Biogeosciences, 1, 123–131, 2004
www.biogeosciences.net/bg/1/123/
SRef-ID: 1726-4189/bg/2004-1-123
European Geosciences Union

Biogeosciences

Carbon isotope anomaly in the major plant C_1 pool and its global biogeochemical implications

F. Keppler[1,2], R. M. Kalin[2], D. B. Harper[1], W. C. McRoberts[1,3], and J. T. G. Hamilton[1,3]

[1]School of Agriculture and Food Science, Queen's University Belfast, Newforge Lane, Belfast BT9 5PX, UK
[2]Environmental Engineering Research Centres, Queen's University Belfast, Belfast BT9 5AG, UK
[3]Department of Agriculture and Rural Development for Northern Ireland, Newforge Lane, Belfast BT9 5PX, UK

Received: 26 July 2004 – Published in Biogeosciences Discussions: 18 August 2004
Revised: 3 December 2004 – Accepted: 9 December 2004 – Published: 13 December 2004

Abstract. We report that the most abundant C_1 units of terrestrial plants, the methoxyl groups of pectin and lignin, have a unique carbon isotope signature exceptionally depleted in ^{13}C. Plant-derived C_1 volatile organic compounds (VOCs) are also anomalously depleted in ^{13}C compared with C_{n+1} VOCs. The results confirm that the plant methoxyl pool is the predominant source of biospheric C_1 compounds of plant origin such as methanol, chloromethane and bromomethane. Furthermore this pool, comprising ca 2.5% of carbon in plant biomass, could be an important substrate for methanogenesis and thus be envisaged as a possible source of isotopically light methane entering the atmosphere. Our findings have significant implications for the use of carbon isotope ratios in elucidation of global carbon cycling. Moreover methoxyl groups could act as markers for biological activity in organic matter of terrestrial and extraterrestrial origin.

1 Introduction

Stable isotope analysis has become a powerful tool for environmental scientists, plant biologists, ecologists and geochemists studying global elemental cycles or past climatic conditions (e.g. Ehleringer et al., 2002; Yakir, 2002; Hayes, 2001; Griffiths, 1998; Lajtha and Michener, 1994). Thus plant species have been photosynthetically characterised as Calvin cycle (C_3), Slack-Hatch cycle (C_4) and Crassulacean acid metabolism (CAM) categories using carbon isotope signatures (Griffiths, 1998; O'Leary, 1981). Moreover variations in the carbon isotope composition ($\delta^{13}C$ values) of compounds, produced and destroyed in the global carbon cycle, are often used to investigate biogeochemical cycles and global source-sink relationships, as well as the underlying mechanisms (e.g. Cerling et al, 1997; Sherwood Lollar et al., 2002; Michaelis et al., 2002). Stable isotope techniques are increasingly applied to the study of atmospheric budgets of volatile organic compounds (VOCs). Many C_1 VOCs, such as methanol (CH_3OH), chloromethane (CH_3Cl), bromomethane (CH_3Br), iodomethane (CH_3I), cyanomethane (CH_3CN) and methane (CH_4), play an important role in atmospheric chemistry and possibly climate change (see, for example, Heikes et al., 2002; Montzka et al., 2003; O'Dowd et al., 2002; Sanhueza et al., 2004; Wuebbles and Hayhoe, 2002). Numerous investigations into the atmospheric budget of such C_1 compounds, some employing stable isotope techniques (Rudolph et al., 1997; Tsunogai et al., 1999; Goldstein, 2003; Bill et al., 2004; Harper et al., 2003; Thompson et al., 2002; Whiticar, 1999; Kalin et al., 2001) have been reported but many questions regarding their origin and fate remain unresolved.

Most CH_3OH released from plants is derived from the ubiquitous plant component pectin by both enzymic and abiotic processes (Fall and Benson, 1996; Warneke et al., 1999; Galbally and Kirstine, 2002). Pectin which normally comprises between 7 and 35% of cell wall material in leaves is composed of galacturonic acid monomer units. Between 50 and 90% of the carboxyl groups of the latter are methyl esterified and provide the methyl pool for the reaction. We have also identified pectin as the source of CH_3Cl and other monohalomethanes produced abiotically by senescent and dead leaf material (Hamilton et al., 2003). However, little consideration has been given to the stable isotope signature of the methoxyl pool of pectin, or indeed that of another important plant component lignin, and the impact these might have on the stable isotope compositions of C_1 compounds in the biosphere.

Correspondence to: F. Keppler
(f.keppler@qub.ac.uk)

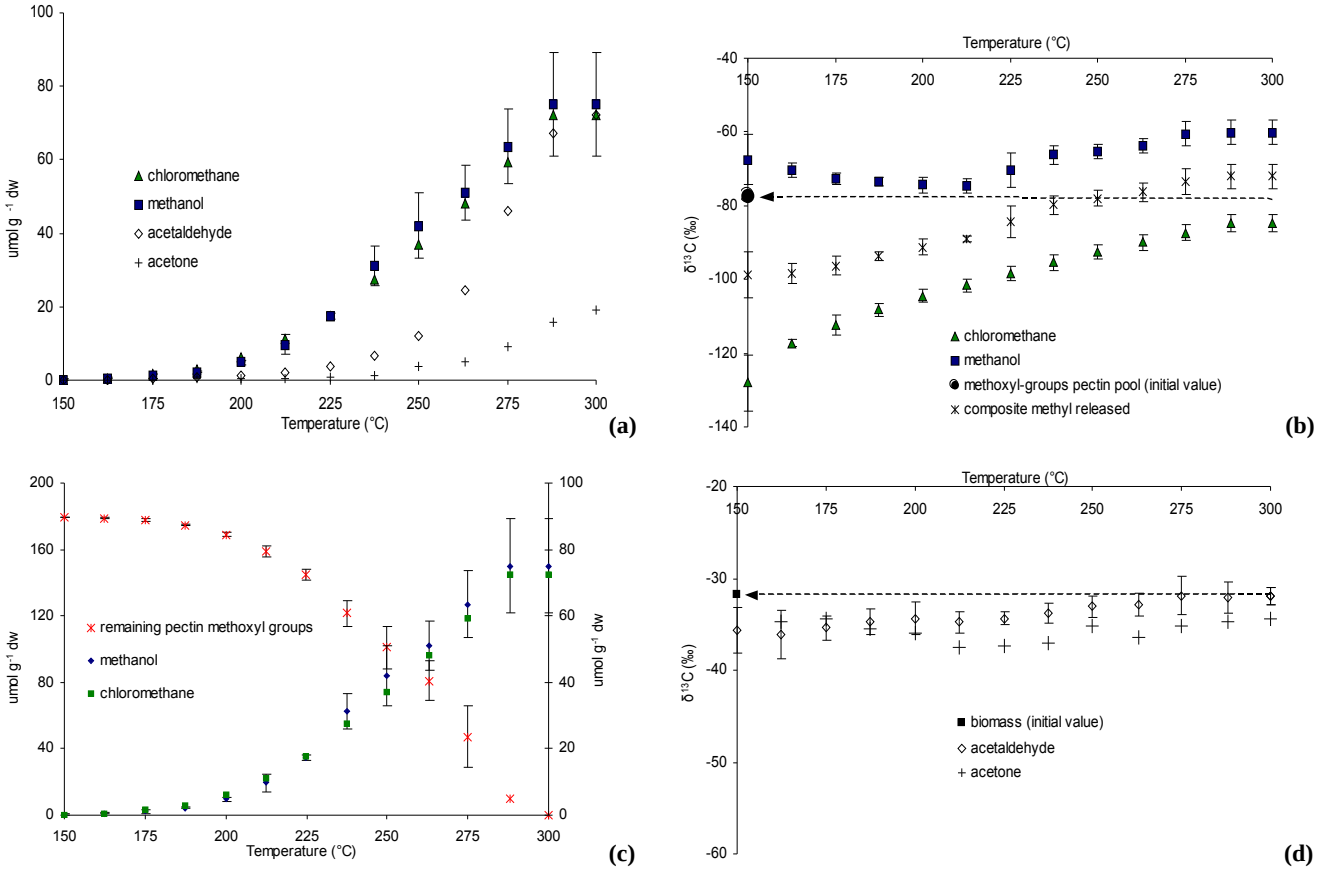

Fig. 1. Amounts and isotopic signatures of several volatile organic compounds formed during progressive heating of lypholised ash leaves. **(a)** Cumulative amounts of methanol, chloromethane, acetaldehyde and acetone formed are shown on a molar basis. Each point is the mean of three replicate analyses of independent samples (n=3). Error bars shown for CH_3OH are typical of SDs for all compounds. **(b)** Carbon isotopic composition of accumulated CH_3OH and CH_3Cl at each temperature during progressive heating. Also shown is the composite $\delta^{13}C$ values calculated on a molar basis for CH_3OH and CH_3Cl released during heating. For reference the measured initial $\delta^{13}C$ of the pectin methoxyl pool is displayed. Vertical bars show SD for triplicate samples. **(c)** Remaining pectin methoxyl pool (PM) after each heating step in relation to the formation of methanol and chloromethane. Left y-axis refers to pectin methoxyl groups and right y-axis to methanol and chloromethane. **(d)** Carbon isotope composition of accumulated acetaldehyde and acetone at various temperatures during progressive heating of ash leaf biomass with reference to the $\delta^{13}C$ value of the original bulk biomass. Error bars shown for acetaldehyde are also typical of those for acetone.

2 Materials and methods

Stable carbon isotope measurements: Carbon isotopic ratios of VOCs were measured by compound specific isotope analysis (GC-MS-IRMS) using a Thermo Finnigan Delta+ isotope ratio mass spectrometer interfaced with a Finnigan DSQ gas chromatograph trace mass spectrometer. The gas chromatograph was equipped with a Poraplot Q column (30 m×0.25, film thickness 8 μm). Bulk $\delta^{13}C$ signatures of dried plant samples were determined using a Eurovector elemental analyzer coupled to a Micromass PRISM III isotope ratio mass spectrometer. Internal precision of $\delta^{13}C$ was ±0.2‰ (2 sigma). Values of $\delta^{13}C$ (‰) relative to that for the Vienna-PDB are defined by the equation $\delta^{13}C\,(‰)=(R_{sample}/R_{standard}-1)\times 1000‰$ with $R={}^{13}C/{}^{12}C$. The isotope difference (Δ) between two pools is defined as $\Delta=\delta^{13}C_{pool1}-\delta^{13}C_{pool2}$.

Heating experiments: For experiments shown in Fig. 1 freeze-dried milled leaf biomass (250–1000 mg) was heated in a glass vessel according to the method of Hamilton et al. (2003) except that temperature programming increments were 12.5°C instead of 25°C. $\delta^{13}C$ values of volatile organic compounds were measured at the end of each temperature increment by GC-MS-IRMS. Results shown in Table 1 are for isothermal heating for 20 min at 225°C of dried plant biomass.

Chloromethane produced from leaves at 40°C to 60°C (Table 2): Freeze-dried milled leaf samples (5 g) placed in glass vial (44 ml) and sealed with Mininert® valves were heated for 8 h at 30°C, then temperature was progressively increased from 40°C to 60°C in 10°C increments. Each temperature

Table 1. $\delta^{13}C$ values[1] of biomass, pectin methoxyl groups and VOCs produced upon heating dried biomass at 225°C for 20 min.

Plant common name (*species*)	Biomass (B) ($\delta^{13}C$)	Pectin methoxyl (PM) ($\delta^{13}C$)	$\Delta^{13}C$ (PM-B) ($\delta^{13}C_{PM}-\delta^{13}C_B$)	Methanol ($\delta^{13}C$)	Chloromethane ($\delta^{13}C$)	Acetaldehyde ($\delta^{13}C$)	Acetone ($\delta^{13}C$)
C_3-leaf							
European ash (*Fraxinus excelsior*)[2]	−31.8±0.2	−77.2±0.1	−45.4	−70.4±2.5	−98.4±2.2	−34.0±1.1	−36.2±2.8
Wych Elm (*Ulmus glabra*)[2]	−28.4±0.1	−61.7±0.2	−33.3	−61.1±1.8	−85.3±3.1	−31.2 ±0.7	−33.2±2.1
Hazelnut (*Corylus avellana*)[2]	−29.1±0.1	−66.3±0.2	−37.2	−64.0±1.8	−96.0±2.1	−33.2±1.3	34.5±1.9
English oak (*Quercus robur*)[2]	−31.4±0.1	−74.4±0.2	−43.0	−75.0	−104.3	−28.9	−31.7
Norway maple (*Acer platanoides*)[2]	−27.6±0.2	−61.3±1.0	−35.7	−58.3	−92.4	−27.0	−21.1
Horse chestnut (*Aesculus hippocastanum*)[2]	−31.7±0.2	−66.3±2.0	−34.6	−60.4	−94.9	−29.5	−29.7
Scots pine (*Pinus sylvestris*)[2]	−27.6±0.1	−53.7±0.2	−26.1	−49.3	−86.8	−24.9	−30.8
Cocksfoot (*Dactylis glomerata*)[3]	−29.3±0.2	−50.7±0.2	−21.4	−52.6	−72.8	−28.1	−34.3
Glasswort *(Salicornia spp)*[4]	−28.6±0.1	−53.7±0.2	−25.1	−42.0	−76.3	−27.1	−34.6
Mean of C_3 plants	**−29.5**	**−62.8**	**−33.5**	**−59.2**	**−89.7**	**−29.3**	**−31.8**
(SD between C_3 plants)	(±1.7)	(±9.2)	(±8.1)	(±10.3)	(±10.3)	(±3.0)	(±4.5)
C_4-leaf tissue							
Maize (*Zea mays*)[4]	−11.0±0.1	−40.5±0.6	−29.5	−39.7	−91.3	−11.6	−16.9
CAM-leaf							
Saltwort *(Batis maritima)*[4]	−25.6±0.4	−63.3±0.4	−37.7	−64.9	−78.3	−22.4	−25.3
Scarlet paintbrush *(Crassula falcata)*[4]	−17.9±0.1	−51.1±0.5	−33.3	−41.1	−81.4	−16.2	−20.8

[1] All values in ‰, either mean of two samples or n=3–5±SD, for sample preparation and analytical measurements see Methods.
[2] Leaves were collected at Crossgar, N. Ireland in October 2002.
[3] Leaves were collected at Crossgar, N. Ireland in July 2003.
[4] Greenhouse-grown in N. Ireland 2003.

step was held for 8h. $\delta^{13}C$ of chloromethane was measured by GC-MS-IRMS at the end of each temperature step. Carbon isotope signatures for chloromethane at 30°C could not be measured because amounts were below the detection limit of the analytical method.

For experiments shown in Fig. 4 leucine methyl ester hydrochloride (100 mg) was heated in a glass vessel according to the method of Hamilton et al. (2003) except that temperature programming increments were 5°C instead of 25°C. $\delta^{13}C$ of chloromethane was measured by GC-MS-IRMS at the end of each temperature step.

Incubation experiments with fresh plant tissue: Fresh leaves (15–30 g) were detached from the plant and immediately placed in glass vials (44 ml) and sealed with caps containing a PTFE lined silicone septa. Samples (n=3–6) were incubated in the dark for 18 h at 25°C and VOCs were measured by GC-MS-IRMS. Table 3 shows the mean values ± SD. Results for C_3 plants are presented as mean values with the SD between species also shown.

Pectin methoxyl groups: Carbon isotope signatures of the pectin methoxyl pool were assessed by measuring $\delta^{13}C$ values of methanol released by alkaline hydrolysis of freeze-dried biomass. Molar NaOH (1 ml) was added to biomass (200 mg) in a 5 ml reaction vial. The vials were sealed with caps containing PTFE lined silicone septa and incubated for 12 h at 50°C to quantitatively hydrolyse ester methoxyl groups to methanol. Control experiments indicated that no chemical fractionation of carbon isotopes in methanol occurred during the analytical procedure.

Lignin methoxyl groups: Carbon isotope signatures of lignin methoxyl groups were assessed by measuring $\delta^{13}C$ values of CH_3I released by HI treatment (for 30 min at 100°C) of the biomass fraction remaining after removal of the pectin methoxyl pool by alkaline hydrolysis. Control experiments with aromatic and aliphatic methyl esters indicated the procedure resulted in quantitative conversion of OCH_3 groups to CH_3I. No significant chemical fractionation of carbon isotopes in CH_3I occurred during the analytical procedure conducted as described.

Sample collection: The origin of the investigated plant tissues is shown below the tables.

3 Results

In this study we have employed compound-specific carbon isotope ratio/mass spectrometry (GC-MS-IR-MS) to measure the $\delta^{13}C$ of two plant C_1 pools, ester methoxyl (largely present as pectin), and aromatic ether methoxyl (predominantly present as lignin), and also the $\delta^{13}C$ of several VOCs derived from fresh plant material at ambient and elevated temperatures. We initially assessed carbon isotope fractionation on the pectin methyl pool in leaf tissue from ash (*Fraxinus excelsior*) by measuring $\delta^{13}C$ in CH_3OH released on alkaline hydrolysis. The $\delta^{13}C$ observed for this esterified methyl pool was −77.2‰, a remarkably large ^{13}C fractionation (Δ=−45.4‰) compared with the overall $\delta^{13}C$ of leaf biomass of −31.8‰ (Table 1). A biochemical rationale for this striking depletion is however possible. Carboxyl groups in pectin are esterified by the enzyme pectin *O*-methyltransferase (PMT) using S-adenosylmethionine (SAM) as methyl donor. Work on purine alkaloids in several

Table 2. $\delta^{13}C$ values[1] of chloromethane produced on heating dried leaf tissue at 40, 50 and 60°C.

Plant common name (*species*)	**Chloromethane (CM)** ($\delta^{13}C$)	**Biomass (B)** ($\delta^{13}C$)	**Pectin methoxyl (PM)** ($\delta^{13}C$)	**$\Delta^{13}C$ (CM-PM)** ($\delta^{13}C_{PM}-\delta^{13}C_B$)
C_3-leaf[2]				
European ash (*Fraxinus excelsior*)		−27.9±0.2	−73.7±1.0	
40°C	−147.0			−73.3
50°C	−142.6			−68.9
60°C	−129.0			−55.3
Wych elm (*Ulmus glabra*)		−30.8±0.1	−69.2±0.3	
40°C	−138.9			−69.7
50°C	−130.4			−61.2
60°C	−126.9			−57.7
Cocksfoot (*Dactylis glomerata*)		−29.3±0.2	−50.7±0.2	
40°C	−119.2			−68.5
50°C	−113.5			−62.8
60°C	−110.3			−59.6

[1] All values in ‰, analytical measurements see Methods.
[2] Leaves were collected at Crossgar, N. Ireland in July 2003.

Table 3. $\delta^{13}C$ values[1] of biomass, pectin methoxyl pool and VOCs emitted at 25°C from fresh plant tissue.

Plant common name (*species*)	**Biomass (B)** ($\delta^{13}C$)	**Pectin methoxyl (PM)** ($\delta^{13}C$)	**$\Delta^{13}C$ (PM-B)** ($\delta^{13}C_{PM}-\delta^{13}C_B$)	**Methanol** ($\delta^{13}C$)	**Acetaldehyde** ($\delta^{13}C$)	**Ethanol** ($\delta^{13}C$)	**Acetone** ($\delta^{13}C$)
C_3-leaf[2]							
European ash (*Fraxinus excelsior*)	−27.9±0.2	−73.7±1.0	−45.8	−73.5±0.7	−22.7±0.4	−28.5±0.5	−31.3±1.4
Wych elm (*Ulmus glabra*)	−28.7±0.1	−68.9±0.1	−40.2	−82.9±5.9	−25.6±1.9	−30.1±2.0	−26.7±3.1
Hazelnut (*Corylus avellana*)	−33.6±0.2	−64.6±0.4	−31.0	−63.5±2.8	−25.9±2.5	−30.5±1.2	−26.3±2.4
English oak (*Quercus robur*)	−30.8±0.1	−69.2±0.3	−38.4	−76.6±3.9	−23.9±0.5	−29.9±0.3	−28.8±2.9
European beech (*Fagus sylvatica*)	−31.8±0.2	−68.2±1.0	−36.4	−84.2±2.6	−25.8±1.4	−31.3±1.1	−27.5±3.2
Norway maple (*Acer platanoides*)	−33.6±0.1	−63.1±0.6	−29.5	−70.3±0.7	−27.8±1.4	−33.7±0.5	−26.9±1.2
Horse chestnut (*Aesculus hippocastanum*)	−31.7±0.3	−73.4±0.1	−41.7	−71.0±2.4	−20.8±0.8	−26.9±1.2	−23.6±2.4
Scots pine (*Pinus sylvestris*)	−28.2±0.2	−57.3±0.3	−29.1	−60.2±1.5	−24.3±1.7	−23.9±1.4	−31.6±2.5
Cocksfoot (*Dactylis glomerata*)	−29.3±0.2	−50.7±0.2	−21.4	−51.9±0.2	−22.7±1.1	−29.0±1.4	−30.6±1.4
Yorkshire fog (*Holcus lanata*)	−31.3±0.3	−57.1±0.2	−25.8	−65.4±1.3	−27.5±0.9	−31.6±1.0	−26.7±0.5
Glasswort (*Salicornia sp*)[3]	−28.6±0.1	−53.7±0.2	−25.1	−50.4±0.9	−26.8±0.7	−28.5±1.5	−29.4±2.1
Mean of C_3 plants	**−30.5**	**−63.6**	**−33.1**	**−68.2**	**−24.9**	**−29.4**	**−28.1**
(SD between C_3 plant species)	(±2.1)	(±7.9)	(±7.8)	(±11.2)	(±2.2)	(±2.6)	(±2.5)
C_4-leaf							
Maize (*Zea mays*)[3]	−11.0±0.1	−40.5±0.6	−29.5	−52.9±0.8	−9.3±1.3	−15.2±0.5	−16.6±1.2
CAM-leaf							
Saltwort (*Batis maritima*)[3]	−25.6±0.4	−63.3±0.4	−37.7	−60.0±0.9	−24.4±1.7	−23.8±1.2	−27.8±0.3
Scarlet paintbrush (*Crassula falcata*)[3]	−17.9±0.1	−51.1±0.9	−33.2	−55.9±2.1	−10.3±0.5	−17.2±0.2	−19.8±0.7

[1] All values in ‰±SD (n=3–5), analytical measurements see Methods.
[2] Fresh leaves were collected at Crossgar, N. Ireland in July 2003.
[3] Greenhouse-grown in N. Ireland 2003.

plant species has suggested that the methyl pool in SAM is significantly depleted ($\delta^{13}C \leq -39$‰) relative to the carbohydrate pool ($\delta^{13}C = -27$‰) (Weilacher et al., 1996). Moreover, enzymic transmethylation involving SAM can entail a substantial kinetic isotope effect (KIE); thus the reaction catalysed by catechol *O*-methyltransferase displays a large fractionation factor (ε=90) (Hegazi et al., 1979). A similar KIE in the enzymic methylation of pectin by PMT utilising ^{13}C-depleted SAM as the methyl donor could account for the magnitude of the ^{13}C depletion observed in the pectin methoxyl pool.

We next investigated the effect of progressive heating of leaf tissue of ash from 150 to 300°C on the $\delta^{13}C$ of volatiles released (Fig. 1) in order to determine their interrelationship and to calculate the mass balance involved. The main VOCs produced were CH_3Cl, CH_3OH, acetaldehyde and acetone

(Fig. 1a). The $\delta^{13}C$ values for both CH_3Cl and CH_3OH (Fig. 1b) were strikingly depleted with respect to biomass (Δ between -30 and -100‰). Emissions of CH_3Cl exhibited a $\delta^{13}C$ of -128‰ at 150°C (-147‰ at 40°C, see Table 2), ^{13}C signatures, which, to the best of our knowledge, are the lightest isotopic values ever observed in a terrestrial carbon compound produced during natural processes. A comparison of the $\delta^{13}C$ of the pectin methyl pool with the composite $\delta^{13}C$ value calculated on a molar basis for CH_3Cl and CH_3OH released during heating is displayed in Fig. 1b. This isotopic mass balance shows that when production of CH_3Cl and CH_3OH had ceased the composite $\delta^{13}C$ value for these compounds closely corresponded with the $\delta^{13}C$ value of the pectin methyl pool. Alkaline hydrolysis of the residual material clearly showed that the pectin methoxyl pool had been volatilised by 300°C (Fig. 1c), indicating that this is the major source of both CH_3Cl and CH_3OH. Measurements conducted using a model system of purified pectin also confirmed that isotopic mass balance was achieved with respect to CH_3Cl, CH_3OH and pectin methoxyl during the heating cycle (see Fig. 2). Furthermore it is evident that relative to the pectin methoxyl pool CH_3Cl is always highly depleted whilst CH_3OH normally exhibits slight but significant enrichment. The $\delta^{13}C$ values determined for acetaldehyde and acetone at all stages of the heating programme clearly reflected the isotope signature of bulk leaf biomass (Fig. 1d), unequivocally distinguishing their origin from that of the C_1 compounds. An explanation for the unprecedented depletion of ^{13}C in CH_3Cl released during heating of leaf tissue must await elucidation of the mechanism of the solid state reaction of halide ion with pectin (Hamilton et al., 2003).

To determine whether our findings with ash leaves could be replicated with other species we conducted further studies on leaf tissue from trees, grasses and halophytes including plants from C_3, C_4 and CAM plant categories involving heating of dried biomass isothermally at 225°C (Table 1). For all species examined we observed a large fractionation between the pectin methoxyl pool and bulk biomass ($\Delta\sim-33$‰, range -21 to -45‰). In general the isotope signatures for CH_3OH reflected those of the pectin methoxyl pool whilst those of CH_3Cl were considerably more depleted in ^{13}C ($\Delta\sim-30$‰). Signatures of C_2 VOCs mirrored in general those of bulk biomass. Although the Br^-, I^- and CN^- content of most plant tissues are insufficient to permit measurements on emissions of the corresponding substituted methanes, experiments performed with purified apple pectin supplemented with the different ions revealed that CH_3Br, CH_3I and CH_3CN released on heating were also highly depleted in ^{13}C.

We extended our measurements to VOCs released from freshly collected plant material at ambient temperatures. Methanol, ethanol, acetaldehyde and acetone were naturally released in sufficient quantities for analytical measurements. The results were similar to those obtained at higher temperatures (Table 3). Thus CH_3OH, like the pectin methoxyl

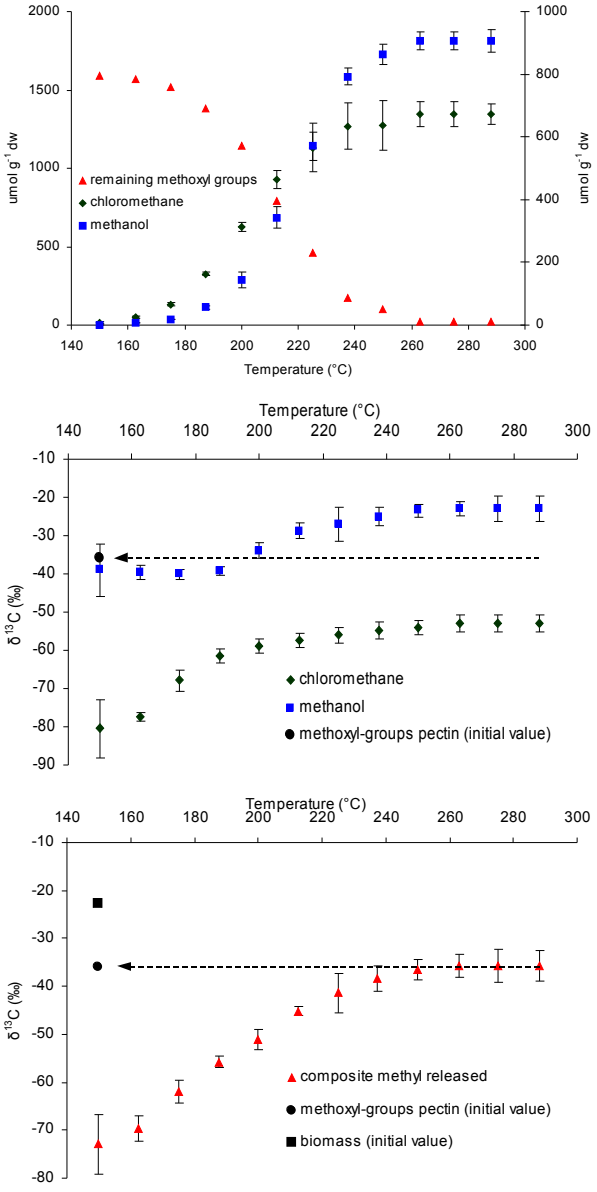

Fig. 2. Amounts and isotopic signatures of chloromethane and methanol formed during progressive heating from 150 to 300°C of pectin with added chloride ion (for preparation see Hamilton et al., 2003). **(a)** Cumulative amounts of chloromethane and methanol and residual pectin methoxyl are shown on a molar basis. Each point is the mean of three replicate analysis of independent samples (n=3). Left y-axis refers to pectin methoxyl groups and right y-axis to methanol and chloromethane. **(b)** Carbon isotopic composition of accumulated CH_3OH and CH_3Cl at each temperature during progressive heating. For reference the measured initial $\delta^{13}C$ of the pectin methoxyl pool is displayed. Vertical bars show SD for triplicate samples. **(c)** Composite $\delta^{13}C$ values calculated on a molar basis for CH_3OH and CH_3Cl released during progressive heating. For reference the measured initial $\delta^{13}C$ of the pectin methoxyl pool and the original bulk pectin is displayed. Vertical bars show SD for triplicate samples.

Table 4. $\delta^{13}C$ values[1] of biomass and the lignin and pectin methoxyl pools of plant tissues.

Plant common name (*species*)	**Biomass (B)** ($\delta^{13}C$)	**Lignin methoxyl (LM)** ($\delta^{13}C$)	**$\Delta^{13}C$ (LM-B)** ($\delta^{13}C_{LM}-\delta^{13}C_B$)	**Pectin methoxyl (PM)** ($\delta^{13}C$)	**$\Delta^{13}C$ (PM-B)** ($\delta^{13}C_{PM}-\delta^{13}C_B$)
C_3-wood[2]					
European ash *(Fraxinus excelsior)*	−24.5±0.7	−36.9±1.2	−12.4	−43.1±0.6	−18.6
English oak *(Quercus robur)*	−29.4±0.2	−41.1±1.3	−11.7	−44.2±1.2	−14.8
Sweet osmanthus *(Osmanthus fragans)*	−27.3±0.1	−41.7±1.2	−14.7	−53.3±1.1	−26.0
Geronggang (*Cratoxylum sp*)	−26.5±0.3	−39.5	−13.0	n.d.	–
Tasmanian oak *(Eucalyptus delegatensis)*	−26.3±0.2	−37.7	−11.4	−45.4±0.7	−18.1
Dark red meranti *(Shorea sp)*	−28.2±0.2	−44.0	−15.8	−45.6±0.3	−17.4
Utile *(Entandrophragma utile)*	−27.1±0.2	−39.5	−12.4	n.d.	–
Mean of wood	**−27.1**	**−40.1**	**−13.0**	**−46.3**	**−19.2**
C_3-leaf[3]					
European ash *(Fraxinus excelsior)*	−27.9±0.2	−65.5	−37.6	−73.7±1.0	−45.8
English oak *(Quercus robur)*	−30.8±0.1	−62.2	−31.4	−69.2±0.3	−38.4
European beech (*Fagus sylvatica*)	−31.8±0.2	−66.2	−34.4	−68.2±1.0	−36.4
Norway maple (*Acer platanoides*)	−33.6±0.2	−61.4	−27.8	−63.1±0.6	−29.5
Scots pine (*Pinus sylvestris*)	−27.6±0.1	−51.7	−24.1	−53.7±0.3	−26.1
Cocksfoot grass (*Dactylis glomerata*)	−29.3±0.2	−53.5	−24.2	−50.7±0.2	−21.4
Mean of C_3-leaves	**−30.2**	**−60.1**	**−29.9**	**−63.1**	**−32.9**
C_4-leaf					
Sugar cane (*Saccharum officinarum)*[4]	−11.9±0.1	−42.1	−30.2	−36.0±0.9	−24.1
Savanna grass (*Hyparrhenia sp*)[5]	−12.8±0.2	−33.1	−20.2	n.d.	–
Maize (*Zea mays*)[6]	−11.0±0.1	−47.3	−36.3	−40.5±0.6	−29.5
CAM-leaf					
Saltwort *(Batis maritima)*[6]	−25.6±0.4	−52.4	−25.7	−63.3±0.4	−37.3
Scarlet paintbrush *(Crassula falcata)*[6]	−17.9±0.1	−49.6	−31.7	−51.1±0.5	−33.2
Mean of C_3, C_4 and CAM leaves			**−29.0**		**−32.2**

[1] All values in ‰, either mean of two samples or n=3–5±SD;
n.d.: not detectable, for sample preparation and analytical measurements see Methods.
[2] Wood samples were collected from Cameroon, Indonesia, Malaysia, and N. Ireland.
[3] Leaves were collected at Crossgar, N. Ireland in July 2003.
[4] Sampled from South Africa.
[5] Sampled from Cote d'Ivory.
[6] Greenhouse-grown in N. Ireland 2003.

pool, was highly depleted in ^{13}C ($\Delta\sim-33$‰ relative to bulk biomass). Carbon isotope signatures of ethanol and acetone were close to that of bulk biomass whilst acetaldehyde showed slight enrichment in ^{13}C ($\Delta\sim5$‰). $\delta^{13}C$ values for CH_3Cl could not be measured (except for the halophytes) as amounts were below the detection limit of the analytical method. As has been shown previously CH_3Cl formation in fresh leaves with a high water content is generally low (Hamilton et al., 2003).

In addition to the pectin methoxyl pool the other important C_1 pool in plant cell walls is represented by aromatic ether methoxyl groups which can comprise up to 18% of lignin. Lignin is a major component of wood (up to 31%) and is also found in smaller quantities in leaves and grasses ($\sim$5%). We therefore measured $\delta^{13}C$ values of the lignin methoxyl pool in plant tissue from several species after conversion to CH_3I with HI subsequent to removal of the pectin methyl pool by alkaline hydrolysis (Table 4). Depletion in ^{13}C of lignin methoxyl groups in wood ($\Delta\sim-13$‰) relative to bulk biomass was substantial although not as dramatic as that observed for lignin methoxyl groups in leaves (mean of all leaves $\Delta\sim-29$‰, range -20 to -38‰). These findings explains the widely reported ^{13}C depletion of lignin relative to other major plant components (Benner et al., 1987; Schweizer et al., 1999; Fernandez et al., 2003; Hobbie and Werner, 2004) which has previously been attributed to ^{13}C fractionation in aromatic amino acids involved in lignin biosynthesis. Thus assuming a methoxyl content of 15–20% a depletion of -13‰ in methoxyl carbon readily explains the observed 2–3‰ difference between $\delta^{13}C$ of lignin and bulk biomass of wood. Similarly the much larger depletion observed in methoxyl carbon in leaf tissue provides an explanation for the 3–7‰ ^{13}C depletion of lignin relative to bulk biomass in leaves of both C_3 and C_4 plants. Archaeological and fossil wood specimens are often used to provide information on palaeoenvironmental and palaeoclimatic conditions in the geological record (van Bergen and Poole, 2002). Hence alterations to wood which involve cleavage of the isotopically light methoxyl groups will be critically important in interpretation of the significance of the isotope signatures of individual wood components.

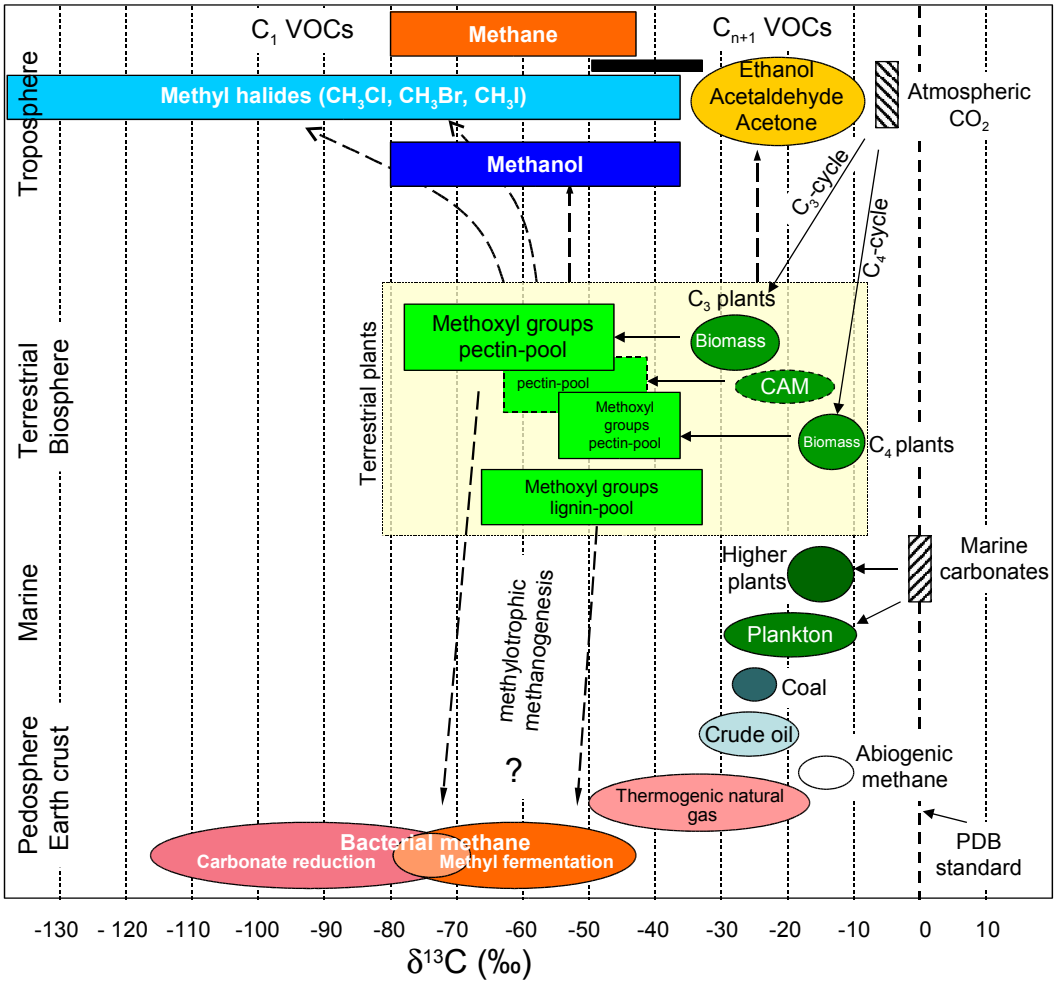

Fig. 3. Schematic diagram displaying ^{13}C depletion of methoxyl groups relative to bulk biomass of terrestrial plants and their relationship to biospheric C_1 and C_{n+1} VOCs. Data for carbon isotopic composition of major carbon pools were taken from Whiticar (1996). Carbon isotope signatures for VOCs are related to sources, degradation steps after formation are not taken into account. The black box shows tropospheric $\delta^{13}C$ values of CH_3Cl and CH_3Br measured by Rudolph et al., 1997, Tsunogai et al., 1999, Thompson et al., 2002, and Bill et al., 2004.

4 Discussion

We have summarised our findings on the $\delta^{13}C$ values of methoxyl pools in plants and some plant derived C_1 VOCs in Fig. 3 where they are related to fractionations reported in the literature for bulk biomass of various categories of plant and other terrestrial carbon sources (Whiticar, 1996). The depletion between bulk plant biomass and plant methoxyl pools ranges from -11 to -46‰ with the pectin C_1 pool generally more depleted than the lignin C_1 pool. The fractionation associated with the methoxyl pools is retained and even further enhanced during their conversion to C_1 VOCs. For biogenic VOC emissions from living vegetation, the more labile pectin pool rather than the lignin pool is likely to be the major source. However during biomass burning both pectin and lignin C_1 pools will contribute to C_1 VOC production. In soils under aerobic conditions demethylation of pectin and lignin, both enzymically (e.g. Fall and Benson, 1996; Ander and Eriksson, 1985) and abiotically (e.g. Dec et al., 2001; Keppler et al., 2000) is very probably an important source of several C_1 VOCs. Under anoxic conditions C_1 VOCs can act as substrates for methylotrophic methanogenic bacteria forming CH_4 (Whiticar, 1999; Cicerone and Oremland, 1988). Therefore, we hypothesise that C_1 compounds from both pectin and lignin C_1 pools could be an important source for methanogenesis. It has been assumed to date that the carbon signature of a substrate for methanogens in a specific environment is broadly similar to that of the bulk organic matter present, provided severe substrate depletion has not occurred (Whiticar, 1999). If, however in the upper horizon of wetlands, peat bogs and rice paddies, methanogens are utilising methanol and other C_1 substrate from the plant methoxyl

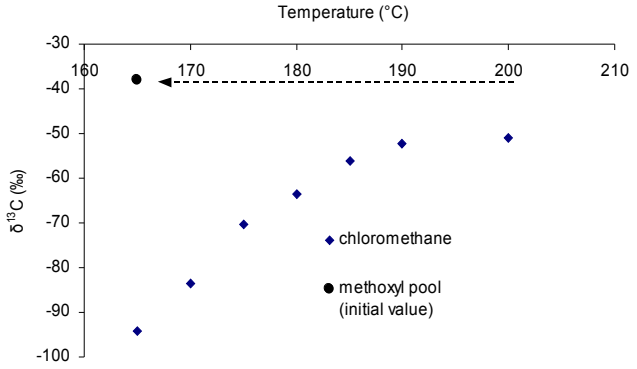

Fig. 4. Isotopic signatures of accumulated chloromethane during progressive heating from 160 to 200 ° C of leucine methyl ester hydrochloride. For reference the measured initial $\delta^{13}C$ of the methoxyl pool is displayed.

pool with $\delta^{13}C$ values averaging −50‰, the production of CH_4 more highly depleted than previously envisaged might be expected. Indeed $\delta^{13}C$ values for CH_4 derived from the plant methoxyl pool may be of the same order as the lowest observed for CH_4 produced by bacterial carbonate reduction (Whiticar, 1999; Cicerone and Oremland, 1988). Since approximately 2.5% of carbon in plant biomass is methoxyl carbon (Galbally and Kirstine, 2002), any isotopically-based discussion of global carbon cycling must give consideration to this isotopically anomalous C_1 pool.

Our findings may also have some relevance to the search for ancient life on earth and for extraterrestrial life. The striking depletion of $\delta^{13}C$ in methoxyl carbon consequent on the biochemistry of C_1 metabolism in plants may well extend to many other organisms which utilise S-adenosylmethionine as a methyl donor in *O*-methyltransferase reactions and could even serve to distinguish biologically formed methyl esters and ethers from those generated abiotically. Conversely, the exceptional ^{13}C fractionation during abiotic production of CH_3Cl from biomass (which we have reproduced by heating hydrochlorides of methyl esters of amino acids, (see Fig. 4) suggest that caution is necessary in interpreting such fractionation as unequivocal evidence of life.

Based on our data the fractionation of carbon isotopes by the principal *O*-methyltransferase enzymes in plants appears to be of the same order as that achieved by ribulose biphosphate carboxylase-oxygenase (Rubisco) in photosynthesis. Hitherto it has been assumed that within a given photosynthetic category the carbon isotope signature of specific chemical components and specific intramolecular sites within such components does not differ from the carbon isotope signature of bulk biomass by more than 12‰ (Weilacher et al., 1996; Hobbie and Werner, 2004). Our results indicate a ^{13}C depletion relative to bulk biomass of up to 45 for methoxyl carbon in plants, the largest carbon isotope fractionation ever observed in the plant kingdom. This isotope anomaly should prove not only an invaluable tool in tracing the path of such C_1 carbon in the environment but also provide a new insight into the global cycling of many C_1 atmospheric trace gases and the biochemical pathways involved.

Acknowledgements. The authors thank W. Michaelis, J. Raven, and T. Röckmann for helpful advice regarding the manuscript; W. Meier-Augenstein, A. Downey, K. Redeker, E. Tujek, B. Ferguson, T. Kennedy for technical assistance and the European Commission for a Marie Curie-Research Training Grant (MCFI-2002-00022) awarded to F. Keppler. Supported by grants from EPSRC GR/R03099/01, QUB SRIF for Environmental Engineering and Biotechnology, and INI TDP Centre of Excellence. Finally we would like to thank two anonymous reviewers for their critical and valuable comments.

Edited by: J. Middelburg

References

Ander, P. and Eriksson, K.-E.: Methanol formation during lignin degradation by Phanerochaeta chrysosporium, Appl. Microbial. Biotechnol., 21, 96–102, 1985.

Benner, R., Fogel, M. L., Sprague, E. K., and Hodson, R. E.: Depletion of ^{13}C lignin and its implications for stable isotope studies, Nature, 329, 708–710, 1987.

Bill, M., Conrad, M. E., and Goldstein, A. H.: Stable carbon isotope composition of atmospheric methyl bromide, Geophys. Res. Lett., 31, L04109, doi:10.1029/2003GL018639, 2004.

Cerling, T. E., Harris, J. M., MacFadden, B. J., et al.: Global vegetation change through the Miocene/Pliocene boundary, Nature, 389, 153–158, 1997.

Cicerone, R. J. and Oremland R. S.: Biogeochemical aspects of atmospheric methane, Global Biogeochem. Cycles, 2, 299–327, 1988.

Dec, J., Haider, K., and Bollag, J.-M.: Decarboxylation and demethoxylation of naturally occurring phenols during coupling reactions and polymerization, Soil Science, 166, 660–671, 2001.

Ehleringer, J. R., Bowling, D. R., Flanagan, L. B., et al.: Stable isotopes and carbon cycle processes in forests and grasslands, Plant Biol., 4, 181–189, 2002.

Fall, R. and Benson, A. A.: Leaf methanol – the simplest natural product from plants, Trends in Plant Science, 1, 296–301, 1996.

Fernandez, I., Mahieu, N., and Cadisch, G.: Carbon isotopic fractionation during decomposition of plant materials of different quality, Global Biogeochem. Cycles, 17, doi:10.1029/2001GB001834, 2003.

Galbally, I. E. and Kirstine, W.: The production of methanol by flowering plants and the global cycle of methanol, J. Atmos. Chem., 43, 195–229, 2002.

Goldstein, A. H. and Shaw, S. L.: Isotopes of volatile organic compounds: an emerging approach for studying atmospheric budgets and chemistry, Chem. Rev., 103, 5025–5048, 2003.

Griffiths, H. (Ed.): Stable Isotopes and the Integration of Biological, Ecological and Geochemical Processes, Bios Scientific Publishers, Oxford, 1998.

Hamilton, J. T. G., McRoberts, W. C., Keppler, F., Kalin, R. M., and Harper, D. B.: Chloride methylation by plant pectin: an ef-

ficient environmentally significant process, Science, 301, 206–209, 2003.

Harper, D. B., Hamilton, J. T. G., Ducrocq, V., et al.: The distinctive isotopic signature of plant-derived chloromethane: possible application in constraining the atmospheric chloromethane budget, Chemosphere, 52, 433–436, 2003.

Hayes, J. M.: Fractionation of the isotopes of carbon and hydrogen in biosynthetic processes, Rev. Mineral Geochem., 43, 225–277, 2001.

Hegazi, M. F., Borchardt, R. T., and Schowen, R. L.: α-Deuterium and carbon-13 isotope effects for methyl transfer catalyzed by catechol -methyltransferase, S_N2-like transition state, J. Am. Chem. Soc., 101, 4359–4364, 1979.

Heikes, B. G., Chang, W., Pilson, M. E. Q., et al.: Atmospheric methanol budget and ocean implication, Global Biogeochem. Cycles, 16, doi:10.1029/2002GB001895, 2002.

Hobbie, E. A. and Werner, R.: Intramolecular, compound-specific, and bulk carbon isotope patterns in C_3 and C_4 plants: a review and synthesis, New Phytologist, 161, 371–385, 2004.

Kalin, R. M., Hamilton, J. T. G., Harper, D. B., et al.: Continuous flow stable isotope methods for sudy of $\delta^{13}C$ fractionation during halomethane production and degradation, Rapid Commun Mass Spectrom., 15, 357–363 2001.

Keppler, F., Eiden, R., Niedan, V., Pracht, J., and Schöler, H. F.: Halocarbons produced by natural oxidation processes during degradation of organic matter, Nature, 403, 298–301, 2000.

Lajtha, K. and Michener, R. B. (Eds.): Stable Isotopes in Ecology and Environmental Science, Blackwell Scientific Publications, Oxford, 1994.

Michaelis, W., Seifert, R., Nauhaus, K., et al.: Microbial reefs in the Black Sea fueled by anaerobic oxidation of methane, Science, 297, 1013–1015, 2002.

Montzka, S. A. and Frazer, P. J.: Controlled Substances and Other Source Gases, in: WMO Scientific Assessment of Ozone Depletion, Global Ozone Research and Monitoring Project, Report No. 47, World Meterological Organization, Geneva, (http://www.wmo.ch/web/arep/reports/ozone_2002/06_chapter1.pdf), 2003.

O'Dowd, C. D., Jimenez, J. C., Bahreini, R., et al.: Marine aerosol formation from biogenic iodine emissions, Nature, 417, 632–636, 2002.

O'Leary, M. H.: Carbon isotope fractionations in plants, Phytochemistry, 20, 553–567, 1981.

Rudolph, J., Lowe, D. C., Martin, J., and Clarkson, T. S.: A novel method for compound specific determination of d13C in volatile organic compounds at ppt levels in ambient air, Geophys. Res. Lett., 24, 659–662, 1997.

Sanhueza, E., Holzinger, R., Kleiss, B., Donoso, L., and Crutzen, P. J.: New insights in the global cycle of acetonitrile: release from the ocean and dry deposition in the tropical savanna of Venezuela, Atmos. Chem. Phys., 4, 275–280, 2004, **SRef-ID: 1680-7324/acp/2004-4-275**.

Schweizer, M., Fear, F., and Cadisch G.: Isotopic (^{13}C) fractionation during plant residue decomposition and its implications for soil organic matter studies, Rapid Commun Mass Spectrom., 13, 1284–1290, 1999.

Sherwood Lollar, B., Westgate, T. D., Ward, J. A., et al.: Abiogenic formation of alkanes in the Earth's crust as a minor source for global hydrocarbon reservoirs, Nature, 416, 522–524, 2002.

Thompson, A. E., Anderson R. S., Rudolph, J., and Huang, L.: Stable carbon isotopes signatures of background tropospheric chloromethane and CFC113, Biogeochemistry, 60, 191–211, 2002.

Tsunogai, U., Yoshida, N., and Gamo T.: Carbon isotopic compositions of C2-C5 hydrocarbons and methyl chloride in urban, coastal, and marittime atmospheres over the western North Pacific, J. Geophys. Res., 104, 16 033–16 039, 1999.

van Bergen, P. F. and Poole, I.: Stable carbon isotopes of wood: a clue to palaeoclimate?, Palaeogeogr, Palaeoclimatol, Palaeoecol, 182, 31–45, 2002.

Warneke, C., Karl, T., Judmaier, H., et al.: Acetone, methanol, and other partially oxidised volatile organic emissions from dead plant matter by abiotical processes: significance for atmospheric HO_x chemistry, Global Biogeochem. Cycles, 13, 9–17, 1999.

Weilacher, T., Gleixner, G., and Schmidt, H. L.: Carbon isotope pattern in purine alkaloids a key to isotope discriminations in C_1 compounds, Phytochemistry, 41, 1073–1077, 1996.

Whiticar, M. J.: Stable isotope geochemistry of coals, humic kerogens and related natural gases, Int. J. Coal. Geol., 32, 191–215, 1996.

Whiticar, M. J.: Carbon and hydrogen isotope systematics of bacterial formation and oxidation of methane, Chem. Geol., 161, 291–314, 1999.

Wuebbles, D. J. and Hayhoe, K.: Atmospheric methane and global change, Earth-Science Reviews, 57, 177–210, 2002.

Yakir, D.: Sphere of influence, Nature, 416, 795, 2002.