussion

Our experiment was primarily designed to quantify the flux of desorbing methane from a standard organic material, and to assess whether such a flux could account for any aerobic methane fluxes from plant material reported in the literature. We found that methane concentrations changed in chambers with filter papers at the same rate as in empty chambers, implying a desorptive flux below the detection limit of our experimental set-up. We, therefore, could not support the hypothesis of Kirschbaum et al. (2007) that methane desorption might be responsible for observed apparent methane fluxes under aerobic conditions.

In designing the experiment, we chose measurement materials and conditions where methane desorption might be relatively large compared to de-novo methane release. Hence, we used filter papers rather than living plant tissues and did not expose our material to high light and especially not to UV radiation. Methane desorption should occur at similarly negligible rates under experimental conditions where other fluxes might be more important.

Our observations could indicate (a) that adsorption at low methane concentration is quantitatively much smaller than would be expected based on a linear dependence of adsorption on the surrounding methane concentration; or (b) that the amounts adsorbed by plant materials are negligible without activation; or (c) that desorption is very fast and completed during the initial 10 min of chamber flushing; or (d) that it proceeds at exceedingly slow rates for extended periods. Whatever the explanation for these negligible observed fluxes, our tests exclude methane desorption as a quantitatively important artefact contributing to any observed aerobic methane fluxes.

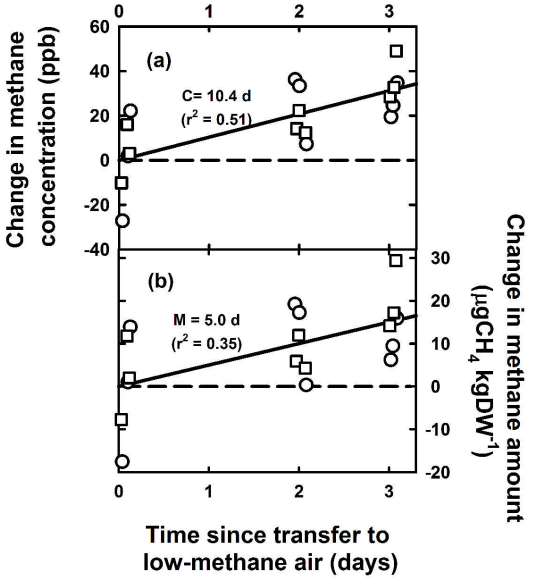

Fig. 3. Methane concentration change **(a)** and corresponding calculated methane fluxes **(b)** as a function of time after incubating plant samples of *Achillea millefolium* in methane-free air. Samples had been pre-incubated in normal atmospheric methane. Data in (b) are calculated after first subtracting the apparent flux into empty chambers ($3.8\,ppb\,d^{-1}$) measured in that experiment. Lines are regression lines, with relevant coefficients shown in the figure.

At the same time, we also observed no, or at most very low, aerobic methane release from living plant tissues. The observed methane flux from living leaves was, at most, a small fraction of the flux reported by Keppler et al. (2006). We know of no explanation for these very different findings.

There was some apparent leakage of methane into our chambers, possibly through the rubber seals of the chamber or the septum used for extracting samples. Leakage could have occurred either during the extraction of a sample or during the intervening period between sampling. Such a flux of methane might be driven by pressure differences between the chamber and the surrounding room air due to small diurnal temperature changes and changes in atmospheric pressure with synoptic changes in weather systems.

This leakage into chambers could possibly be higher for chambers with plant material because the air in those chambers would have built up to 100% relative humidity so that the displacement of gas by water vapour and any condensation/re-evaporation during small diurnal temperature changes would have created additional pressure changes in these chambers. It is thus possible that there would have been greater leakage into chambers with plant materials than into empty chambers (or chambers with filter papers). Actual emission rates from leaves could therefore have been either very small (if leakage was the same as into empty chambers) or zero if leakage rates into chambers with leaves were higher than into chambers without leaves.

While apparent rates emitted by leaves could have been slightly increased by the possibility of enhanced leakage, it seems unlikely that there were any artefacts that would have negated actual fluxes. One possible mechanism for the removal of methane could have been the presence of methanotrophic bacteria, which oxidise methane and release carbon dioxide and water. We tested for that possibility by exposing some of our plant material to about 13 000 ppb methane and recorded the methane concentration for the following three days (data not shown). No significant change in methane concentration was observed which effectively eliminated the presence of methanotrophs as a complicating factor in our experiments.

Consistent with our findings, Dueck et al. (2007) also found no, or extremely small, methane efflux from measurements on their plant materials, although a subsequent investigation by Vigano et al. (2008) indicated that a small amount of methane had been produced by the plant material in the experiment of Dueck et al. (2007). In another recent study, Beerling et al. (2008) used a different experimental protocol and were also unable to detect any aerobic methane release from their plant materials. It is possible that some of the differences between the high rates reported by Keppler et al. (2006) and other workers might have been caused by artefacts such as the apparent storage of soil-derived methane in plant stems that had been identified by Wang et al. (2008).

Wang et al. (2008) looked at 40 different species and found no methane emission from most of the species once potential artefacts had been excluded. *Artemisia frigida*, however, emitted methane at about 0.9 $ngCH_4\,kgDW^{-1}\,s^{-1}$. It is possible that the observations of Wang et al. (2008) might relate not to species but to some specific aspect of the growth condition or physiological state of plant materials that had been investigated. When we measured emissions from a related species, *Artemisia absinthium*, we found rates that were at most one tenth of the rates reported by Wang et al. (2008).

Vigano et al. (2008) and McLeod et al. (2008) reported that aerobic methane release was strongly stimulated by exposure to UV radiation. Vigano et al. (2008) and Keppler et al. (2008) also reported similar stimulation of aerobic methane release by high temperatures in excess of 80°C. Extrapolation of these findings to low-UV and low-temperature conditions indicates only very low release rates. This recent work has shown that at least under high UV or high temperature conditions, aerobic methane release can, indeed, be repeatably observed.

With the range of observations now available, including the observations of matching isotopic signature between plant material and released methane (Keppler et al., 2006), the detailed investigations by Wang et al. (2008), the identification of a link to UV exposure (Vigano et al., 2008; McLeod et al., 2008) and the elimination of a range of possible artefacts (Kirschbaum et al., 2007 and the present study), it does seem likely that it is indeed possible for methane to be produced by plants under aerobic conditions, at least by some plant materials and under some conditions. Divergent findings by Dueck et al. (2007), Wang et al. (2008), Beerling et al. (2008), and the investigation of live plant materials in the present study may simply indicate that different species have different methane production potentials, or that methane production varies strongly with environmental or physiological conditions.

The work of Vigano et al. (2008) and McLeod et al. (2008) has strongly implicated UV (UV-B and to a lesser extent UV-A) exposure as an agent of methane release from dead plant materials, with rates as high as 1000 $ngCH_4\,kgDW^{-1}\,s^{-1}$. Keppler et al. (2006), in their original work, observed higher rates from intact plants than dead plant material, which suggests that a possible second mechanism might operate in living tissues. Ghyczy et al. (2008) recently proposed a mechanism that implicated methane release from choline and other compounds with methyl groups during transient oxygen deprivation. The hypothesis was supported by a number of experiments in chemical solutions and mitochondrial extracts from rat livers. While the hypothesis was not tested with plant cells, it would seem plausible that the same process could operate in plant mitochondria.

Keppler et al. (2008) and McLeod et al. (2008) recently corroborated the role of methyl groups as the likely precursor for methane release under aerobic conditions and the likely role of reactive oxygen species. McLeod et al. (2008) found that methane release from pectin was strongly stimulated by exposure to UV radiation, but that stimulation was lost again if reactive-oxygen scavenging chemicals were included in their incubations as well.

The different studies published recently, and our work reported here, all add to the understanding of possible aerobic methane release. The different observations still do not form a completely coherent picture, but plausible explanations to resolve some of the apparently conflicting findings have recently emerged. It has now become possible to reliably prove the existence of aerobic methane release under UV exposure in repeatable experiments. The involvement of methyl groups, first postulated by Keppler et al. (2006), has now been demonstrated more directly (Keppler et al., 2008; McLeod et al., 2008). There has been much progress in understanding the mechanisms underlying methane release

over just the past few months. Nonetheless, further work is still needed to further characterise the environmental and physiological conditions that stimulate or suppress aerobic methane release and to fully be able to appreciate the quantitative significance of aerobic methane release in a global context.

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References

Beerling, D. J., Gardiner, T., Leggett, G., McLeod, A., and Quick, W. P.: Missing methane emissions from leaves of terrestrial plants, Global Change Biol., 14, 1–6, 2008.

Biloe, S., Goetz, V., and Guillot, A.: Optimal design of an activated carbon for an adsorbed natural gas storage system, Carbon, 40, 1295–1308, 2002.

Carpita, N., Sabularse, D., Montezinos, D., and Delmer, D. P.: Determination of the pore size of cell walls of living plant cells, Science, 205, 1144–1147, 1979.

Carpita, N. C. and Gibeaut, D. M.: Structural models of primary cell walls in flowering plants: consistency of molecular structure with the physical properties of the walls during growth, The Plant Journal, 3, 1–30, 1993.

Celzard, A. and Fierro, V.: Preparing a suitable material designed for methane storage: A comprehensive report, Energy and Fuels, 19, 573–583, 2005.

Cheng, A.-L. and Huang, W.-L.: Selective adsorption of hydrocarbon gases on clays and organic matter, Org. Geochem., 35, 413–423, 2004.

Crutzen, P. J., Sanhueza, E., and Brenninkmeijer, C. A. M.: Methane production from mixed tropical savanna and forest vegetation in Venezuela, Atmos. Chem. Phys. Discuss., 6, 3093-3097, 2006,
http://www.atmos-chem-phys-discuss.net/6/3093/2006/.

do Carmo, J. B., Keller, M., Dias, J. D., de Camargo, P. B., and Crill, P.: A source of methane from upland forests in the Brazilian Amazon, Geophys. Res. Lett., 33, L04809, doi:10.1029/2005GL025436, 2006.

Dueck, T. A., de Visser, R., Poorter, H., Persijn, S., Gorissen, A., de Visser, W., Schapendonk, A., Verhagen, J., Snel, J., Harren, F. J. M., Ngai, A. K. Y., Verstappen, F., Bouwmeester, H., Voesenek, L. A. C. J., and van der Werf, A.: No evidence for substantial aerobic methane emission by terrestrial plants: A ^{13}C-labelling approach, New Phytol., 175, 29–35, 2007.

Ferretti, D. F., Miller, J. B., White, J. W. C., Lassey, K. R., Lowe, D. C., and Etheridge, D. M.: Stable isotopes provide revised global limits of aerobic methane emissions from plants, Atmos. Chem. Phys., 7, 237–241, 2007,
http://www.atmos-chem-phys.net/7/237/2007/.

Frankenberg, C., Bergamaschi, P., Butz, A., Houweling, S., Meirink, J. F., Notholt, J., Petersen, A. K., Schrijver, H., Warneke, T., and Aben, I.: Tropical methane emissions: A revised view from SCIAMACHY onboard ENVISAT, Geophys. Res. Lett., 35, L15811, doi:10.1029/2008GL034300, 2008.

Frankenberg, C., Meirink, J. F., Bergamaschi, P., Goede, A. P. H., Heimann, M., Körner, S., Platt, U., van Weele, M., and Wagner, T.: Satellite chartography of atmospheric methane from SCIAMACHY onboard ENVISAT: Analysis of the years 2003 and 2004, J. Geophys. Res., 111, D07303, doi:10.1029/2005JD006235, 2006.

Frankenberg, C., Meirink, J. F., van Weele, M., Platt, U., and Wagner, T.: Assessing methane emissions from global space-borne observations, Science, 308, 1010–1014, 2005.

García-Pérez, E., Parra, J. B., Ania, C. O., García-Sánchez, A., van Baten, J. M., Krishna, R., Dubbeldam, D., and Calero, S.: A computational study of CO_2, N_2, and CH_4 adsorption in zeolites, Adsorption, 13, 469–476, 2007.

Ghyczy, M., Torday, C., Kaszaki, J., Szabó, A., Czóbel, M., and Mihály, B.: Hypoxia-induced generation of methane in mitochondria and eukaryotic cells – an alternative approach to methanogenesis, Cell. Physiol. Biochem., 21, 251–258, 2008.

Harrison, D., Seakins, P. W., and Lewis, A. C.: Simultaneous monitoring of atmospheric methane and speciated nonmethane hydrocarbon concentrations using Peltier effect sub-ambient preconcentration and gas chromatography, J. Environ. Monitor., 2, 59–63, 2000.

Higaki, S., Oya, Y., and Makide, Y.: Emission of methane from stainless steel surface investigated by using tritium as a radioactive tracer, Chem. Lett., 35, 292–293, 2006.

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

Keppler, F., Hamilton, J. T. G., McRoberts, W. C., Vigano, I., Braß, M., and Röckmann, T.: Methoxyl groups of plant pectin as a precursor of atmospheric methane: Evidence from deuterium labelling studies, New Phytol., 178, 808–814, 2008.

Kirschbaum, M. U. F., Bruhn, D., Etheridge, D. M., Evans, J. R., Farquhar, G. D., Gifford, R. M., Paul, K. I., and Winters, A. J.: A comment on the quantitative significance of aerobic methane release by plants, Funct. Plant Biol., 33, 521–530, 2006.

Kirschbaum, M. U. F., Niinemets, U., Bruhn, D., and Winters, A. J.: How important is aerobic methane release by plants?, Functional Plant Science and Biotechnology, 1, 138–145, 2007.

Lozano-Castello, D., Alcaniz-Monge, J., de la Casa-Lillo, M. A., Cazorla-Amoros, D., and Linares-Solano, A.: Advances in the study of methane storage in porous carbonaceous materials, Fuel, 81, 1777–1803, 2002.

McLeod, A. R., Fry, S. C., Loake, G. J., Messenger, D. J., Reay, D. S., Smith, K. A., and Yun, B.-W.: Ultraviolet radiation drives methane emissions from terrestrial plant pectins, New Phytol., 180, 124–132, doi:10.1111/j.1469-8137.2008.02571.x, 2008.

Pignatello, J. J. and Xing, B.: Mechanisms of slow sorption of organic chemicals to natural particles, Environ. Sci. Technol., 30, 1–11, 1996.

Pollmann, J., Helmig, D., Hueber, J., Tanner, D., and Tans, P. P.: Evaluation of solid adsorbent materials for cryogen-free trapping – gas chromatographic analysis of atmospheric C2-C6 nonmethane hydrocarbons, J. Chromato. A, 1134, 1–15, 2006.

Ramaswamy, V., Boucher, O., Haigh, J., Hauglustaine, D., Haywood, J., Myhre, G., Nakajima, T., Shi, G. Y., and Solomon, S.: Radiative forcing of climate change, in: Climate Change 2001: The Scientific Basis, Contribution of Working Group I to the Third Assessment Report of the Intergovernmental Panel on Climate Change, edited by: Houghton, J. T., Ding, Y., Griggs, D. J., Noguer, M., van der Linden, P. J., Dai, X., Maskell, K., and Johnson, C. A., Cambridge University Press, Cambridge, UK, 349–416, 2001.

R Development Core Team: R-A language and environment for statistical computing, version 2.7.1. R Foundation for Statistical Computing, Vienna, Austria, ISBN 3-900051-07-0 (http://www.R-project.org), 2008.

Saha, B. B ., Koyama, S., El-Sharkawy, I. I., Habib, K., Srinivasan, K., and Dutta, P.: Evaluation of adsorption parameters and heats of adsorption through desorption measurements, Journal of Chemical Engineering Data, 52, 2419–2424, 2007.

Sanhueza, E. and Donoso, L.: Methane emission from tropical savanna *Trachypogon* sp. grasses, Atmos. Chem. Phys., 6, 5315–5319, 2006, http://www.atmos-chem-phys.net/6/5315/2006/.

Shao, X., Wang, W., Xue, R., and Shen, Z.: Adsorption of methane and hydrogen on mesocarbon microbeads by experiment and molecular simulation, J. Phys.l Chem. B, 108, 2970–2978, 2004.

Thammakhet, C., Thavarungkul, P., Brukh, R., Mitra, S., and Kanatharana, P.: Microtrap modulated flame ionization detector for on-line monitoring of methane, J. Chromatogr. A, 1072, 243–248, 2005.

Vigano, I., van Weelden, H., Holzinger, R., Keppler, F., McLeod, A., and Röckmann, T.: Effect of UV radiation and temperature on the emission of methane from plant biomass and structural components, Biogeosciences, 5, 937–947, 2008, http://www.biogeosciences.net/5/937/2008/.

Walton, K. S., Cavalcante, C. L., and Levan, M. D.: Adsorption equilibrium of alkanes on a high surface area activated carbon prepared from Brazilian coconut shells, Adsorption, 11, 107–111, 2005.

Wang, Z.-P., Han, X.-G., Wang, G. G., Song, Y. and, Gulledge, J.: Aerobic methane emission from plants in the inner Mongolia steppe, Environ. Sci. Technol., 42, 62–68, 2008.

Wegrzyn, J. and Gurevich, M.: Adsorbent storage of natural gas, Appl. Energ., 55, 71–83, 1996.

Zhang, T. and Krooss, B. M.: Experimental investigation on the carbon isotope fractionation of methane during gas migration by diffusion through sedimentary rocks at elevated temperature and pressure, Geochim. et Cosmochim. Ac., 65, 2723–2742, 2001.