Stable Carbon Isotope Geochemistry

Key Reading:

Suggested Reading:

Stable Carbon Isotopes

There are 2 stable isotopes of carbon:

<table>
<thead>
<tr>
<th>Isotope</th>
<th>Abundance</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^{12}$C</td>
<td>98.89 %</td>
</tr>
<tr>
<td>$^{13}$C</td>
<td>1.11 %</td>
</tr>
</tbody>
</table>

- Ratio $^{13}$C/$^{12}$C = $1.225 \times 10^{-2}$ (on average)
- However, this ratio varies slightly among different carbonaceous materials

Nomenclature:

\[ \delta^{13}C \text{ (permil, ‰)} = \left[ \frac{\text{[13C/12C]}_{std}/\text{[13C/12C]}_{std} - 1} {\text{[13C/12C]}_{std}} \right] \times 1000 \]

Standard reference materials:

<table>
<thead>
<tr>
<th>$^{13}$C/$^{12}$C</th>
<th>Symbol</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.123x10^{-2}</td>
<td>PDB</td>
</tr>
<tr>
<td>1.1218x10^{-2}</td>
<td>NBS-20</td>
</tr>
</tbody>
</table>
Processes Controlling Isotope Composition of Sedimentary Organic Matter

Production

Primary production
• - Photosynthesis - phytoplankton, higher plants, cyanobacteria.

Secondary production
• - Chemoautotrophy - sulfide oxidizers, methanogens.

Recycling

Aerobic recycling
• - Respiration - aerobic heterotrophic bacteria
• - Methane recapture - methanotrophs

Secondary production
• - Fermentation

Notation and nomenclature

\[ \delta - \text{Notation} \]

\[ \delta = 1 \text{‰} \]

\[ + \quad \text{enriched} \quad \text{heavier} \quad \text{higher} \quad \text{positive} \]

\[ - \quad \text{depleted} \quad \text{lighter} \quad \text{lower} \quad \text{negative} \]
Isotope fractionation effects

- An isotope effect (a physical phenomenon) leads to fractionation (an observable quantity)

**Fractionation factor:**
- By convention, the magnitude of the equilibrium isotope effect is expressed as a fractionation factor:
  - e.g. for: $^{13}{\text{CO}}_{2}(g) + H^{12}{\text{CO}}_{3}^{-}(aq) = ^{12}{\text{CO}}_{2} (g) + H^{13}{\text{CO}}_{3}^{-}(aq)$
- The fractionation factor, $\alpha$, is expressed as:
  $\alpha_{\text{HCO}_3^-/\text{CO}_2} = \frac{(^{13}\text{C}/^{12}\text{C})_{\text{HCO}_3^-}}{(^{13}\text{C}/^{12}\text{C})_{\text{CO}_2}}$

A related expression is the "difference fractionation factor"
$\epsilon \equiv \Delta^{13}\text{C} = \delta^{13}\text{C}_{\text{product}} - \delta^{13}\text{C}_{\text{reactant}}$

2 types of isotope effect:
- (i) Equilibrium isotope effects
- (ii) Kinetic isotope effects

Equilibrium isotope effects

1. Rule of thumb - the heavy isotope ($^{13}\text{C}$) is concentrated in the chemical compound in which it is bound most strongly.
2. The expression for the isotopic exchange reaction is written just as for any chemical reaction and the equilibrium constant ($K$) is determined in the same way.
   - e.g. for the reaction: $^{13}\text{CO}_2(g) + H^{12}\text{CO}_3^{-}(aq) = ^{12}\text{CO}_2 (g) + H^{13}\text{CO}_3^{-}(aq)$
   $K = \frac{[^{12}\text{CO}_2(g)][H^{13}\text{CO}_3^{-}(aq)]}{[^{13}\text{CO}_2(g)][H^{12}\text{CO}_3^{-}(aq)]}$

- The major equilibrium isotope system affecting organic carbon isotope compositions is the inorganic carbonate buffer system. At seawater pH:

<table>
<thead>
<tr>
<th>$\Delta^{13}\text{C}$</th>
<th>$\delta^{13}\text{C}_{\text{CO}_2}$</th>
<th>$\delta^{13}\text{C}_{\text{CO}_2}$</th>
<th>$\delta^{13}\text{C}_{\text{H}_2\text{CO}_3}$</th>
<th>$\delta^{13}\text{C}_{\text{HCO}_3^-}$</th>
<th>$\delta^{13}\text{C}_{\text{CO}_2}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>-7</td>
<td>-7</td>
<td>-6</td>
<td>+2</td>
<td>+1</td>
<td>-1.7</td>
</tr>
<tr>
<td>$\Delta^{13}\text{C}$</td>
<td>$\delta^{13}\text{C}_{\text{CO}_2}$</td>
<td>$\delta^{13}\text{C}_{\text{CO}_2}$</td>
<td>$\delta^{13}\text{C}_{\text{H}_2\text{CO}_3}$</td>
<td>$\delta^{13}\text{C}_{\text{HCO}_3^-}$</td>
<td>$\delta^{13}\text{C}_{\text{CO}_2}$</td>
</tr>
<tr>
<td></td>
<td>+1</td>
<td>+8</td>
<td>-1</td>
<td>-1.7</td>
<td>-0.5</td>
</tr>
</tbody>
</table>

- N.B. The major fractionation effect is the hydration of CO$_2$.
- (i.e. bicarbonate is enriched in $^{13}\text{C}$ relative to CO$_2$ in solution by ca. 8‰)

- In equilibrium isotope effects, the difference between the reactant and product depends only on temperature, and not the distribution of material between product and reactant.
- e.g., while relative abundances of CO$_2$(aq) and HCO$_3^-$ varies as a function of pH, isotope differences only vary with temperature.
Kinetic Isotope Effects

- Many reactions involving organic compounds result in kinetic isotope effects.
- The effect results from different rates of conversion of reactants with $^{13}$C and $^{12}$C to a product. Activation energy for light isotopic species is smaller, and thus in general the species with the lighter isotope will react faster.
- By convention the rate constant for the species with the light isotope is placed as the numerator and almost always the ratio is >1. This is called the standard (or "normal") isotope effect.
- Fractionation factors ($\Delta^{13}$C) can be determined as for equilibrium isotope effects.
- Two processes which give rise to kinetic isotope effects:
  - Transport processes
  - Chemical processes

Kinetic isotope effect terminology:
- Normal = Light isotopic species reacts more rapidly.
- Inverse = Heavy isotopic species reacts more rapidly.
- Primary = Isotopic substitution at a position to which a chemical bond is made or broken influences the reaction rate.
- Secondary = Isotopic substitution at a remote position influences the reaction rate.

Isotope Fractionation in Biological Processes

Single carbon substrates ($\text{CO}_2, \text{CH}_4$)
- Fixation of $\text{CO}_2$ by primary producers (photosynthesis)
- Fixation of $\text{CO}_2$ by chemoautotrophs (sulfide oxidisers, methanogens)
- Processing of intermediates in methanogenesis
- Assimilation of $\text{C}_1$ compounds by methylotrophs

Multi-carbon substrates
- Assimilation of organic molecules by heterotrophic bacteria
- Catabolic metabolism of consumers at all levels
- Biosynthesis in all organisms

Fig. 2. An overview of a cellular carbon budget and of pathways leading to biosynthetic products. Kinetic isotope effects are denoted by $\epsilon$ and are arbitrarily numbered to indicate their independence. Isotopic compositions are denoted by similarly numbered $\delta$ terms. The circled numbers indicate branch points.
Isotope Fractionation during Photosynthesis, $\varepsilon_p$

In photosynthesis $^{12}\text{CO}_2$ is preferentially taken up relative to $^{13}\text{CO}_2$. There are two stages when kinetic isotope effects can occur:

1. *Transport (diffusion) processes*
   - Gas phase diffusion (i.e. Atmospheric CO$_2 \rightarrow$ dissolved CO$_2$ in leaf)
     - Approx. fractionation factor: 4.4 ‰ (i.e., depletion = -4.4 ‰)
     - Only important for emergent (vascular) plants where air/leaf interaction occurs.
   - Liquid phase diffusion of CO$_2$ or HCO$_3^-$
     - Approx fractionation factor: 0.8 ‰ (relatively minor)

2. *Chemical (Enzymatic) processes*
   - Four pathways:
     i. C$_3$ (Calvin-Benson)
     ii. C$_4$ (Hatch-Slack)
     iii. CAM
     iv. Bacterial

(i) The C$_3$ (Calvin-Benson) pathway

- Most common for terrestrial (vascular) plants and phytoplankton (also cyanobacteria).
- All trees use C$_3$ pathway

*Characteristics:*
- optimum growth temperature: 20-35°C
- CO$_2$ compensation point: 0.004%
- light saturation 3,000 ft.cdl
- max. photosynthetic rate: slow
- enzyme: Ribulose-1,5-biphosphate (RuBP) carboxylase-oxygenase (*RUBISCO*)
  - $\Delta^{13}\text{CO}_2$: -23 to -41 ‰
  - ave. $-27^\circ$ ‰ for land plants
  - ave. $-25^\circ$ ‰ for unicellular phytoplankton

*This difference reflects either differences in carbon transport/fixation mechanisms (see below) or different isotope effect for RUBISCO between emergent and aquatic plants.*

Overall reaction:

$$6\text{CO}_2 + 12\text{NADPH} + 18\text{ATP} \rightarrow C_6\text{H}_12\text{O}_6 + 12\text{NADP}^+ + 18\text{ADP}$$

(energy and reduction power come from h$\nu$ splitting of H$_2$O mediated by chlorophyll)
Carbon isotopic fractionation during C₃ photosynthesis

Model describing the isotopic fractionation, $\Delta$, in C₃ plants:

$$\Delta = a + \left(\frac{c_i}{c_a}\right)(b - a)$$

where

- $a$ is the isotope effect associated with diffusion of CO₂ into the plant (~ 0.8 ‰)
- $b$ is the fractionation associated with carboxylation (by RUBISCO enzyme)
- $c_i/c_a$ is the concentration ratio of CO₂ internal to CO₂ external.
- When $c_i/c_a = 1$ (i.e. unlimited CO₂) max RUBISCO fractionation, $b$ expressed.
- When $c_i/c_a << 1$ (i.e. limited CO₂) diffusion limited, and only $a$ expressed.

Formation of 2 molecules of 3-phosphoglycerate from ribulose 1,5-bisphosphate

(ii) The C₄ (Hatch-Slack) pathway

- Less common for vascular plants
- Exceptions: sugar cane, corn, bamboo (typical of plants in hot arid climates)
- Tropical grasses, desert plants, salt marsh plants.
- The adaptation allows maximum CO₂ fixation per unit loss of water.

Characteristics:

- optimum growth temp.: 35°C
- CO₂ compensation point: 0.0004% (1/100th of today’s atmosphere)
- light saturation: 10,000 ft cdl.
- max. photosynthetic rate: fast
- enzyme: phosphoenyl pyruvate (PEP) carboxylase
  - N.B. no competing oxygenase activity
  - $\Delta^{13}$CO₂: -0.5 to -3.6 ‰ (ave. -2.2 ‰).
(ii) The C₄ (Hatch-Slack) pathway

Plants using the C₄ pathways utilize PEP carboxylase for the first committed step in CO₂ fixation. The CO₂ fixed by PEP is carried as part of a C₄ acid from the mesophyll into the internal bundle sheath cells, where CO₂ is released again. The bundle sheath approximates a closed system, so most of CO₂ entering cell is fixed by RUBISCO to organic matter (minimal leakage) and internal CO₂ concentrations can be very high (100x atm.). Thus little isotopic fractionation is expressed in this step.

The smaller (ca. 2 ‰) isotope effect associated with the PEP carboxylase enzyme gives C₄ plants more positive δ¹³C values (-8 to -18 ‰).

Environmental Conditions favoring C₄ photosynthesis
Carbon isotopic fractionation during C₄ photosynthesis

Model describing the isotopic fractionation in C₄ plants:

\[ \Delta = a + (b_4 + b_3 \phi - a) \times c/c_a \]

where:
• \( a \) is the isotope effect associated with diffusion of CO₂ into the plant
• \( b_4 \) is the isotopic effect with CO₂ diffusion in bundle sheath cells,
• \( b_3 \) is the fractionation associated with carboxylation (by PEP enzyme)
• \( \phi \) is the leakiness of the plant to CO₂
• \( c/c_a \) is the concentration ratio of CO₂ internal to CO₂ external.

N.B. Recently it has been shown that some algae (diatoms) can use a C₄-like pathway (Reinfelder et al., 2000, Nature 407, 996-999).

(iii) The CAM (crassulacean acid metabolism) pathway

• Used by succulents – cacti, crassulaceae
• Similar to C₄ pathway, but different spatial and temporal packing.
• Plants using this pathway have intermediate isotopic compositions between C₃ and C₄.
• Isotopically speaking, marine algae also generally fall between C₃ and C₄.
• CAM plants open their stomata at night (when temp and water loss is lowest) and produce malate (via PEP).
• The malate is then processed further during the day
• There is higher diffusive loss of CO₂ from CAM plants
(iv) Anoxygenic bacterial photosynthesis

- Doesn’t produce $O_2$; $H_2$ taken from $H_2S$ instead of $H_2O$:

$$CO_2 + 2H_2S \rightarrow CH_2O + S_2 + H_2O$$

- Some bacteria (e.g., purple photosynthetic bacteria) use RuDP pathway to fix $CO_2$ whereas others use an inversion of the Acetyl-CoA decarboxylation reaction - “reverse TCA cycle” (green photosynthetic bacteria).
- The latter is important in Black Sea (see later lecture) - green sulfur bacteria (Chlorobiaceae) - anaerobic photoautotrophs.

$$\text{Acetyl-CoA} + CO_2 \xrightarrow{\text{feridoxin}} \text{pyruvate} + \text{CoA}$$

- Fractionation factors range from -10 to -30 ‰

Isotopic fractionation in aquatic photoautotrophs

- Very complex, and not fully understood. This is because they may use more than one carbon fixation path, and more than one source of inorganic carbon.
- In general as $[CO_2]_{sea}$ decreases (due to high algal densities, elevated temps, fall in $[CO_2]_{atm}$ or increased pH) a shift toward heavier algal carbon is observed.
- Isotopic fractionation in aquatic plants is more complex. Because $CO_2$ diffuses more slowly in water than air, diffusion is often the limiting step.
- Most aquatic plants have some membrane-bound mechanism that actively transports dissolved inorganic carbon (DIC) into the photosynthesizing cells.
- If DIC ($CO_2$ and $HCO_3^-$) concentrations are low, plants can “pump” DIC into cell.
- Plants grown at high DIC conc (5%) exhibit similar $\delta^{13}C$ values to $C_3$ vascular plants.
- Plants grown at low DIC conc (0.03%) exhibit only a 5 ‰ fractionation.
- Model describing the isotopic fractionation in aquatic plants:

$$\Delta = d + b_3 \times (F_3/F_1)$$

- Where:
  - $d$ is the equilibrium isotope effect between $CO_2$ and $HCO_3^-$
  - $b_3$ is the isotopic fractionation associated with carboxylation (by RUBISCO)
  - $F_3/F_1$ is the ratio of $CO_2$ leaking out of the cell to the amount inside the cell.
Active transport (pumping) of DIC into algal cells

Figure 6. Model for dissolved inorganic carbon transport.
Summary of pathways of photosynthetic carbon fixation

In spite of differences in pathways by which carbon is supplied to biosynthetic reaction networks, sources of metabolic intermediates are similar for each system whereby carbohydrates are synthesized by the Calvin Cycle.

Organisms in the lower 2 groups, however, fix carbon at multiple reaction sites, resulting in large isotopic contrasts (both intermolecular and intramolecular).
Isotopic fractionation in aquatic photoautotrophs

Other Considerations:

$\delta^{13}C$ of inorganic carbon source

- In general the $\delta^{13}C$ of a photosynthetic organism will be the sum of the $\delta^{13}C$ of the inorganic source and the $\Delta^{13}C$ of the enzymatic fractionation:

$$\delta^{13}C_{\text{organism}} = \delta^{13}C_{\text{DIC}} + \Delta^{13}C_{\text{enzyme}}$$

- For marine phytoplankton the effects of temperature on the marine DIC pool and on the enzymatic system used to fix CO$_2$ are difficult to separate and isotopic trends with temperature are complex.

Influence of heterotrophic activity on isotope composition

- In general, the following assumption can be made (Hayes et al., 1990):
  - For multicarbon substrates, chemical reactions will not have a large effect on the molecular-average isotope compositions.
  - A grazing organism that ingests particles does not discriminate on the basis of isotope composition. Consequently the isotopic composition of a given particle type should be no different from the starting material(s).
  - The isotopic composition of a heterotroph can vary from that of its carbon source.
    - e.g. for respiratory processes:
      $$\text{CH}_2\text{O} + \text{oxidant (O}_2\text{)} \rightarrow \text{CO}_2 + \text{C in biomass (new tissue)} + \text{reduction product (H}_2\text{O)}$$
  - The isotopic difference between biomass and respired carbon depends on fractionation during metabolism.
The isotopic fractionation during metabolism can be summarized as:

\[ \delta_i = (1 - f_b) \delta_d + f_b \times \delta_b \]

where:
- \( i \) = input carbon
- \( d \) = respired CO2
- \( b \) = biomass
- \( f_b \) = fraction of input carbon converted to biomass ("conversion efficiency")

Before a carbon atom can be lost a carbon-carbon bond must be broken (in most cases):
- \(^{12}\text{C}\) is lost more readily than \(^{13}\text{C}\) (i.e., respired CO2 is \(^{13}\text{C}\)-depleted).
- Therefore carbon retained as biomass is enriched in \(^{13}\text{C}\) relative to that respired.
- The isotope difference is typically 1 to 1.5 ‰ for organisms with low conversion efficiencies (\( f_b = 0.5 - 0.6 \)).
- Water dwelling invertebrates and protozoans have high conversion efficiencies
- Average isotopic shifts per trophic level are expected to be less than 1.5 ‰.
- Fermentative bacteria use biochemical processes that are markedly different from those in respiring heterotrophs. In general, the isotopic characteristics of these processes are poorly known, but have the potential for significant fractionations.

Isotope relationships between animals (and microbes) and diet

\(^{13}\text{C}\) enrichment in marine ecosystems
Stable isotopes as indicators of carbon flow in ecosystems

- Since there is generally considered to be minimal fractionation associated with aerobic heterotrophy, the potential exists to use isotopes as dietary indicators within food webs.
  - "You are what you eat, plus 1‰"
  - Isotope values "integrate" the diet
  - A number of plant sources can be distinguished

Potential problems:
- Individual variability in δ¹³C averages 1 to 2‰ (masks assimilation effect)
- Results are often tissue or biochemical dependent.

But - a molecule passed on with an intact carbon skeleton should retain its original carbon isotopic composition*.
- *Important for molecular isotopic biogeochemistry since "surviving" molecules are what we study

Stable Carbon Isotopes as Source Indicators

- Isotope composition of original plant material is almost unaffected by diagenetic alteration
- Most of the detritus in sediments are plant remains (phytoplankton, vascular plants)
- Much effort has been devoted to distinguishing contributions from these two sources based on δ¹³C values.
- Problems: most problems associated with accurately assigning end member compositions
- Reasons:
  - End-members are unknown
  - End-members although known are temporally variable
  - There are more than 2 end-members

River-ocean transects

Isotope mixing models

A. 2 sources (50:50)
B. 2 sources (30:70), but δ value of one source modified by metabolic processes.
C. 3 potential sources
Carbon Isotopic differences between biochemicals

The immediate product of photosynthesis is glucose. However the metabolic conversion of glucose to other biochemicals often involves isotopic fractionations.

\[ \delta_{\text{biomass}} = f_{\text{NA}} \cdot \delta_{\text{NA}} + f_{\text{Prot}} \cdot \delta_{\text{Prot}} + f_{\text{PS}} \cdot \delta_{\text{PS}} + f_{\text{Lipid}} \cdot \delta_{\text{Lipid}} \]

- where \( f \) = mole fraction as C

- In general: \( \delta_{\text{PS}} > \delta_{\text{NA}} = \delta_{\text{Prot}} > \delta_{\text{Lipid}} \)

\[ \text{Increasingly } ^{13}\text{C depleted} \]

Isotopic variations in vascular plant leaf biochemicals

Fig. 2: Average \( \delta^{13}\text{C} \) (‰ vs PDB) values for total tissue (1), total surface lipid extracts (2) and weighted mean n-alkanes (3) for each of the three plant groups (C₄, CAM and C₃). Error bars represent the standard deviation of the measurements for each fraction for the given plant group.
Depletion of \( ^{13}\text{C} \) in lipids relative to marine algal biomass as a function of cellular composition

Components sum to yield biomass \((X_C = \text{mole fraction})\):

\[
X_{\text{Chla}} + X_{\text{Prot}} + X_{\text{Sacc}} + X_{\text{lip}} = 1; \quad X_{\text{Prot}}/X_{\text{Chla}} = 8.6
\]

Isotopic mass balance:

\[
X_{\text{Chla}}\delta_{\text{Chla}} + X_{\text{Prot}}\delta_{\text{Prot}} + X_{\text{Sacc}}\delta_{\text{Sacc}} + X_{\text{lip}}\delta_{\text{lip}} = \delta_{\text{biomass}}
\]

\[
\delta_{\text{Chla}} = \delta_{\text{Prot}}, \quad \delta_{\text{Prot}} - \delta_{\text{Sacc}} = -1\%_0, \quad \delta_{\text{lip}} - \delta_{\text{Sacc}} = -6\%_0
\]

Differences in \( \delta^{13}\text{C} \) between individual biochemicals

"Compound-Specific Isotope Analysis" (CSIA)

Pioneering work by Abelson and Hoering (1961) on amino acids.

- Isotopic differences among individual amino acids in algae and bacteria have been observed (Macko et al., 1987)
- Isotopic differences among different lipid and pigment molecules have now been reported (Hayes et al., 1987; Freeman et al., 1990, Schouten et al., 1998).
- Isotopic relationships dictated by biosynthetic pathways (starting substrates and number of branching points in pathway).

- For lipids:
  - Acetogenic lipids (based on acetate units)
  - Isoprenoid lipids
    - Mevalonic-acid pathway
    - Methylerythritol phosphate pathway (only recently recognized).
Carbon Isotopic distributions within molecules

- Glucose is considered to be isotopically homogeneous
- Lipids exhibit sawtooth $\delta^{13}C$ distributions down linear carbon chains with the carboxyl-derived carbons from the acetyl-coA being about 6‰ lighter than the methyl-derived carbons.
- Monson and Hayes (1982) demonstrated alternating isotope pattern and related it to
  - (a) overall depletion of $^{13}C$ in fatty acid lipid fraction and
  - (b) the biochemical pathway of fatty acid formation.

Pathways of lipid biosynthesis in eukaryotic organisms

Complexity is introduced by presence of subcellular organelles. Since acetate cannot pass through mitochondrial wall, C$_2$ units produced there by decarboxylation of pyruvate are packaged as C$_6$ species (citrate) for export. Formation and subsequent conversion of C$_6$ to C$_2$ units provides possibilities for isotopic fractionation during biosynthesis of lipids.
Pathways of lipid biosynthesis in prokaryotic organisms

Pathways of isoprenoid synthesis: Isotopic implications

*aka Deoxyxylulose pathway
Synthesis of cyclic isoprenoids (triterpenoids)

- Tetracyclic triterpenoids (steroids)
- Pentacyclic triterpenoids (e.g., hopanoids)

Isotopic distribution within isoprenoids

- Synthesis via MVA pathway
- Synthesis via MEP pathway
Factors bearing on isotopic fractionation in aquatic photoautotrophs

<table>
<thead>
<tr>
<th>Organisms</th>
<th>Isoprenoid biosynthesis</th>
<th>Oxidative pentose phosphate pathway in plastid?</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C_{20}, C_{40}</td>
<td>C_{15}, C_{30}</td>
</tr>
<tr>
<td>“Normal”</td>
<td>MEP</td>
<td>MVA</td>
</tr>
<tr>
<td>Green Algae</td>
<td>MEP</td>
<td>MEP</td>
</tr>
<tr>
<td>Euglenoids</td>
<td>MVA</td>
<td>MVA</td>
</tr>
<tr>
<td>Cyanobacteria</td>
<td>MEP</td>
<td>MEP</td>
</tr>
</tbody>
</table>

C_{20} and C_{40} generally plastidic (formed in chloroplast)
C_{15} and C_{30} commonly cytosolic
MEP = methylerythritol-phosphate pathway
MVA = mevalonic acid pathway

Depletion of $^{13}$C in isoprenoid lipids produced by eukaryotic algae

Schulze et al. (1998), Riegler et al. (1997), Pepp et al. (1998), Rebesell et al. (2003)
Stable carbon isotopic composition in *T. minimum* (freshwater green alga)

Continuous cultures

Batch cultures