All fats are not equal: Considerations when using fatty acid biomarkers in compound-specific stable isotope (CSSI) soil and sediment tracing

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Introduction

Atmospheric CO_2 is the source of C for plants; photosynthesis fixes CO_2, producing organic molecules, a process which discriminates against the heavier ^{13}C isotope over the ^{12}C isotope. Plant species, type, environment, etc. determine the relative incorporation rate of the isotopes, leading to traceable biomarkers i.e. organic molecules with a unique ^{13}C:^{12}C signature.

Deposition of unique biomarkers onto soil from site A e.g. grass field

Deposition of unique biomarkers onto soil from site B e.g. maize crops

Mobilization of biomarkers A from field to river

Mobilization of biomarkers B from field to river

Sediments containing biomarkers from sites A & B mix in the river and are transported and deposited downstream

Statistical analysis determines the contribution of ^{13}C and ^{12}C by each biomarker in the source soils to the panel of biomarkers found in the downstream sediment; percent contribution of site A vs. site B to the total isotopic signature is determined

Various factors contribute to the sensitivity of the biomarkers; some are discussed here.

Natural Variability

Environmental factors may affect $^{13}C_{bulk}$ values between and within species. Less discrimination (more positive $\delta^{13}C$ values) occur due to:

- Water use efficiency (WUE); less water, stomata (see Fig. 1) close to minimize transpiration
- Stomatal conductance (SC); stomata close, less CO_2 flow into the cell
- Plant type; conifers show greater WUE than deciduous trees
- Lower nitrogen (nutrient) availability
- Decrease in temperature; SC is increased
- Branch length; longer branches lead to poorer hydraulic conductance

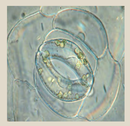
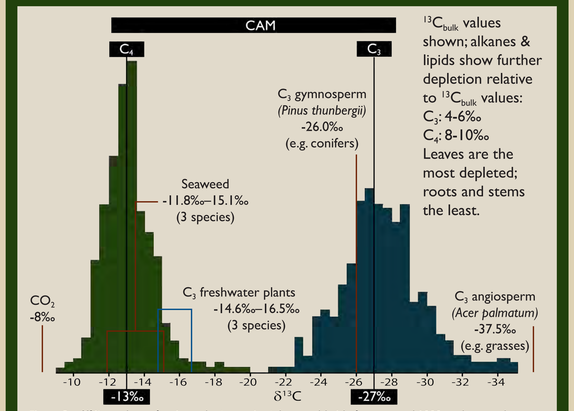


Figure 1. Open stoma found in leaves; CO_2 conductance increases when stomata are open.

Higher rates of photosynthesis increase discrimination:

- As stomatal conductance increases
- During growth; photosynthetic rates increase
- In sunlit versus shaded areas
- Due to the location of foliage in the crown



$^{13}C_{bulk}$ values shown; alkanes & lipids show further depletion relative to $^{13}C_{bulk}$ values: C_3: 4-6‰ C_4: 8-10‰ Leaves are the most depleted; roots and stems the least.

Figure 2. $\delta^{13}C_{bulk}$ values of various plants species taken worldwide from around 1000 analyses and are relative to the V-PDB standard; histogram adapted from O'Leary and Glaser. Species shown are based on $^{13}C_{bulk}$ analysis of plants as reported by Chikaraishi and Naraoka; atmospheric CO_2 shown as a reference.

Significant differences in the ^{13}C:^{12}C ratio occur between C_3 (e.g. conifers) and C_4 (e.g. maize) plant types as a consequence of their respective photosynthetic pathways; C_4 plants show enrichment in ^{13}C (see Fig. 2 for sample bulk/total C ($^{13}C_{bulk}$) depletion values).

Other factors include: light quality; human activity and CO_2 production; microorganism respiration; relative humidity; altitude and location in the hemisphere; air circulation; salinity; and increasing soil strength.

Biomarker Selection

Suitable biomarkers must be recalcitrant, provide a unique isotopic signature, be subject to transport, and be relatively easy to isolate and detect. Soil movement generally occurs at the surface, with the exception of bank erosion; penetration into the soil is also a factor.

Very long chain fatty acids (VLCFAs) and their derivatives are suitable biomarkers. The pathway for the synthesis of C_{16}-C_{18} fatty acids (FAs) from sugar, a product of photosynthesis, is identical in plants and are poor biomarkers (Fig. 3 inset); chain elongation to VLCFAs produces unique biomarkers, including VLCFA derivatives (Fig. 3).

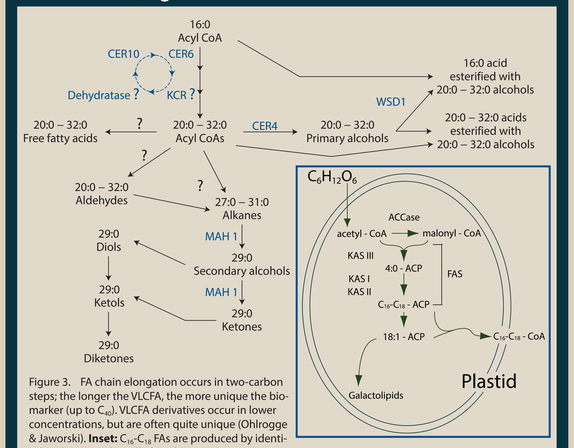


Figure 3. FA chain elongation occurs in two-carbon steps; the longer the VLCFA, the more unique the biomarker (up to C_{36}). VLCFA derivatives occur in lower concentrations, but are often quite unique (Ohlrogge & Jaworski). **Inset:** C_{16}-C_{18} FAs are produced by identical pathways in the cell plastid (Jetter & Kunst).

C_{16}-C_{18} FAs are unreliable biomarkers because of:

- Low biological istopic variability within plant types
- Deeper pentration into the soil than VLCFAs
- Commonality with other organisms
- Re-synthesis by soil microorganisms
- Contributions by suberin i.e. fine root material

Analytical Factors

An advantage of CSSI over quantification techniques is that ^{13}C:^{12}C ratios are simply evaluated; as long as a signal is present and not identical to another sample, the FA may be used as a tracer. The purity of the extract, however, is extremely important even though relative abundance is not.

In CSSI work, analyses are performed by GC-C-IRMS (gas chromatography-combustion-isotope ratio mass spectrometer). A chromatograph of a purified soil extract (blue) and some of the impurities removed (red) is shown in Fig. 4. Purification is essential in minimizing the contribution of unknown compounds to the ^{13}C:^{12}C signal, which leads to increased, undesirable variability when unmixing the signals.

Figure 4. Blue: Purified VLCFAs. Red: Portion of the signal removed by purification. Pressurized solvent extraction on ~55 g of organic-poor soil yielded approximatetely ~10 mg of total extract.

Reliability is improved through: correct GC column selection and injection techniques; use of correct solvents for extraction and purification (e.g. differing VLCFAs do not exhibit identical solubilities in certain solvents, which leads to reduced signal and more interference by impurities), adequate extraction quantities for detection purposes, and methylation techniques (free FAs are ideally converted to esters for analysis on most GC columns; esterification to a methyl ester is common). Some methylation procedures may lead to a loss of signal (Fig. 5).

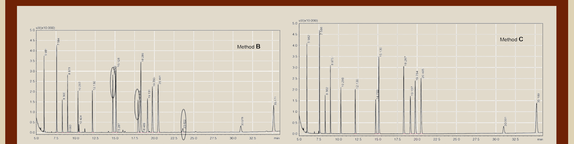


Figure 5. Xu *et al.* examined the methylation of a FA standard using a common methylation technique: methanolic HCl; for example, Method B led to additional peaks and loss in other peaks, whereas Method C matched expected values from the standard used (GC-FID used for purification and detection).

Current Research

Research is currently being carried out in Canada in the Horsefly River (BC) and South Tobacco Creek (MB) watersheds. The objective is to maximize sensitivity using CSSIs in a temperate climate where variability in vegetation types is low, and to identify methods which may lead to increasing realiability, practicality and standardization. A detailed sampling regime has been established. For more information and references, scan the QR code or visit:

web.unbc.ca/~dreiffar/research.html