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"Carbon Isotope Anomaly in the major Plant C_1 Pool and its Biogeochemical Implications"

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Relative Isotope Abundance: δ-notation definition

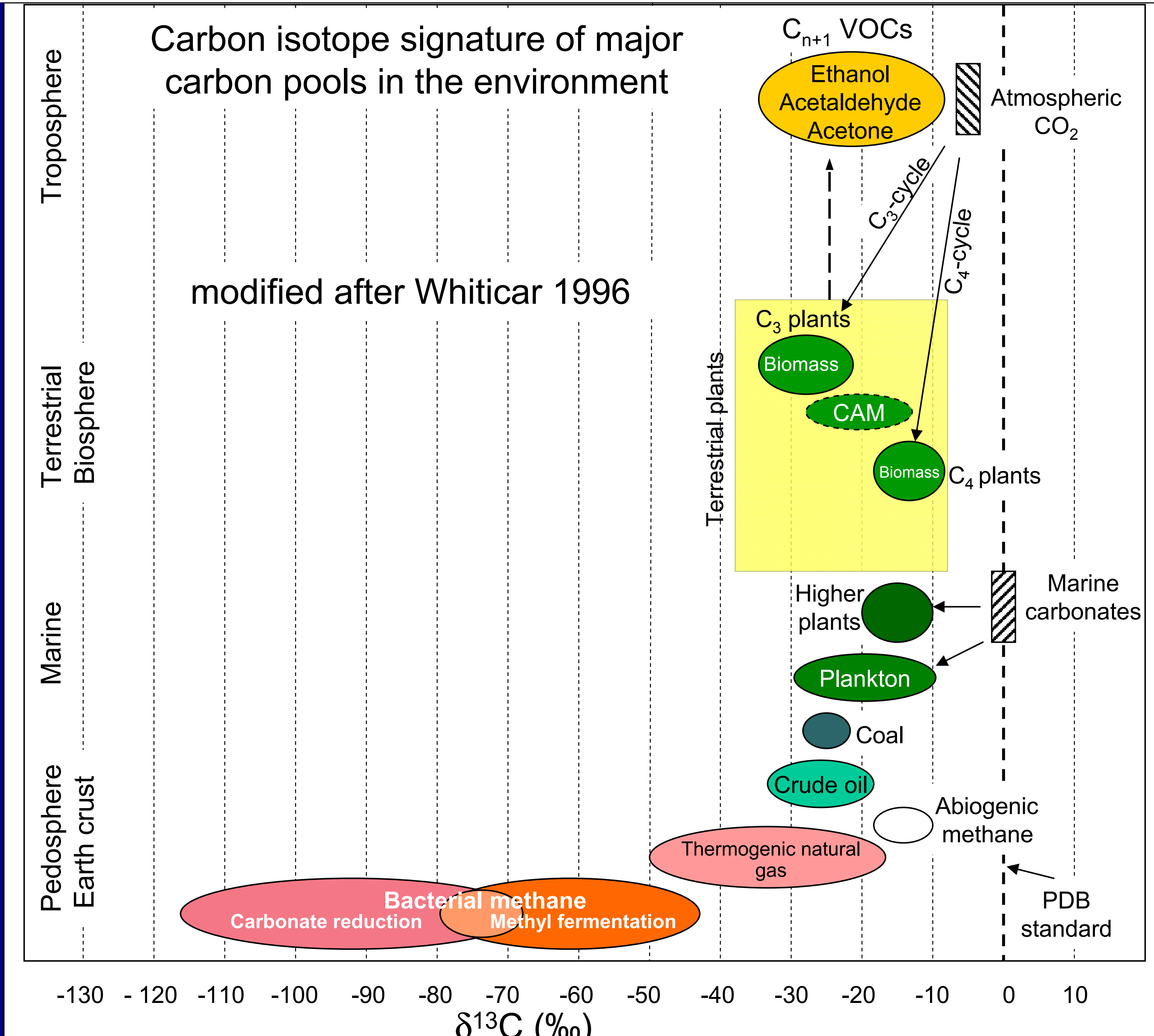
Isotopic compositions are usually expressed in term of δ values which are parts per thousand deviations from a standard. Delta notation: a convenient expression for small differences

$$\delta^{13}C = \left\{ \frac{^{13}R_{Sample} - {}^{13}R_{Standard}}{^{13}R_{Standard}} \right\} \cdot 1000$$

^{13}R = abundance (^{13}C) / abundance (^{12}C)

'Unit': [‰] ('per mil') = per thousand deviation from a reference ratio

Please note: In this presentation carbon isotope ratios ($\delta^{13}C$ are reported on the Vienna-PDB scale!


Carbon isotope signature of major carbon pools in the environment
modified after Whiticar 1996
Troposphere
Terrestrial Biosphere
Marine
Pedosphere Earth crust
C_{n+1} VOCs
Ethanol
Acetaldehyde
Acetone
Atmospheric CO_2
C_3-cycle
C_4-cycle
Terrestrial plants
C_3 plants
Biomass
CAM
Biomass
C_4 plants
Higher plants
Marine carbonates
Plankton
Coal
Crude oil
Abiogenic methane
Thermogenic natural gas
Bacterial methane
Carbonate reduction
Methyl fermentation
PDB standard
-130
- 120
-110
-100
-90
-80
-70
-60
-50
-40
-30
-20
-10
0
10
$\delta^{13}C$ (‰)

Furthermore…

- Differences in bulk $\delta^{13}C$ of different plant parts (e.g. leaves, roots) are common (often in the range of 1–3‰)

- Differences in $\delta^{13}C$ among plant compounds (e.g. cellulose, lignin are also common; often in the range of 1–5‰)

Measurement	C_3 (‰)	C_4 (‰)	Reference	Comments
Cellulose – lignin	3.5		Wilson & Grinsted (1977)	Wood
Cellulose – lignin	4.1 ± 0.6	6.2 ± 0.9	Benner *et al.* (1987)	Various tissues
Cellulose – lignin	4.6	5.1	Schweizer *et al.* (1999)	Leaves
Cellulose – lignin	2.5	4.6	Schweizer *et al.* (1999)	Roots

Table taken from Hobbie & Werner 2004

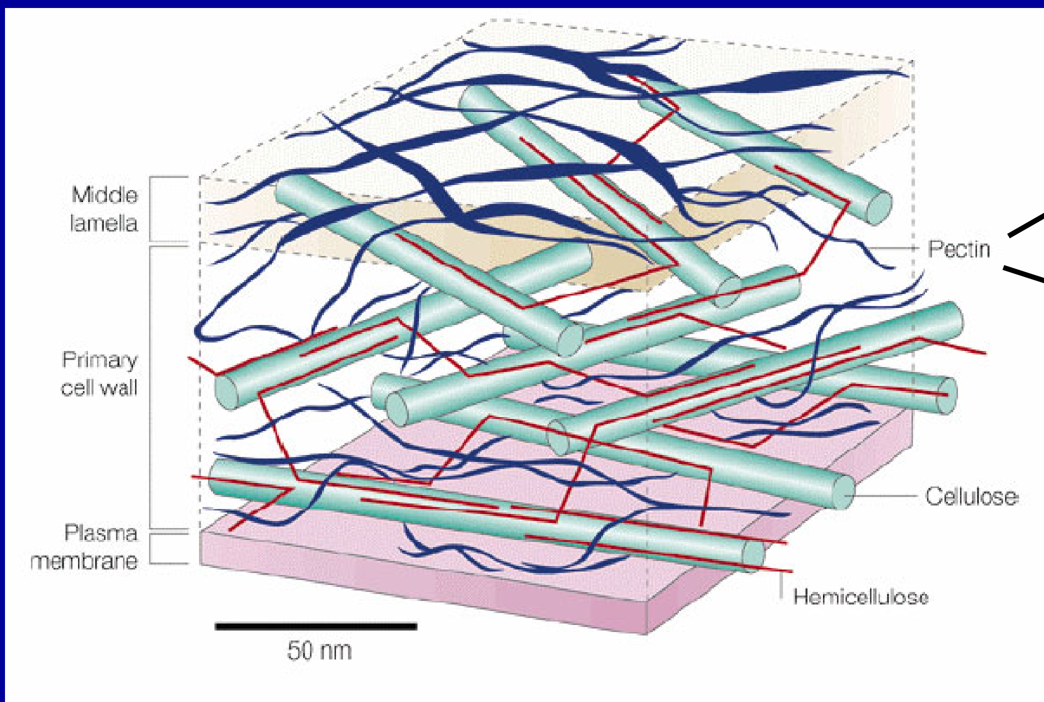
The major chemical component of a living plant is water, but on a dry weight basis, all plant cell walls consist mainly of sugar-based polymers (carbohydrates including cellulose, hemicellulose, pectin) that are combined with lignin with lesser amounts of extractives, protein, starch, and inorganics.

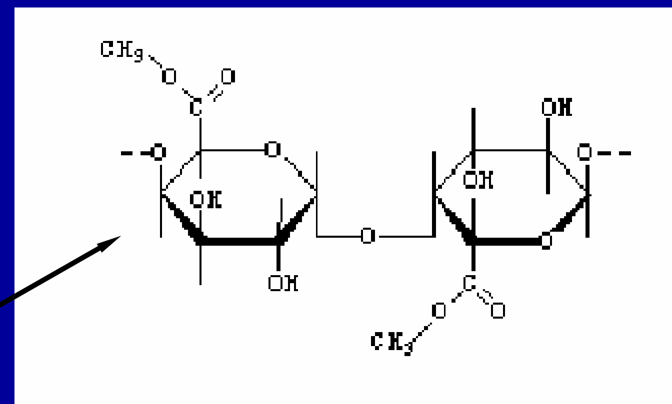
Pectin **Lignin**

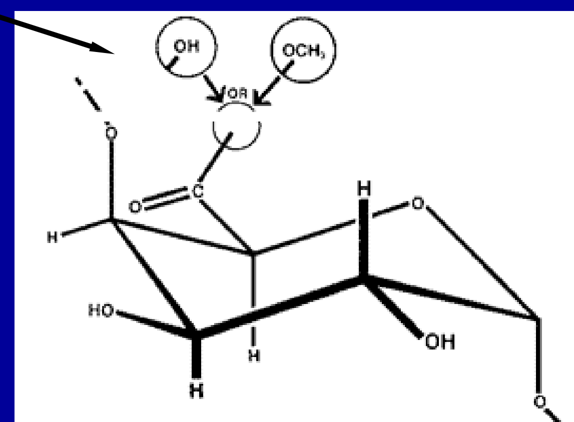
rich in methoxyl groups

Pectin is a major component of the cell wall in plants:

- Polymer of around 200 galacturonic acid molecules
- Many of the carboxyl groups are methylated ($COOCH_3$), 50-90%
- Pectin normally comprises between 7 and 35% of cell wall material in leaves
- Methoxyl groups (OCH_3) constitute up to 6% on a dry weight basis

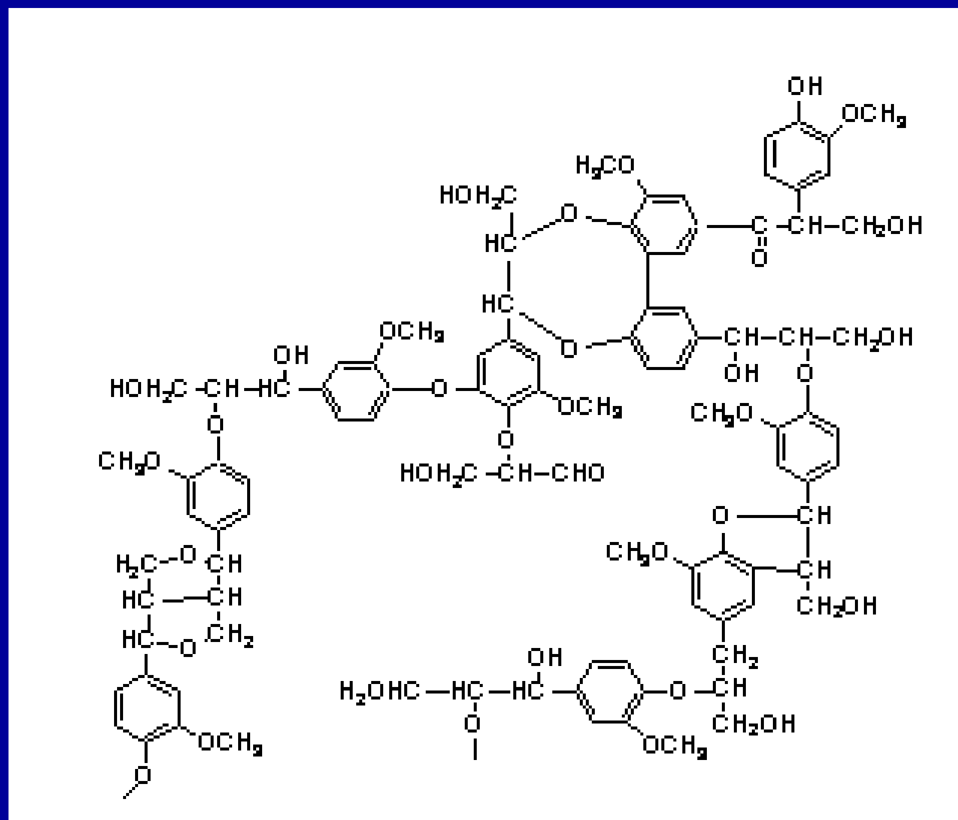






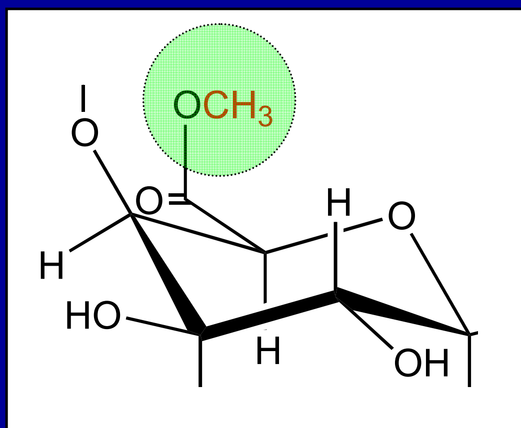
Lignin defines the polymeric material located in the woody cell wall “the glue that holds plant together“

- lignin, cellulose and hemicellulose are the major fractions in wood
- lignin confers strength and rigidity to the woody cell wall of plants
- up to 30-40% on a dry weight basis in woody tissue, less in non-woody tissue
- rich in methoxyl groups (OCH_3), up to 15-20 % on a dry weight basis

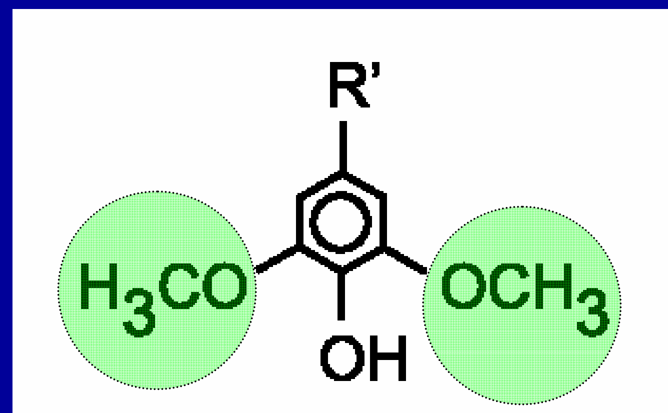


Methoxyl groups in terrestrial plants (including ester and ether groups) comprise *ca* 2.5% of carbon in plant biomass!!!

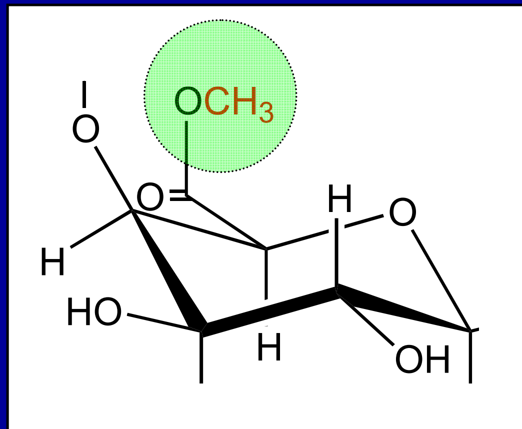
The carbon isotope signature of methoxyl groups in plants?



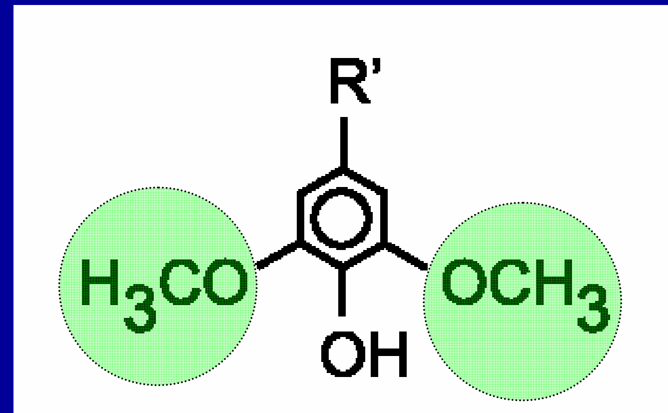
Pectin



Lignin



Pectin



Lignin

+ NaOH

‘akaline hydrolysis’

CH_3OH

+ HI

‘Zeisel method’

CH_3I

GC/MS/IRMS

(compound-specific carbon isotope ratio / mass spectrometry)

$\delta^{13}C$ values of methoxyl groups of plant tissue from woods

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**

Data from Keppler et al. 2004

Please note: the isotope difference between two pools is here defined as

$$\Delta = \delta^{13}C_{pool\ 1} - \delta^{13}C_{pool\ 2}$$

Biochemical rationale?

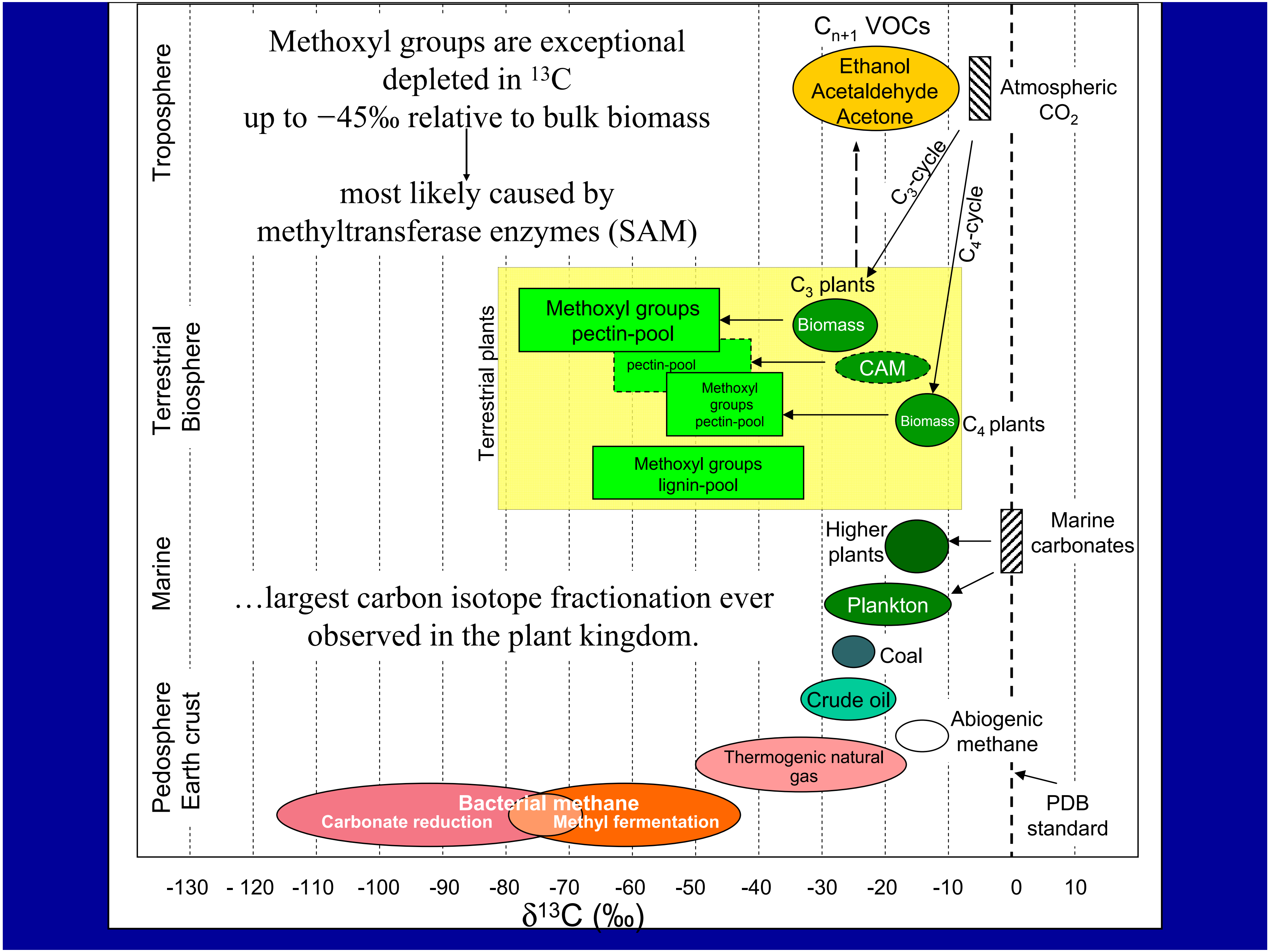
Work on purine alkaloids in several plant species has suggested that the methyl pool in SAM (S-adenosylmethionine) is significantly depleted ($\delta^{13}C \leq -39‰$) relative to the carbohydrate pool ($\delta^{13}C = -27‰$).

Weilacher, T., Gleixner, G. & Schmidt, H.L. Carbon isotope pattern in purine alkaloids a key to isotope discriminations in C1 compounds. *Phytochemistry* **41**, 1073-1077 (1996).

$\delta^{13}C$ values of methoxyl groups of leaf tissue from trees, grasses and halophytes including plants from C_3, C_4 and CAM plant categories

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-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**

Data from Keppler et al. 2004



Methoxyl groups are exceptional depleted in ^{13}C
up to −45‰ relative to bulk biomass
most likely caused by methyltransferase enzymes (SAM)
…largest carbon isotope fractionation ever observed in the plant kingdom.
Troposphere
Terrestrial Biosphere
Marine
Pedosphere Earth crust
C_{n+1} VOCs
Ethanol
Acetaldehyde
Acetone
Atmospheric CO_2
C_3-cycle
C_4-cycle
C_3 plants
Biomass
CAM
Biomass
C_4 plants
Terrestrial plants
Methoxyl groups pectin-pool
pectin-pool
Methoxyl groups pectin-pool
Methoxyl groups lignin-pool
Higher plants
Marine carbonates
Plankton
Coal
Crude oil
Abiogenic methane
Thermogenic natural gas
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Carbonate reduction
Methyl fermentation
PDB standard
-130
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-100
-90
-80
-70
-60
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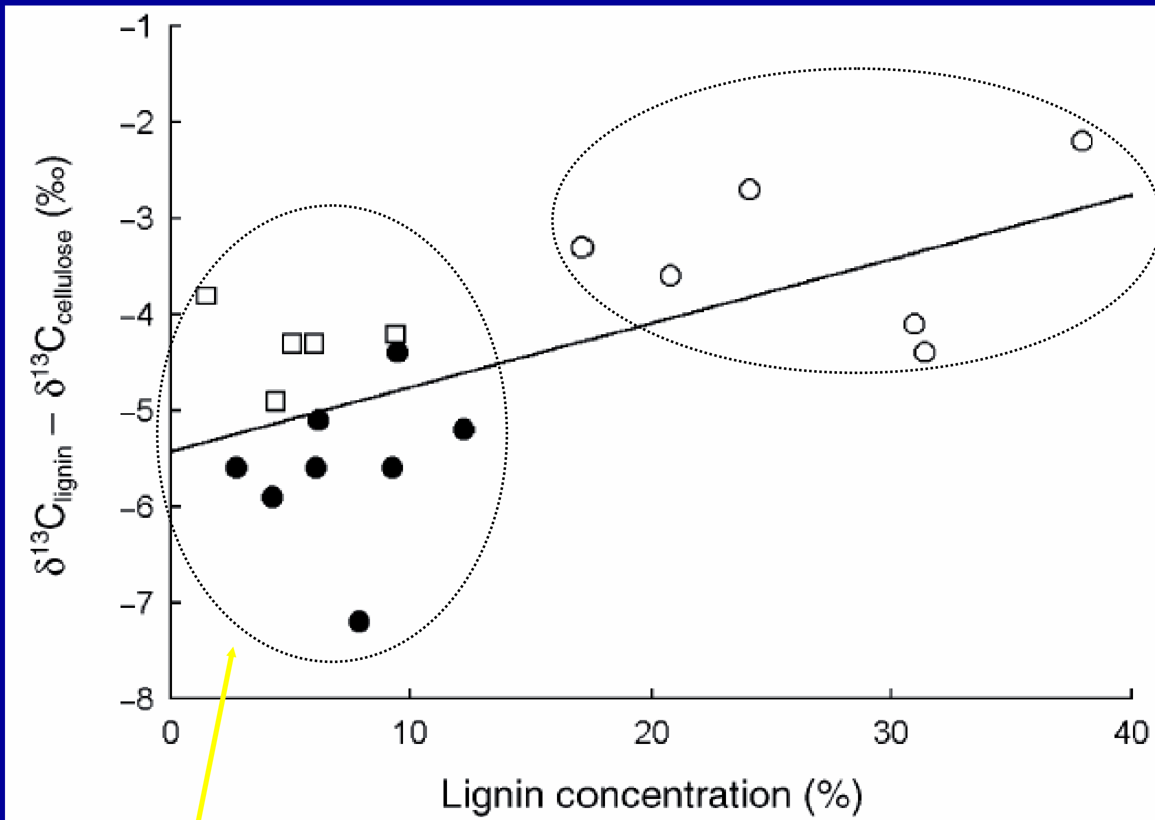
-30
-20
-10
0
10
$\delta^{13}C$ (‰)

Biogeochemical implications?

Explanation for the widely reported ^{13}C depletion of lignin relative to other major plant components (e.g. Benner et al., 1987; Schweizer et al., 1999; Fernandez et al., 2003; Hobbie and Werner, 2004)



Trees:

assuming a methoxyl content of 15-20% in lignin, 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.

Figure taken from Hobbie & Werner 2004

Leaves and grasses:

the much larger depletion observed in methoxyl carbon in leaves and grasses (in the range of −20 to −38‰) 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.

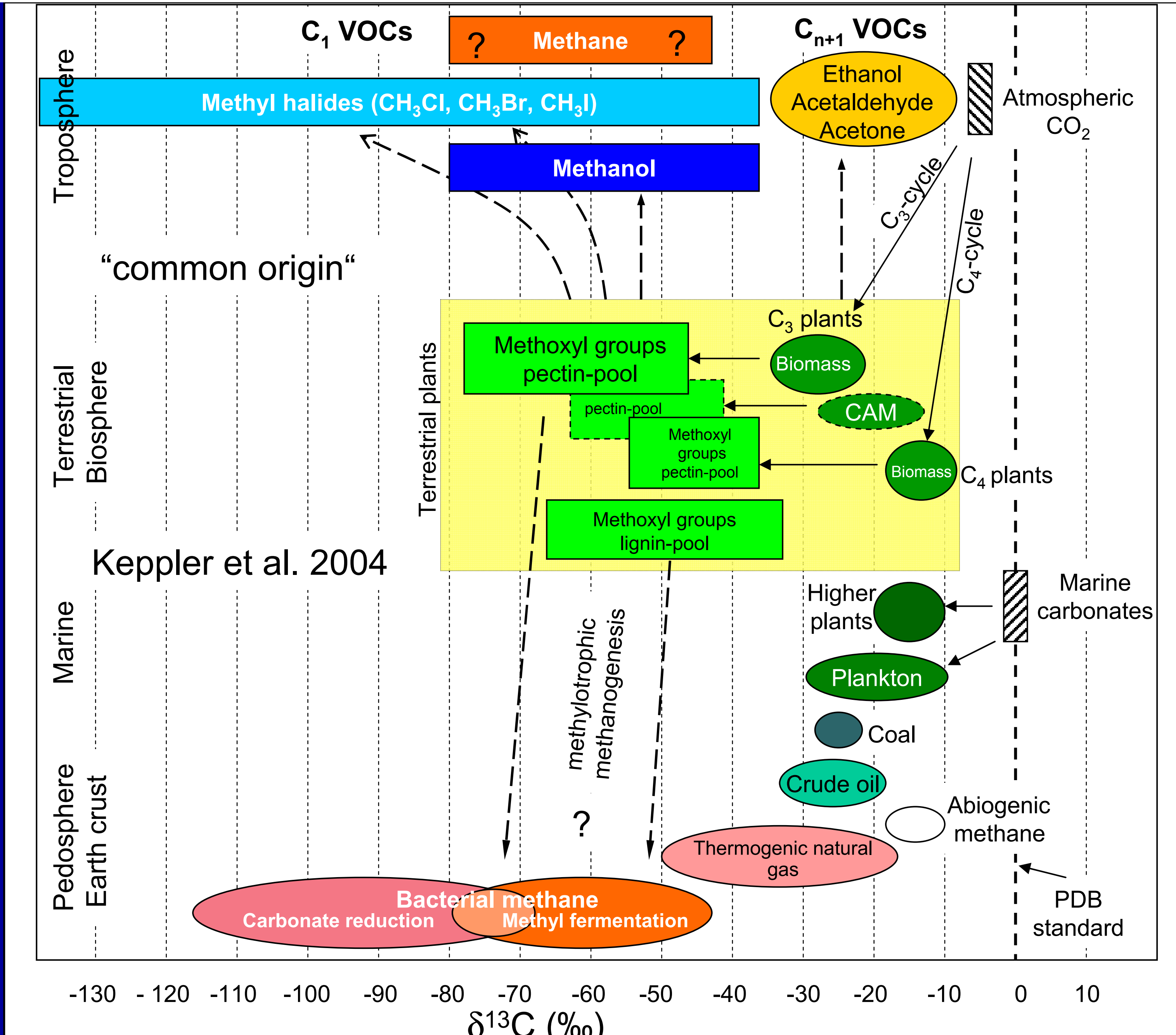
A common origin of C_1 volatiles!

Most biospheric C_1 volatiles such as
methanol,
chloromethane,
bromomethane,
and
possibly methane (?)

have a **common (parent) plant source!**

↓

'methoxyl pool'



C1 VOCs
? Methane ?
Cn+1 VOCs
Troposphere
Methyl halides (CH_3Cl, CH_3Br, CH_3I)
Ethanol
Acetaldehyde
Acetone
Atmospheric CO_2
Methanol
C_3-cycle
C_4-cycle
"common origin"
Terrestrial Biosphere
Terrestrial plants
C_3 plants
Methoxyl groups pectin-pool
Biomass
pectin-pool
CAM
Methoxyl groups pectin-pool
Biomass
C_4 plants
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Keppler et al. 2004
Marine
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Abiogenic methane
?
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0
10
$\delta^{13}C$ (‰)

Conclusion / Outlook

- The 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.

- Methoxyl groups could act as markers for biological activity in organic matter of terrestrial and extraterrestrial origin

(The striking depletion of $\delta^{13}C$ in methoxyl carbon consequent on the biochemistry of C1 metabolism in plants may well extend to many other organisms which utilise S-adenosylmethionine as a methyl donor in *O*-methyltransferase reactions)

"Acknowledgment"

David Harper

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Bob Kalin

Colin McRoberts

... and many helpful hands

Reminder:

Poster by

F. Keppler, D. Harper, T. Röckmann and J. Hamilton

entitled:

"New insight into the atmospheric chloromethane budget gained using stable carbon isotope ratios"

European Commission DG Research - Improving The Human Research Potential

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