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The stable isotope signature of methane emitted from plant material under UV irradiation

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ABSTRACT

Recent experiments have shown that dry and fresh leaves, other plant matter, as well as several structural plant components, emit methane upon irradiation with UV light. Here we present the source isotope signatures of the methane emitted from a range of dry natural plant leaves and structural compounds. UV-induced methane from organic matter is strongly depleted in both ^{13}C and D compared to the bulk biomass. The isotopic content of plant methoxyl groups, which have been identified as important precursors of aerobic methane formation in plants, falls roughly halfway between the bulk and CH_4 isotopic composition. C3 and C4/CAM plants show the well-established isotope difference in bulk ^{13}C content. Our results show that they also emit CH_4 with different $\delta^{13}C$ value. Furthermore, $\delta^{13}C$ of methoxyl groups in the plant material, and ester methoxyl groups only, show a similar difference between C3 and C4/CAM plants. The correlation between the $\delta^{13}C$ of emitted CH_4 and methoxyl groups implies that methoxyl groups are not the only source substrate of CH_4.

Interestingly, δD values of the emitted CH_4 are also found to be different for C3 and C4 plants, although there is no significant difference in the bulk material. Bulk δD analyses may be compromised by a large reservoir of exchangeable hydrogen, but no significant δD difference is found either for the methoxyl groups, which do not contain exchangeable hydrogen. The δD difference in CH_4 between C3 and C4 plants indicates that at least two different reservoirs are involved in CH_4 emission. One of them is the OCH_3 group, the other one must be significantly depleted, and contribute more to the emissions of C3 plants compared to C4 plants. In qualitative agreement with this hypothesis, CH_4 emission rates are higher for C3 plants than for C4 plants.

1. Introduction

Greenhouse gases are important for the radiative balance of the earth, and their increase over the past centuries, related to anthropogenic activities, is of concern (IPCC, 2007). Therefore, their global cycles have been studied intensively over the past decades. Neglecting water vapour, methane is the second most important greenhouse gas after CO_2 (Collins et al., 2006) with a radiative forcing of about 0.5 W m^{-2} (IPCC, 2007). It is the most simple and most abundant reduced organic compound in the atmosphere and an important component in atmospheric chemistry.

According to established literature, reduced compounds like CH_4 can be formed only in anoxic environments. Major sources include, for example, wetlands, rice paddies and ruminants, where CH_4 is formed by methanogens in the absence of oxygen (Breas et al., 2002; Houweling et al., 2000). Recently, it was suggested that plants can emit methane under aerobic conditions (Keppler et al., 2006). The scientific community has been arguing over the following years on the existence of this source and possible causes and effects (Beerling et al., 2008; Bergamaschi et al., 2006; Butenhoff and Khalil, 2007; Dueck and van der Werf, 2008; Dueck et al., 2007; Houweling, 2006; Kirschbaum et al., 2007; Nisbet et al., 2008; Sharpatyi, 2007; Xie et al., 2009).

Recent findings clearly demonstrated the important role of UV radiation and temperature in CH_4 production from organic plant matter under atmospheric oxygen concentrations (McLeod et al., 2008; Vigano et al., 2008), and the role of reactive oxygen species

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involved (Messenger et al., 2009). Lab experiments under stress conditions have been carried out showing how emissions vary from uncut to cut plants (Wang et al., 2009). New field studies on the Mongolian and Tibetan plateaus reported measurements of methane emitted from plants, although with uncertainty (Cao et al., 2008; Wang et al., 2008). Furthermore, boreal and tropical ecosystems were investigated (Sinha et al., 2007).

According to the latest published data, emissions of CH_4 have been observed from living plants even under low light (without UV) conditions by using a stable isotope approach (Bruggemann et al., 2009). Methane production in oxygenated environments has recently also been reported in sea water (Ingall, 2008; Karl et al., 2008). As an interesting note, experiments of organic matter degradation under high UV levels typically present on Mars showed methane as the most commonly observed reaction product (Stoker and Bullock, 1997).

Strong CH_4 emissions from plants were regarded as a possible explanation for elevated CH_4 concentrations detected by satellite observations over tropical forest areas (Frankenberg et al., 2005). New spectroscopic data have recently led to a downscaling of these missing CH_4 emissions (Frankenberg et al., 2008). However, still one third of the global CH_4 source strength is allocated to tropical regions.

Isotope studies are an established useful tool for quantifying the relative emissions of CH_4 from different sources (Brenninkmeijer et al., 2003; Pataki et al., 2003). In the biosphere, isotopic signatures can be used to characterize a multitude of individual compounds (Phillips and Gregg, 2001) and isotope studies have led to a deeper insight into the main photosynthetic pathways described as Calvin Cycle (C3), Slack–Hatch cycle (C4) and Crassulacean acid metabolism (CAM), respectively (Farquhar et al., 1989; Hobbie and Werner, 2004; Smith and Ziegler, 1990; Ziegler et al., 1976).

Stable isotopes are often used to investigate biogeochemical cycles (Goldstein and Shaw, 2003) where plants are important emitters of trace gases and thus affect atmospheric chemistry (Lelieveld et al., 2008). Vegetation is a direct emitter of biogenic volatile organic compounds (VOCs) during the plant lifecycle (Goldstein and Shaw, 2003; Loreto et al., 2008) and an indirect emitter, if we consider plant biomass degradation (Keppler et al., 2000).

Strong ^{13}C depletions relative to the bulk biomass have been measured for methoxyl groups (OCH_3) present in pectin and lignin (Keppler et al., 2004). The fractionation associated with the methoxyl pools is retained and even further enhanced during their conversion to C1 VOCs such as methanol (CH_3OH), chloromethane (CH_3Cl), bromomethane (CH_3Br) and iodomethane (CH_3I) (Keppler et al., 2004). Methane is the most reduced C_1 plant-derived organic compound and isotopic labeling studies on pectin and polygalacturonic acid (PGA), have recently established that methoxyl groups are important precursors of CH_4 that is emitted from these compounds under UV irradiation and heating (Keppler et al., 2008).

The work of Keppler et al. (2006) already indicated that plants emit methane with a carbon isotope signature that is characteristic for the C3 and C4 photosynthetic pathways, respectively.

We have determined the $\delta^{13}C$-CH_4 (carbon) and δD-CH_4 (deuterium) isotopic signatures of CH_4 emitted from different dry plant materials exposed to UV light. These isotope source signatures will be compared to the isotopic composition of the bulk biomass and methoxyl groups as potential precursors of CH_4.

2. Experimental methods

2.1. UV irradiation experiments

The study of Vigano et al. (2008) explains in detail the effect of UV radiation and temperature on CH_4 emissions from plant biomass. The experiments presented here follow a similar experimental approach, but a static quartz glass reactor of 100 ml was used so that sufficiently high CH_4 levels could build up to derive the source isotope signatures by applying different irradiation times as will be described in 3.1. Several leaves, as well as structural plant compounds, (Table 1) were dried at 60 °C overnight (for at least 10 h) in an oven. Then they were enclosed in the reactor and preflushed with 100 ml min^{-1} of compressed air at ambient methane level ($\approx$1.9 ppm, $\delta^{13}C$-$CH_4 \approx -48‰$, δD-$CH_4 \approx -90‰$) for at least 15 min. An OSRAM Vitalux lamp (300 W) served as UV source. This type of lamp is commonly employed for medical purposes, with a UVA/UVB content comparable to solar radiation if the source is kept at appropriate distance. The total unweighted UVB radiation was determined with a calibrated Waldmann UV meter (Waldmann, Schwenningen, Germany). The relative spectral distribution measurements and the calibration of the Waldmann device were performed with a calibrated standard UV–visible spectro-radiometer (model 752, Optronic Laboratories Inc, Orlando, FL, USA).

The lamp irradiated a leak tight 100 ml Suprasil finger (UV transparent) and to increase the amount of CH_4 emitted, the UVB intensity was 5 times higher (20 W m^{-2}) than typical natural tropical levels (4 W m^{-2}). For one sample, it was verified that the isotope signature did not depend on UV intensity, i.e., the same isotope signature was derived using 4 W m^{-2} and 20 W m^{-2}.

When the lamp was turned on, the amount of CH_4 increased linearly with irradiation time. Temperature inside the quartz finger was monitored with a micro-thermocouple placed inside the vial and always kept below 30 °C by using a powerful ventilator that was located outside the vial.

2.2. Continuous flow isotope ratio mass spectrometry for measurements of $\delta^{13}C$ and δD of methane

After irradiation, the gas was sampled by expansion of the air in the Suprasil finger into a 100 ml evacuated glass bottle from which sub-samples were successively injected into a continuous flow-isotope ratio mass spectrometry (CF-IRMS) system for high precision analysis of $\delta^{13}C$-CH_4 (reproducibility $\approx$ 0.1‰) and δD-CH_4 (reproducibility $\approx$ 2‰) (Keppler et al., 2008; Vigano et al., 2008). The grab samples were taken at different times in order to allow determination of the source isotopic composition using a Keeling plot analysis with at least 3 points (Fig. 1a and b).

The CF-IRMS analysis provides isotope values and concentrations derived from the peak areas of the chromatogram. The source signatures were then determined from the *y*-axis intercept in a Keeling plot (δ value versus inverse peak area). The method allows determining single signatures within 6–8 h with an accuracy of $\pm$0.5‰ for $\delta^{13}C$-CH_4 and $\pm$5‰ for δD-CH_4.

$\delta^{13}C$ and δD values are the relative deviations of the $^{13}C/^{12}C$ and D/H ratio in a sample relative to the respective recognized international standards: $\delta^{13}C$ refers to Vienna PeeDee Belemnite (VPDB) and δD refers to Vienna Standard Mean Oceanic Water (VSMOW).

2.3. Bulk isotope analysis

Bulk $\delta^{13}C$ analyses were performed at the Earth Sciences Dept. of Utrecht University (The Netherlands) and bulk δD was determined at the Stable Isotope Laboratory of the Max-Plank Institute for Biogeochemistry in Jena (Germany).

Carbon isotopes of the bulk leaves were analyzed on a Fisons NA1500NCS Elemental Analyser coupled to a Thermo Delta$^+$XL isotope ratio mass spectrometer. A labstandard (Graphite Quartsite) was used for calibration. That in turn was calibrated with NBS-22 and USGS-24. Stdev of the lab-standards are <0.1‰,

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Table 1
List of materials analyzed with relative isotopic analysis (‰) and emission rates (ng g DWh^{-1}).

Plants-Compounds		$\delta^{13}C$-CH_4	bulk $\delta^{13}C$	$\delta^{13}C$-OCH_3	Pectin-$\delta^{13}C$-OCH_3	δD-CH_4	bulk δD	δD-OCH_3	ER
Lignin	Commercial	−53.30	−23.32	−16.76	n.m.	−201.7	−150.6	−306.5	n.d.
Pectin	Commercial	−55.69	−26.99	n.m.	n.m.	−393.0	−29.3	n.m.	n.d.
Cellulose	Commercial	−81.14	−25.69	n.m.	n.m.	−428.9	−67.4	n.m.	n.d.
Perennial ryegrass (*Lolium perenne*)	C3	−64.50	−27.92	−49.20	−56.22	−418.3	−99.7	−241.9	504.5
Sweet vernal grass (*Anthoxanthum odoratum* L.)	C3	−67.10	−29.49	−47.86	−69.78	−410.7	−79.3	−242.5	497.6
Madagascar dragon tree (*Dracaena marginata*)	C3	−56.38	n.m.	n.m.	n.m.	n.m.	n.m.	n.m.	573.8
Oil tree (*Pentaclethra macroloba*)	C3	−73.50	−31.36	−64.15	−75.17	−414.4	−80.5	−236.7	450.5
Weeping fig (*Ficus benjamina*)	C3	−69.74	−29.69	−50.39	−63.05	−356.7	−72.2	−170.1	586.3
(*Crinum lily*)	C3	−67.03	−28.39	−52.92	−54.13	n.m.	−59.9	−179.1	128.8
Planes (*Platanus orientalis* L.)	C3	−65.33	n.m.	−49.22	−55.50	n.m.	−108.2	−240.4	314.0
Maple (*Acer pseudoplatanus*)	C3	−67.57	n.m.	−62.95	−66.23	−343.4	−97.7	−218.2	677.2
Rosmary (*Rosmarinus officinalis*)	C3	−64.65	n.m.	n.m.	n.m.	n.m.	n.m.	n.m.	60.8
Ash (*Fraxinus xanthoxyloides*)	C3	−67.05	n.m.	−73.09	−77.10	−356.2	−102.8	−216.4	222.6
Salvia (*Salvia argentea*)	C3	−62.72	n.m.	n.m.	n.m.	n.m.	n.m.	n.m.	225.1
Ginger (*Costus scaber*)	C3	−64.90	n.m.	n.m.	n.m.	−360.8	n.m.	n.m.	222.2
Barbados nut (*Jatropha curcas*)	C3	−72.50	n.m.	n.m.	n.m.	n.m.	n.m.	n.m.	38.9
Octopus tree (*Schefflera actinophylla*)	C3	−72.15	n.m.	n.m.	n.m.	−343.1	n.m.	n.m.	329.0
Palma imperial (*Ceratozamia mexicana*)	C3	−68.09	−29.24	−40.75	−52.47	n.m.	−56.5	−239.3	381.7
Bananas (*Musa acuminata*)	C3	−60.52	−29.53	−52.38	−50.11	−403.7	−66.3	−220.6	292.9
Bamboo (*Phyllostachys aurea*)	C3	−60.54	−32.40	−43.12	−54.96	n.m.	−98.3	−190.9	299.8
Scarlet star (*Guzmania lingulata*)	CAM	−61.70	n.m.	n.m.	n.m.	n.m.	n.m.	n.m.	257.2
Snake plant (*Sansevieria trifasciata*)	CAM	−50.59	−15.94	−41.56	−45.89	−283.6	−37.4	−96.4	192.1
Shirley temple airplant (*Tillandsia xerographica*)	CAM	−52.50	−17.10	−28.22	−33.03	−256.6	−31.5	−163.1	338.9
Spanish moss (*Tillandsia usneoides*)	CAM	−55.58	n.m.	−48.69	−47.95	−267.0	−59.1	−209.9	104.3
Switchgrass (*Panicum virgatum*)	C4	−55.90	−12.90	−28.14	−36.56	−260.1	−77.3	−190.9	40.9
Sorghum a (*Sorghum bicolor*)	C4	−48.43	n.m.	−24.90	−47.95	−274.3	−76.1	−220.6	133.1
Orzaga (*Atriplex halimus*)	C4	−47.55	n.m.	−52.79	−51.52	−346.5	−90.4	−216.0	158.7
Red amarant (*Amaranthus cruentus*)	C4	−59.92	n.m.	−56.37	−57.28	−318.9	−77.1	−187.1	117.7
Sorghum b (*Sorghum drummondii*)	C4	−47.55	−13.60	−30.84	−31.33	n.m.	−66.7	−194.3	250.5
Lemon grass (*Cymbopogon flexuosus*)	C4	−52.30	−13.30	−22.72	−30.28	−325.2	−78.3	−205.3	179.3
Maize (*Zea mais*)	C4	−42.37	−11.78	−19.23	−26.21	−279.1	−84.3	−214.2	285.2
Cotton flower (*Gossypium hirsutum*)[a]	C3	−69.90	n.m.	n.m.	n.m.	−653.3	−25.4	n.m.	n.d.

(n.m.: not measured; n.d.: not determined).
Standard deviation for δD-CH_4 and $\delta^{13}C$-CH_4 signatures are: 5‰ and 0.5‰ respectively.
Standard deviation for bulk $\delta^{13}C$ analyses is ~0.5‰, while for bulk δD is ~5‰.
Standard deviation for methoxyl groups analyses is ~0.5‰.
Emission rates are derived from the peak area of the Isotope Ratio Mass Spectrometer by using ISODAT® software; standard deviation is ~20 ng g DWh^{-1}.
[a] Cotton flowers from Tadzhikistan.

The method for bulk δD analysis using a High Temperature Conversion (HTC) mass spectrometric technique closely follows the procedure described for $\delta^{18}O$ determination in bulk material by Brand et al. (2009). Samples are weighed into Ag-foil and positioned into an Autosampler ('Zero-Blank', Costec, Milan, Italy). Upon actuation, the packets drop into a helium flushed high temperature reactor (HTO, Hekatech, Wegberg, Germany) kept at 1450 °C. The reactor has an outer SiC tube and an inner tube made from glassy carbon with reversed carrier gas flow to ensure that the full helium flux passes through the core of the reactor (Gehre et al., 2004). In addition, the inner tube is filled with glassy carbon chips up to the highest temperature zone where the reaction to CO, H_2, and carbon plus further unspecified reaction products and residues takes place. From the reactor, the sample gas passes a fine filter containing NaOH on pumice ('Ascarite') and $Mg(ClO_4)_2$ before entering a packed column (5-Å) gas chromatograph at 70 °C for separating H_2 and CO. An open split interface ('ConFlo III', (Werner et al., 1999)) provides the connection to a Delta$^+$XL stable isotope mass spectrometer (both from Thermo-Fisher Scientific, Bremen, Germany). By monitoring the ion currents at m/z 3 and 2, the hydrogen isotopic composition is analyzed. As primary reference for reporting on the VSMOW scale we used NBS-22 oil with an assigned value of −118.5‰. Using this material, a local reference material 'PET-J1' (polyethylene, 'Uvasol', purchased from Merck, Darmstadt, Germany) was calibrated with a δD value of −80.75‰ versus VSMOW. Aliquots of these materials were interspersed together with the samples and analyzed in the same sequence. The reported sample results are based on the relative differences to the working reference material results. As a quality control reference we included one sample of IAEA-CH7 polyethylene in the daily sequence of measurements. IAEA-CH7 has an assigned value of −100.3‰ on the VSMOW scale.

2.4. Isotope analysis of plant methoxyl groups

The $^{13}C/^{12}C$ and D/H isotope ratios of the plant methoxyl groups were analyzed using a new method, which was recently published by Greule et al. (2008) and Greule et al. (2009). In brief, methoxyl groups of plant material are cleaved off with hydriodic acid (HI) at 130 °C in closed reaction vials. Subsequently, a headspace analysis of the gaseous product methyl iodide (CH_3I) is carried out using gas chromatography pyrolysis/combustion isotope ratio mass spectrometry (GC-C/P-IRMS). This rapid and precise method enables the determination of both $\delta^{13}C$ and δD values of plant methoxyl groups without apparent isotopic discrimination.

Plant methoxyl groups exist in two different types of chemical bondage, ester methoxyl groups (mainly appearing in pectin) and ether methoxyl groups (main sources in nature is lignin). To determine the $\delta^{13}C$ values of pectin methoxyl groups (pectin-$\delta^{13}C$-OCH_3) the plant material was treated with a 1-M sodium hydroxide solution at 50 °C to quantitatively hydrolyze ester methoxyl groups to methanol (Keppler et al., 2004). The methanol containing liquid phase was separated from the residual sample and heated with HI at 130 °C. In the following the generated CH_3I was analyzed as described by Greule et al. (2009).

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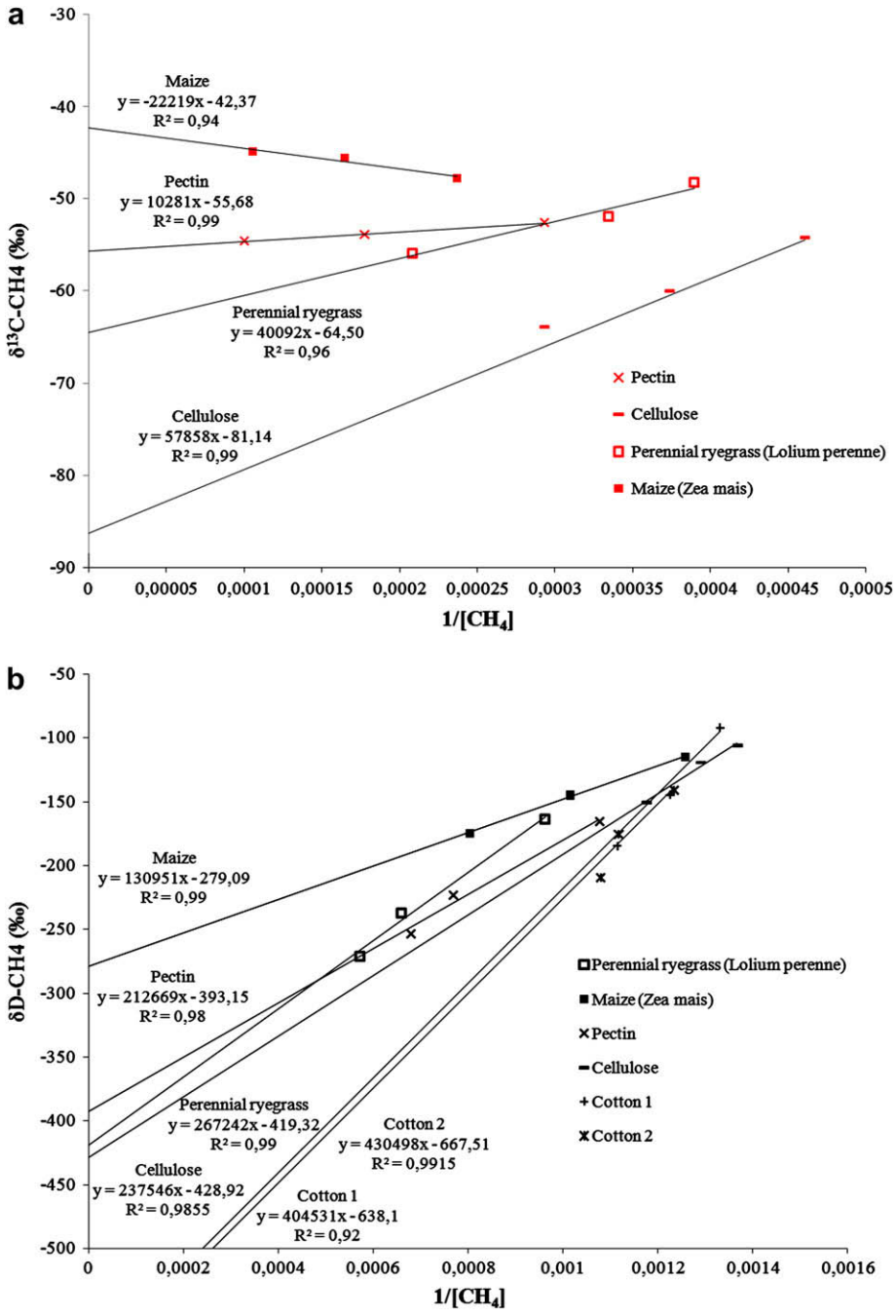

Fig. 1. Examples of keeling plot for different plant material and compounds analyzed. The equations of the regression lines show the intercept at $[CH_4] \rightarrow \infty$. (a) for $\delta^{13}C$ analyses and (b) for δD. Cotton experiment repeated twice for δD.

2.5. Materials

The full list of materials investigated is given in Table 1. Most of the plant material was obtained from the Botanical Garden of Utrecht University; some leaves were collected during summer (June–August) from regular outside plants or plants grown inside the greenhouse. The same day of the collection they were dried as previously described, and stored in a vacuumed exicator in order to keep stable the moisture content. The organic compounds used for experiments were obtained from Sigma® (apple pectin, purity 98%, CAS number 9000-69-5, cellulose microcrystalline, purity 95%, CAS number 9004-34-6, lignin, purity 95%, CAS number 8068-05-1). Typical amounts used in our experiments ranged from 0.1 to 2 g of dry material.

3. Isotopic composition of CH_4

3.1. Determining the source isotopic signatures

During the static experiments the methane mixing ratio increased proportionally with irradiation time and this change in mixing ratio usually leads to a change in the isotopic composition. Typically we irradiated the samples for at least three different time periods (1 h, 2 h, and 3 h). Before and after every run the reactor

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was flushed for 15 min with 100 ml min^{-1} of conventional compressed air ($\approx$1.8 ppm CH_4). Keeling plots of isotopic composition versus inverse mixing ratio, 1/[CH_4], yield good linear correlations ($R^2 > 0.9$) and the isotope source signatures are derived by extrapolating to $[CH_4] \Rightarrow \infty$, which corresponds to the *y*-axis intercept of the linear fit to the δ versus 1/[CH_4} data. Fig. 1a and b show some examples of Keeling plots.

In the following sections the results of isotopic signatures and the median values of isotope discriminations have been discerned for C3 plants and for C4–CAM plants.

Notwithstanding the differences in C4 and CAM plants metabolisms, here for simplicity, they have been grouped together in the data evaluation based on the fact that they have almost similar isotope values measured (Table 3). Separate discussions on CAM plants have been done for cases where values are considered to be outliers (especially for δD analyses).

3.2. δ^{13}C-CH_4 signatures from plants and structural compounds

More than twenty species from a wide variety of plants were analyzed and for each one a Keeling plot analysis was carried out to determine the δ^{13}C-CH_4 source signature as explained in 4.1. The results in Table 2 and Fig. 2 show that it is possible to define two main groups of plants with different δ^{13}C (CH_4) values, which relate to their photosynthetic pathways. The group with more depleted δ^{13}C values contains the C3 species measured, while more enriched values are observed for the C4 species and CAM plants. A similar difference is well established for the ^{13}C of the bulk biomass (see below). Outliers were observed for Madagascar dragon tree, banana, bamboo and maize. The Madagascar dragon tree, banana and bamboo leaves that were analyzed produced CH_4 with δ^{13}C-CH_4 values characteristic of the C4 group, although they are C3 plants, while our maize sample was particular enriched within the C4 group (Table 1). In order to avoid biases from such outliers, for the following calculations we use median values instead of average values.

Median δ^{13}C-CH_4 values for C3 and C4–CAM are $-67.0 \pm 4.5‰$ ($\pm1\sigma$) and $-52.3 \pm 5.5‰$ ($\pm1\sigma$) respectively. The study of Keppler et al. (2006) reported average δ^{13}C-CH_4 values for C3 and C4 plants of $-58.2‰$ and $-49.5‰$, respectively for CH_4 produced from fresh and dried material at 30–40 °C. The values of Keppler et al. are slightly enriched relative to our signatures obtained with UV light. However it should be noticed that here, we have analyzed different plant species under different conditions of radiation and temperature which might be element of discrepancies. The general relation, that CH_4 from C3 plants is lighter than from C4 plants, is in good qualitative agreement. The difference may be due to the different experimental setups. Heat and UV irradiation may produce CH_4 with different isotopic composition. A recent study using isotopically labeled pectin has shown that the fraction of CH_4 derived from methoxyl groups of pectin is larger for heating than for UV irradiation and that in the latter there must be a substantial additional reservoir (Keppler et al., 2008). In this context, Messenger et al., 2009 suggested that acetyl esters (*O*-acetyl groups) are a potential minor CH_4 source and may account for discrepancies in previous labeling experiments of Keppler et al. (2008).

Table 2
Median values for δ values and emission rates of C3 and C4/CAM plants.

	C3	C4–CAM
δ^{13}C-CH_4 (‰)	−67.0(±4.5)	−52.3 (±5.5)
$\delta^{13}C_{bulk}$ (‰)	−29.5(±1.5)	−13.5(±2.0)
δ^{13}C-OCH_3 (‰)	−50.4 (±9.6)	−29.5(±13.4)
δ^{13}C-$OCH_{3pectin}$ (‰)	−56.2(±9.4)	−36.5 (±11.0)
δD-CH_4 (‰)	−360(±32)	−279(±32)
δD_{bulk} (‰)	−80(±18)	−77(±19)
δD-OCH_3 (‰)	−220(±26)	−200(±37)
ER (ng gDWh^{-1})[a]	301 (±187)	180 (±88)

[a] Emission rates in nano-gram per gram of dry weight per hour. Standard deviations given in brackets.

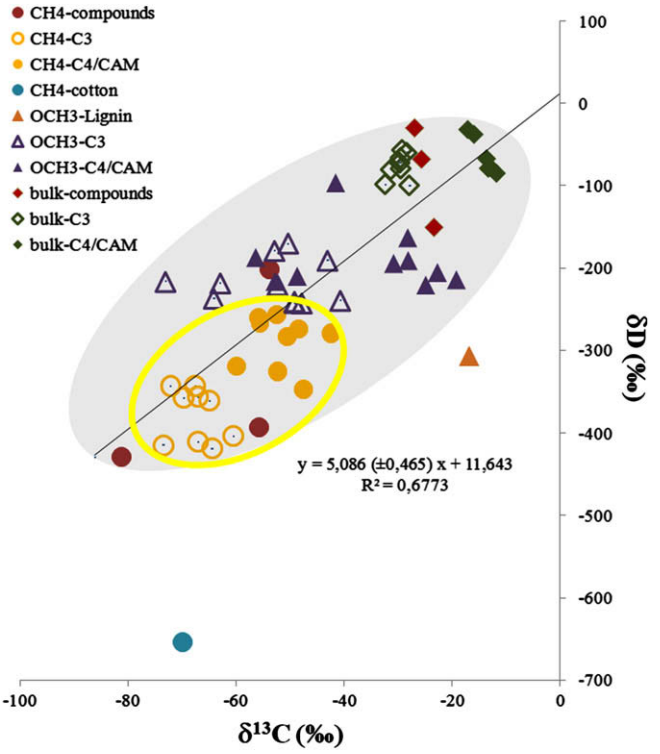

Fig. 2. General scatter plot of δ^{13}C versus δD for most of the measurements, showing the relation 1:5 between carbon and hydrogen fractionation in plants. Into the yellow circle are positioned the CH_4 signatures. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Isotopic signatures of UV-induced CH_4 were obtained also from analyses of plant structural compounds; these were purchased from commercial suppliers (Sigma®) and not directly extracted from fresh plant material. Apple pectin and lignin liberate CH_4 with δ^{13}C values of $-55.7‰$ and $-52.3‰$ respectively, which are similar to those found for C4 plants (Table 1). In contrast, cellulose produces very depleted CH_4 with $\delta^{13}C = -81.1‰$, the lowest value observed in this study. Earlier irradiation experiments on cellulose had shown non-zero CH_4 emissions, although far less then emissions observed from lignin and pectin (Vigano et al., 2008). The lower emission rates could in principle be a cause for the stronger fractionation, however, the CH_4 formation pathway for cellulose must be different, as this molecule has no methoxyl groups which are hypothesized to be important (but not the only) precursors for CH_4 emission from lignin and pectin (Keppler et al., 2008). More specific kinetic studies on the formation mechanism for cellulose may help understand the additional formation pathways for other compounds, too.

3.3. δD-CH_4 signatures from plants and structural compounds

δD-CH_4 source signatures were determined from a subset of the same samples with the same approach. δD-values for C3 and C4–CAM plants fall again in two separate groups with median δD values of $-360 \pm 32‰$ ($\pm1\sigma$) and $-279 \pm 32‰$ ($\pm1\sigma$) respectively (Table 2 and Fig. 2). In contrast to ^{13}C, a similar δD difference is not found in

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the bulk material, and thus the difference indicates a difference in the production pathways of CH_4 from the different plant types.

Our results establish CH_4 liberated from plants under UV irradiation as one of the more depleted CH_4 sources in Nature (Fig. 3). In fact deuterium signatures of methane range from values of $\approx -120‰$ for methane from coal mining down to $\approx -400‰$ for methane emitted from termites (Fischer et al., 2008; Quay et al., 1999; Schaefer and Whiticar, 2008; Whiticar, 1993). Deuterium-depleted CH_4 is generally viewed as clear signature for microbial production. Our results show that non-microbial photochemical processes (Vigano et al., 2008) can also form D-depleted CH4. The measured values are slightly (C4 plants) or even much (C3 plants) lower than the value of $-260‰$ that was recently estimated for plant-derived CH_4 in a study targeted at constraining the global methane budget using stable isotopes (Whiticar and Schaefer, 2007).

δD-CH_4 values were also measured from commercially available plant structural compounds. CH_4 emitted from lignin is significantly enriched in deuterium relative to CH_4 from plant material (δD-$CH_4 = -201‰$). The δD-CH_4 value from pectin, on the other hand is in the range of the C3 plants (δD-$CH_4 = -393‰$) and cellulose has a δD value slightly lower than the natural plant samples (δD-$CH_4 = -429‰$). The absolute values of these samples are not conclusive, however, since the D source for those materials is not known and may be different from the plant samples and between the compounds.

Nevertheless, the CH_4 formation from cellulose deserves some further discussion: cellulose is a chain/polymer of D-glucose molecules, which contain hydroxymethyl-groups $-CH_2OH$ that chemically differ from methoxyl groups $-OCH_3$. If CH_4 can also be formed from such functional groups, this has at least two important implications: (i), two hydrogen atoms are added to the CH_2 group instead of one to the CH_3 group; and (ii), the reaction kinetics must be different.

The fact that for all compounds the emitted CH_4 is isotopically so much lighter than the bulk material and the methoxyl groups (see chapter below) implies either strong kinetic isotope effects or a strongly depleted source for the 4th H atom that is incorporated when the CH_4 molecule is formed. The light δD value for cellulose implies that the isotopic composition of the additional D atoms is light. It would be interesting to carry out an isotope labeling study on cellulose to identify the carbon and hydrogen source for CH_4 generated from this molecule.

An unexpected strong depletion was observed for cotton flower (cotton fibers) from two repeated measurements, which showed an average value of δD $\approx -653‰$ (Table 1). Cotton fibers, also previously investigated under UV irradiation (Vigano et al., 2008), consist mostly of cellulose. As discussed above the δD-CH_4 value of cellulose is the most depleted of all investigated structural compounds (δD $\approx -429‰$), but still, the strong depletion of δD of CH_4 emitted from cellulose cannot explain the extremely low values observed for cotton flower.

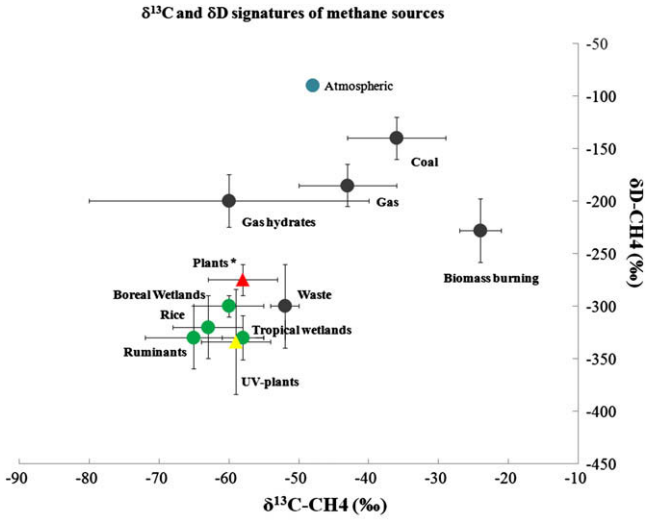

Fig. 3. Data are from Quay et al., 1999 and Whiticar, (1993). Mainly non-microbial sources are indicated by grey dots, mainly microbial sources by green dots. Plants signatures are indicated with triangles: red for the old evaluations, yellow for the new UV signatures here described. The error bars indicate the spread of reported values. Actual atmospheric δ values are indicated with a blue dot. *δ^{13}C data are from Keppler et al., 2006 while the δD is in the range of the projections made by Whiticar and Schaefer, 2007 and Fischer et al., 2008. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

4. Relation between ^{13}C and D content of bulk plant material and methoxyl groups

Methoxyl groups are generally depleted in ^{13}C and D versus the bulk biomass (Fig. 2), and the CH_4 emitted is even further depleted. It is important to note that plant methoxyl groups exist in two different types of chemical bondage; ester methoxyl groups (mainly appearing in pectin) and ether methoxyl groups (main sources in nature is lignin). Recently it has shown that pectin methoxyl groups are an important precursor of CH_4 formation when dried plant matter is exposed to UV light (Keppler et al., 2008; McLeod et al., 2008). Moreover, it was found that δ^{13}C values of pectin methoxyl groups differ from δ^{13}C values measured for the total methoxyl pool (composite of pectin and lignin OCH_3 groups) (Keppler et al., 2004, Greule et al., 2009). However, this appears not to be the case for δD values of pectin and lignin OCH_3 groups measured in the same plant sample. This is why in this study we measured both δ^{13}C values of total methoxyl pool (pectin and lignin OCH_3 groups) and the methyl ester pool (pectin-OCH_3 groups). The data in Fig. 2 show a considerable scatter, but a linear square fit yields a slope ≈ 5, which indicates that the overall fractionation from the bulk biomass to CH_4 is ≈ 5 times stronger for δD than for δ^{13}C.

For a better understanding of isotope fractionation in the formation of CH_4 from plant biomass, we introduce the isotope discrimination between two substances a and b defined as $\Delta_{a-b} = \delta_a - \delta_b$ (Table 4), which quantifies the difference in isotopic content between two reservoirs due to kinetic isotope effects (Criss and Farquhar, 2008).

4.1. $\delta^{13}C$

Fig. 4a shows a scatter plot of δ^{13}C of CH_4, total methoxyl pool (OCH_3 groups) and the methyl ester pool (pectin-OCH_3 groups), with the bulk ^{13}C content of the biomass. For all signatures, the average ^{13}C content in the C3 plants is lower than in the C4 and CAM plants. δ^{13}C of pectin-OCH_3 groups is slightly lower than δ^{13}C of all OCH_3 groups, and δ^{13}C of CH_4 is clearly the lowest. The slopes of the linear fits through the data are largely determined by the average δ^{13}C difference between C3 and C4 plants. The slope of the CH_4-bulk correlation is 1.01 ± 0.15, indicating that the difference in the bulk ^{13}C content is transferred 1:1 to the CH_4 emitted. Surprisingly, the slope of the OCH_3-bulk and ester OCH_3-bulk correlations is >1. Supposedly, similar processes as those leading to the ^{13}C difference in the bulk biomass between the two plant types cause even stronger ^{13}C differences in the OCH_3 groups. Although there is a large scatter in the ^{13}C content measured in these compartments, the difference from slope 1 is significant at the 2σ level. The observed signals are also much larger than the

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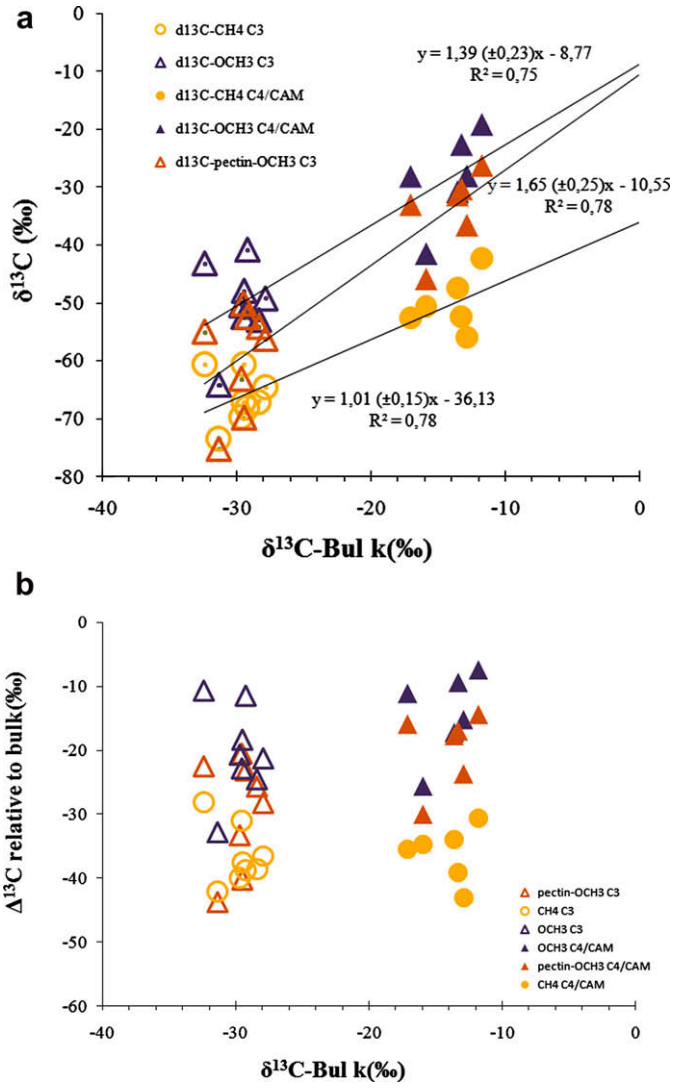

Fig. 4. (a) Scatter plot between δ^{13}C-bulk and δ^{13}C of the CH_4 emitted and of the methoxyl groups. (b) Scatter plot of the δ^{13}C discriminations for CH_4, OCH_3 groups and pectin-OCH_3 groups relative to bulk δ^{13}C.

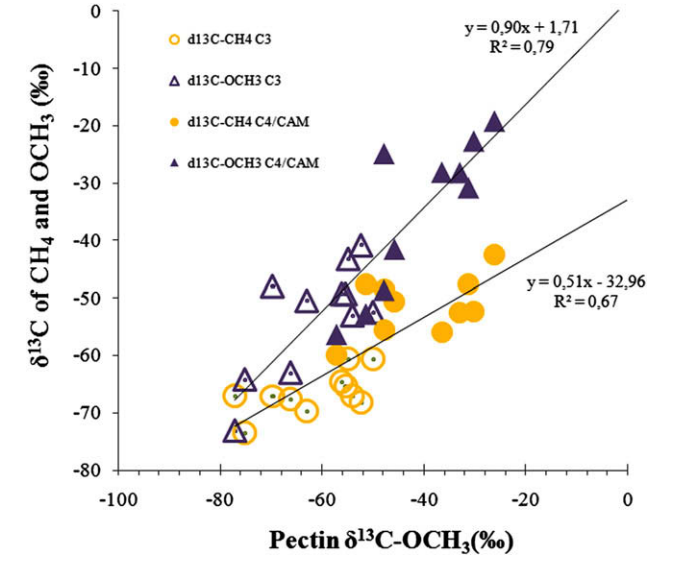

Fig. 5. Relationship between δ^{13}C of methoxyl groups and CH_4.

Table 3
Average values for δ values of C4 and CAM plants.

	C4	CAM
δ^{13}C-CH_4 (‰)	−50.7(±5.6)	−55.1(±4.8)
$\delta^{13}C_{bulk}$ (‰)	−12.9(±0.8)	−16.5(±0.8)
δ^{13}C-OCH_3 (‰)	−33.6(±14.8)	−39.5(±10.4)
δ^{13}C-$OCH_{3pectin}$ (‰)	−40.1(±12.1)	−40.5 (±10.5)
δD-CH_4 (‰)	−300(±34)	−270(±13)
δD_{bulk} (‰)	−78(±7)	−42(±14)
δD-OCH_3 (‰)	−204(±13)	−156(±57)

Standard deviations given in brackets.

measurement uncertainty (see above). The origin of the large scatter within the two groups is unknown at present.

In Fig. 4b the same data are presented as isotope discrimination relative to the δ^{13}C value of the bulk biomass. The discrimination of the produced CH_4 is similar for the two plant categories with levels around −36‰. For both, total OCH_3 and pectin-OCH_3 groups, however, isotope discrimination in C3 is stronger than in C4 plants. Again, the origin of this enhanced discrimination, as well as of the scatter within the two groups, is not known at present.

In Fig. 5 we investigate in more detail the relation between δ^{13}C in the OCH_3 groups and in CH_4. First, as expected, pectin-OCH_3 groups correlate well with the total methoxyl pool, and the slope of the linear fit is indistinguishable from 1 at the 1σ level. However, the correlation slope between δ^{13}C in CH_4 and the pectin-OCH_3 groups, which are considered an important source substrate for CH_4 formation, is only 0.5. This directly implies that pectin-OCH_3 groups cannot be the only source substrate for CH_4 formation, as already concluded by (Keppler et al., 2008). The additional C source must be characterized by a ^{13}C difference between C3 and C4/CAM plants that is smaller than the one for the OCH_3 groups in order to explain the smaller slope.

4.2. δD

δD-bulk values do not display significant isotopic difference between C3 and C4–CAM plants, with median values for δD-bulk of -80 ± 18‰ ($\pm 1\sigma$) and -77 ± 19‰ ($\pm 1\sigma$), respectively (Table 1 and Fig. 2). CAM plants exhibit slightly enriched δD values (Table 3). The plants from the greenhouse have been grown with water of similar isotopic composition and the absence of a δD difference between C3 and C4–CAM plants indicates a not evident isotope fractionation in the metabolic pathways for both groups. However, such a δD difference does exist in the CH_4 produced (see above), which implies either a different fractionation in the CH_4 formation mechanism between the plant categories, or differences in the source substrate (which is then not represented well by bulk biomass). In fact, δD measurements on bulk biomass are problematic to interpret, because they represent a mix of exchangeable and non-exchangeable hydrogen. The isotope discrimination for δD

Table 4
Carbon and deuterium Δ isotope discrimination.

Δ Isotope discrimination (‰) (δa–δb)[a]	C3	C4–CAM
Δ^{13}C(CH_4-bulk)	−37.5 (±3.0)	−38.8(±3.7)
Δ^{13}C (CH_4-OCH_3)	−16.6 (±7.1)	−22.7(±9.4)
Δ^{13}C (CH_4-$OCH_{3pectin}$)	−10.8(±6.9)	−15.7(±8.3)
Δ^{13}C (bulk-OCH_3)	20.8 (±5.5)	21.2 (±7.7)
Δ^{13}C (bulk-$OCH_{3pectin}$)	26.7 (±5.4)	23.1 (±6.5)
Δ^{13}C (OCH_3-$OCH_{3pectin}$)	10.9 (±9.5)	7.0 (±12.2)
Δ_D (CH_4-bulk)	−280(±25)	−202(±26)
Δ_D (CH_4-OCH_3)	−140(±32)	−100(±34)
Δ_D (bulk-OCH_3)	137 (±22)	123 (±28)

[a] Subtraction between median values of Table 1. Standard deviations given in brackets.

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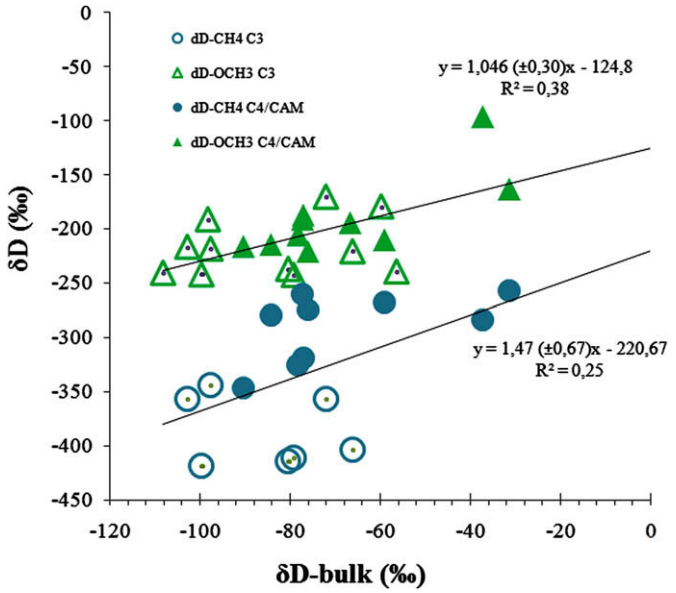

Fig. 6. Relationship between δD bulk and δD of CH_4 and methoxyl groups.

between CH_4 and bulk biomass is significantly more negative for C3 plants ($\Delta D = -280 \pm 25‰$ ($\pm 1\sigma$)) than for C4–CAM plants ($\Delta D = -202 \pm 26‰$ ($\pm 1\sigma$)) (see Table 4), while for carbon, the isotope discrimination is similar for the two plant categories. As discussed for the absolute δD values, the reason for the difference between C3 and C4–CAM plants must either be a kinetic fractionation, or an isotope difference in the precursor compounds, which is not captured in the bulk δD, or both.

The incorporation of hydrogen in plants depends on various factors: water adsorption, evapotranspiration rates and on the type of metabolic pathways specific from specie to specie. Within the same group the isotopic fractionations for hydrogen can be diverse due to different kinetic isotope effects (Ziegler et al., 1976). In fact, a correlation plot between δD-bulk and δD-CH_4 shows a lot of scatter (Fig. 6). It is noted that $\delta D(CH_4)$ vary over a much wider range than δD of the bulk biomass (180‰ versus 90‰, respectively). The principle feature is the strong enrichment in $\delta D(CH_4)$ from C4–CAM plants compared to C3 plants. The slope of a linear fit is largely determined by the slightly enriched CAM plants mentioned above.

Methoxyl groups contain non-exchangeable hydrogen (Greule et al., 2008; Keppler et al., 2007, 2008) and have been identified as a source substrate for CH_4 formation. Fig. 6 shows that δD values of the OCH_3 groups are not significantly different for C3 and C4 plants, whereas again the CAM plants stand out slightly with elevated δD values for both bulk and OCH_3 groups. These CAM plants again mainly determine the slope of the linear fit line through all data points, whereas the remaining data show no significant correlation. The absence of a strong δD difference in the OCH_3 groups of C3 and C4–CAM plants indicates that the difference observed in the CH_4 also does not originate from the OCH_3 precursors. Since it is unlikely that kinetic fractionation processes alone could account for the differences between the plant categories, this also implies that there must be an additional isotopically distinct reservoir that acts as substrate for CH_4. We propose that the difference of $\delta D(CH_4)$ between the plant groups originates from different relative fractions of CH_4 derived from OCH_3 groups and another isotopically distinct reservoir (with possible additional kinetic fractionations). For example, if this additional reservoir is more depleted than the OCH_3 groups, then C3 plants would produce relatively more CH_4 from this reservoir, and relatively less from OCH_3 groups, compared to C4 plants.

5. Emission rates

The concentration increase in the static setup allows for a straightforward calculation of CH_4 emission rates. It should be kept in mind that the UV intensity in all experiments was 5 times higher than the typical tropical levels, so the absolute emission rates can't be transferred to natural conditions. Nevertheless, we can examine differences between the individual species under constant high UV conditions. The precision in the determination of the emission rates from individual plants is ±20 nano-gram per gram of dry weight per hour (ng $gDWh^{-1}$).

Table 1 and Fig. 7 show that on first approach, emission rates are higher from C3 plants compared to C4–CAM plants with median values of 301 ng $gDWh^{-1}$ and 180 ng $gDWh^{-1}$ respectively.

Fig. 7 shows the deuterium and carbon isotope discrimination between methane and the different plant compounds ($\Delta = \delta CH_4 - \delta_{plant}$) as a function of emission rate.

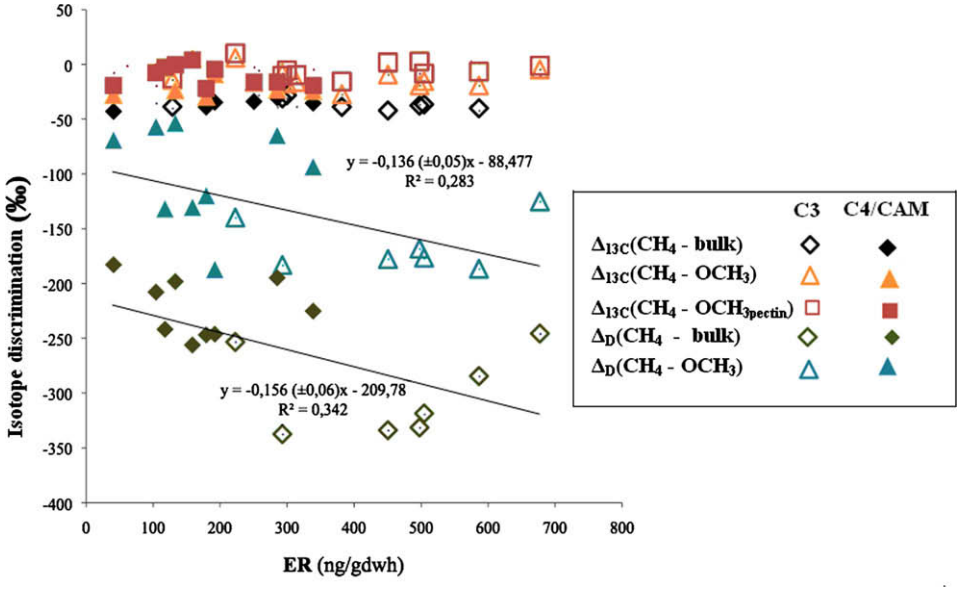

Fig. 7. Isotope discriminations for C3 and C4/CAM plants versus CH_4 emission rates in nano-gram per gram of dry weight per hour.

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The fact that C3 and C4 plants differ in ER and in $\Delta_{CH_4\text{-bulk}}$ leads to a correlation between these quantities. This is in qualitative agreement with the hypothesis presented above, that C3 emit relatively more from an additional source substrate than the OCH_3 groups, which then needs to be depleted in D. Further research should be carried out to identify this additional substrate.

6. Conclusions and outlook

The formation of methane when plant compounds are irradiated with UV light has now been reported from several research groups (Keppler et al., 2008; McLeod et al., 2008; Messenger et al., 2009; Vigano et al., 2008). The experiments presented above constitute further support for this CH_4 formation pathway from organic matter under aerobic conditions and provide several new insights.

Overall, CH_4 produced from plants under UV irradiation is strongly depleted in both D and ^{13}C. Thus isotopically light CH_4 is not an unambiguous fingerprint for bacterial sources, but can also be produced photochemically from plant matter. The CH_4 is even more depleted than the exceptionally depleted methoxyl groups of pectin, which only contain non-exchangeable hydrogen. The correlation of $\delta^{13}C$ in methoxyl groups and CH_4, as well as the strong difference in $\delta D(CH_4)$ between C3 and C4 plants require that at least one other source substrate is involved in CH_4 formation, and that CH_4 from this substrate is strongly depleted in δD. C3 plants would then emit relatively more CH_4 from this substrate than C4 plants, and in fact the average emission rates from C3 plants are higher than from C4 plants.

The isotopic signatures (both $\delta^{13}C$ and δD) of CH_4 produced from plant material irradiated with UV light provide new parameters for the assessment of the role of this source in the global CH_4 budget, since its effect on the isotope budget can be included. If vegetation is an important source of CH_4, it should be included in the interpretation of historic CH_4 isotope changes as reconstructed from ice core data (Ferretti et al., 2005) on glacial-interglacial time scales. Recent isotope studies indicate the potential importance of shifting patterns of C3 and C4 vegetation for the isotope content of atmospheric CH_4 (Schaefer and Whiticar, 2008).

The overall scatter in CH_4 emitted from different plant species is considerable, and the origin of variations within the individual plant groups (C3, C4, CAM) is unclear. The study could be extended to include more plant species in order to improve the understanding of the processes that lead to methane emission upon UV irradiation and also to classify the Biomes characterized by different plant communities. Furthermore, the difference in $\delta D(CH_4)$ between C3 and C4/CAM plants in the absence of a difference in the bulk material is intriguing, and the hypothesis of a second (depleted) substrate should be examined by using isotope labeling studies. The selective discrimination of hydrogen and carbon in plants with different metabolism (C3, C4 or CAM) may be used as additional tool for classification techniques.

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