Mass Spectrometry of Soils

edited by

Thomas W. Boutton
Texas A&M University
College Station, Texas

Shin-ichi Yamasaki
National Institute of Agro-Environmental Sciences
Tsukuba, Ibaraki, Japan

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Stable Carbon Isotope Ratios of Soil Organic Matter and Their Use as Indicators of Vegetation and Climate Change

THOMAS W. BOUTTON Texas A&M University, College Station, Texas

1. INTRODUCTION

Stable isotope ratios of elements in both organic and inorganic components of nature often record and integrate information relating to (1) the kinds of processes that formed those components, (2) the rates of those processes, and (3) the environmental conditions prevailing at the time those components were formed. As a result, a wealth of environmental and ecological information can often be obtained from stable isotope analyses [1-10].

Because carbon plays a central role in the structure and function of the earth-atmosphere-biosphere system, considerable emphasis has been placed on understanding the isotopic biogeochemistry of this element. A summary of the natural distribution of stable carbon isotopes ($^{13}$C/$^{12}$C) in different components of the environment (Fig. 1) reveals substantial variation in the ratio of these two isotopes. This natural variation is extremely useful in tracing and quantifying sources, sinks, and flux rates within the biogeochemical carbon cycle.

With respect to the plant-soil system, the natural stable carbon isotope ratio ($^{13}$C/$^{12}$C) of soil organic carbon contains information regarding the presence or absence of plant species with the C3 (low $^{13}$C/$^{12}$C) and C4 (high $^{13}$C/$^{12}$C) pathways of photosynthesis in past plant communities and their relative contribution to community net primary productivity through time. Because these different photosynthetic pathway types are physiologically and ecologically distinctive, any changes in relative C3-C4 importance imply alteration of both ecosystem structure and function. Changes in relative C3-C4 productivity through time are often attributable to changes in disturbance regime and/or climate. Thus, isotopic measurements of soil organic carbon have been utilized to document the effects of land use practices on...
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II. STABLE CARBON ISOTOPES: BACKGROUND AND METHODOLOGY

Carbon has two naturally occurring stable isotopes, $^{12}$C and $^{13}$C. Approximately 98.89% of all carbon in nature is $^{12}$C, and 1.11% of all carbon is $^{13}$C. The ratio of these two stable isotopes ($^{13}$C/$^{12}$C) in natural materials varies slightly around these average values as a result of isotopic fractionation during physical, chemical, and biological processes. This carbon isotope variation is relatively small in vegetation and soil organic matter, with the most enriched materials (those highest in $^{13}$C) differing from the least enriched (those lowest in $^{13}$C) by approximately 2% or 20 parts per thousand.

To utilize these small isotopic variations, $^{13}$C/$^{12}$C ratios of soil organic matter must be measured with high precision. These measurements are usually made with a gas isotope ratio mass spectrometer equipped with a mass beam collector. Briefly, organic matter is quantitatively converted to CO$_2$ by combustion at ~900°C in an O$_2$ atmosphere, the CO$_2$ is isolated and purified by cryogenic distillation or gas chromatography, and the isotopic composition of the CO$_2$ is determined by simultaneous collection of masses 44 ($^{12}$CO$_2$), 45 ($^{13}$CO$_2$), and 46 ($^{14}$CO$_2$) in the mass spectrometer. The isotopic composition of CO$_2$ from the sample is compared to that derived from a standard with a known $^{13}$C/$^{12}$C ratio. Details of sample preparation procedures and gas isotope ratio mass spectrometry have been reviewed recently [23–27] and will not be addressed further.

Stable carbon isotope ratios are usually expressed in delta notation as

$$
\delta^{13}C = \left( \frac{R_{\text{sample}} - R_{\text{standard}}}{R_{\text{standard}}} \right) \times 1000
$$

where $\delta^{13}C$ is the difference between the $^{13}$C/$^{12}$C ratio of the sample and that of the standard, and $R$ is the mass 45/44 ratio of the sample or standard gas [28,29]. Although the $\delta^{13}C$ value is a ratio of ratios and is dimensionless, it is customarily assigned units of parts per thousand (or per mil, ‰) because it is multiplied by 1000. Correction factors are usually applied to the $\delta^{13}C$ values to account for the

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Figure 1 Stable carbon isotope ratios ($\delta^{13}C$) of the major components of terrestrial, marine, and freshwater ecosystems. Data are generalized and exceptions may be noted within specific ecosystems. (From Ref. 11, with permission from Academic Press.)
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1. Fractionation due to photobiontic pathways: As a result of the biochemical properties of the primary photosynthetic pathways, the δ13C values of S1, S2, and S3 are expected to be -15 to -25‰.

2. Fractionation due to the soil organic carbon pool: The δ13C values of S1, S2, and S3 are expected to be -20 to -30‰.

3. Fractionation due to the existence of different photosynthetic pathways: The δ13C values of S1, S2, and S3 are expected to be -10 to -20‰.

4. Fractionation due to the incorporation of carbon derived from organic matter: The δ13C values of S1, S2, and S3 are expected to be -30 to -40‰.

5. Fractionation due to the use of atmospheric CO2: The δ13C values of S1, S2, and S3 are expected to be -10 to -20‰.

The δ13C values of HCO3- and CO32- are expected to be -15 to -25‰.

The δ13C values of CaCO3 and MgCO3 are expected to be -5 to 0‰.

The δ13C values of H2CO3 and CO2 are expected to be -10 to -20‰.

The δ13C values of C3 plants are expected to be -10 to -20‰.

The δ13C values of C4 plants are expected to be -15 to -25‰.

The δ13C values of CH4 are expected to be -50 to -60‰.

The δ13C values of CO2 are expected to be -10 to -20‰.

The δ13C values of N2O are expected to be -30 to -40‰.

The δ13C values of H2O are expected to be -10 to -20‰.

The δ13C values of H2O2 are expected to be -20 to -30‰.

The δ13C values of H2O2 are expected to be -30 to -40‰.

The δ13C values of H2O2 are expected to be -40 to -50‰.

The δ13C values of H2O2 are expected to be -50 to -60‰.

The δ13C values of H2O2 are expected to be -60 to -70‰.

The δ13C values of H2O2 are expected to be -70 to -80‰.

The δ13C values of H2O2 are expected to be -80 to -90‰.

The δ13C values of H2O2 are expected to be -90 to -100‰.

The δ13C values of H2O2 are expected to be -100 to -110‰.

The δ13C values of H2O2 are expected to be -110 to -120‰.

The δ13C values of H2O2 are expected to be -120 to -130‰.

The δ13C values of H2O2 are expected to be -130 to -140‰.

The δ13C values of H2O2 are expected to be -140 to -150‰.

The δ13C values of H2O2 are expected to be -150 to -160‰.

The δ13C values of H2O2 are expected to be -160 to -170‰.

The δ13C values of H2O2 are expected to be -170 to -180‰.

The δ13C values of H2O2 are expected to be -180 to -190‰.

The δ13C values of H2O2 are expected to be -190 to -200‰.

The δ13C values of H2O2 are expected to be -200 to -210‰.

The δ13C values of H2O2 are expected to be -210 to -220‰.

The δ13C values of H2O2 are expected to be -220 to -230‰.

The δ13C values of H2O2 are expected to be -230 to -240‰.

The δ13C values of H2O2 are expected to be -240 to -250‰.

The δ13C values of H2O2 are expected to be -250 to -260‰.

The δ13C values of H2O2 are expected to be -260 to -270‰.

The δ13C values of H2O2 are expected to be -270 to -280‰.

The δ13C values of H2O2 are expected to be -280 to -290‰.

The δ13C values of H2O2 are expected to be -290 to -300‰.

The δ13C values of H2O2 are expected to be -300 to -310‰.

The δ13C values of H2O2 are expected to be -310 to -320‰.

The δ13C values of H2O2 are expected to be -320 to -330‰.

The δ13C values of H2O2 are expected to be -330 to -340‰.

The δ13C values of H2O2 are expected to be -340 to -350‰.

The δ13C values of H2O2 are expected to be -350 to -360‰.

The δ13C values of H2O2 are expected to be -360 to -370‰.

The δ13C values of H2O2 are expected to be -370 to -380‰.

The δ13C values of H2O2 are expected to be -380 to -390‰.

The δ13C values of H2O2 are expected to be -390 to -400‰.

The δ13C values of H2O2 are expected to be -400 to -410‰.

The δ13C values of H2O2 are expected to be -410 to -420‰.

The δ13C values of H2O2 are expected to be -420 to -430‰.

The δ13C values of H2O2 are expected to be -430 to -440‰.

The δ13C values of H2O2 are expected to be -440 to -450‰.

The δ13C values of H2O2 are expected to be -450 to -460‰.

The δ13C values of H2O2 are expected to be -460 to -470‰.

The δ13C values of H2O2 are expected to be -470 to -480‰.

The δ13C values of H2O2 are expected to be -480 to -490‰.

The δ13C values of H2O2 are expected to be -490 to -500‰.
Table 1: List of Some Environmental and Biological Factors That Have the Potential to Influence $^{13}C$/$^{12}C$ of $C_4$ and $C_3$ Plants Though Their Effects on $p_c$.

<table>
<thead>
<tr>
<th>Environmental Biological</th>
<th>Factor</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2: Effect of Water Availability on $\Delta$ Values of Phaseolus vulgaris and Phaseolus acutifolius Grown in a Common Garden

$$\Delta \, (\%)$$

<table>
<thead>
<tr>
<th></th>
<th>HW</th>
<th>LW</th>
<th>Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Both species$^a$</td>
<td>18.9 (0.1)</td>
<td>18.1 (0.2)</td>
<td>0.8</td>
</tr>
<tr>
<td>Phaseolus vulgaris$^a$</td>
<td>19.5 (0.1)</td>
<td>18.9 (0.2)</td>
<td>0.6</td>
</tr>
<tr>
<td>Phaseolus acutifolius$^a$</td>
<td>18.4 (0.1)</td>
<td>17.3 (0.2)</td>
<td>1.1</td>
</tr>
</tbody>
</table>

Note: Plants in HW were drip irrigated daily, whereas those in LW received no supplemental water. Numbers in parentheses are standard errors. Differences between species ($p < 0.01$) and water treatment ($p < 0.05$) were significant, and there was a significant species $\times$ water interaction ($p < 0.05$).

$^a$ $n = 48$.

Source: Ref. 38.

$P.$ acutifolius in response to water limitation. These results illustrate the importance of genetic variation and genotype $\times$ environment interactions in determining carbon isotope discrimination by $C_3$ plants.

Soil nitrogen availability also influenced carbon isotope discrimination by both Phaseolus species [38], with greater $\Delta$ values in the low nitrogen compared to the high nitrogen treatment (Table 3). Plants with high nitrogen concentrations in their leaves usually have high levels of carbon-fixing enzymes and, therefore, enhanced ability to capture CO$_2$. Enhanced photosynthetic capacity would increase consumption of intercellular CO$_2$ and decrease $p_c$, thus reducing $\Delta$ in

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Table 3: Effect of Nitrogen Fertilization on $\Delta$ Values of Phaseolus vulgaris and Phaseolus acutifolius Grown in a Common Garden

<table>
<thead>
<tr>
<th></th>
<th>HN</th>
<th>LN</th>
<th>Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Both species$^a$</td>
<td>18.3 (0.2)</td>
<td>18.8 (0.2)</td>
<td>0.5</td>
</tr>
<tr>
<td>Phaseolus vulgaris$^b$</td>
<td>18.8 (0.2)</td>
<td>19.6 (0.2)</td>
<td>0.8</td>
</tr>
<tr>
<td>Phaseolus acutifolius$^a$</td>
<td>17.7 (0.2)</td>
<td>18.0 (0.2)</td>
<td>0.3</td>
</tr>
</tbody>
</table>

Note: Each plant in the HN treatment received 10 g of nitrogen fertilizer (46:0:0) at the late seedling stage, whereas those in the LN treatment received none. Numbers in parentheses are standard errors. Differences between species ($p < 0.01$) and nitrogen treatments ($p < 0.05$) were significant, and there was a significant species $\times$ nitrogen interaction ($p < 0.05$).

$^a$ $n = 48$.

Source: Ref. 38.

Plants receiving supplemental nitrogen. $\Delta$ values of $P.$ vulgaris were greater than those of $P.$ acutifolius in both treatments, and $P.$ vulgaris was more responsive to nitrogen nutrition than $P.$ acutifolius (Table 3). Again, genetic differences between these two species and a genotype $\times$ environment interaction were important in determining carbon isotope discrimination by plants experiencing different levels of nitrogen nutrition.

The impacts of other environmental and biological factors (Table 1) on carbon isotope discrimination by $C_3$ plants have been reviewed [53, 54], and their effects on $\Delta$ are similar in magnitude to those demonstrated above for soil water and nitrogen availability. All of these factors appear to influence $\Delta$ either by altering the rate of diffusion of CO$_2$ into the leaf (as in the soil water experiment above) or by altering the rates of CO$_2$ consumption by photosynthesis within the leaf (as in the nitrogen experiment above).

2. Fractionation by $C_4$ Plants

In contrast to the more ubiquitous $C_3$ species, $C_4$ species comprise only about 5% of all plant species [55], are found in only 18 plant families, and are somewhat restricted in geographic distribution. Nearly half of all $C_4$ species are grasses of tropical and subropical origin [39], and all extensive areas of $C_4$ vegetation (e.g., grasslands, savannas, salt marshes) are grass dominated [35, 36, 40–42]. Approximately 1% of the terrestrial surface is covered by $C_4$ plants [42], and they are responsible for 20–30% of total global photosynthesis [42, 43]. Both the geographic distribution [44–46] and relative productivity [47] of $C_4$ grass species are correlated strongly with temperature, although the seasonal distribution and amount of
Figure 2. δ¹³Cleaf values of leaves from six grass species growing in a tropical savanna ecosystem in Masai Mara Game Reserve, Kenya. Each point is the mean ± S.D. of six replicate samples. Only the youngest fully expanded leaves were sampled to minimize isotopic variation due to developmental status. Soil moisture was highest during the May–June rainy season. (From Ref. 54, with permission from Springer-Verlag.)

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CAM plants are relatively minor components of most ecosystems, they will not be considered further in this chapter.

B. Landscape-Level Variation in the Carbon Isotope Composition of Plants

From the discussion above, it is clear that there are numerous environmental factors (Table 1) capable of inducing small changes in plant δ¹³Cleaf in addition to the large fractionations associated with the different photosynthetic pathway types. Most of these environmental factors will change across a landscape in response to slope, aspect, elevation, soil type, and other physical/chemical characteristics of a landscape that are capable of inducing environmental gradients. Thus, variations in δ¹³Cleaf might be anticipated across a landscape.

Variation in δ¹³Cleaf values of C3 plants along soil moisture gradients has been described in a variety of both natural and managed ecosystems. For example, Elderfield and Cooper (58) found that mean foliar δ¹³Cleaf Values of C3 desert plants increased from −26.5% to −24% along a toposequence of decreasing water availability from a wash bottom (where plants would have higher ρpH) to a dry slope (where plants would have lower ρpH). δ¹³Cleaf values of C3 tree species in deciduous forests in North America and Europe have been shown to increase by 1–2‰ along gradients from mesic low-lying landscape positions to drier upland positions (79,60). Similarly, δ¹³Cleaf values of field-grown wheat, pea, and canola crops were significantly higher in the more upland portions of an agroecosystem than those in the lower-lying landscape positions (61). Thus, topographic effects on soil moisture availability appear to have a small but statistically significant and predictable influence on δ¹³Cleaf of C3 plants.

C. Carbon Isotope Variation Associated With Plant Growth Form and Life Span

In addition to the major differences in plant δ¹³Cleaf attributable to the three photosynthetic pathway types, there are some trends in plant δ¹³Cleaf related to plant growth form and life span. Among plants growing in the same habitat, evergreen species have δ¹³Cleaf values that are 1–3‰ higher than deciduous species (58,59,62), and trees have δ¹³Cleaf values that are 1–5‰ higher than shrubs (58,63). In desert ecosystems, it appears that shrub-like (2–5-year) plant species have δ¹³Cleaf values that are 3–5‰ lower than those of long-lived (>50 years) species (58,64). It is not clear if these differences due to life span apply to other ecosystem types. These growth-form and life-span differences in δ¹³Cleaf probably result from complex relationships between the intrinsic biological characteristics of the plants and the array of environmental conditions they experience.
D. Carbon Isotope Variation in Plant Tissues and Biochemical Fractions

Variation in $\delta^{13}C_{\text{cell}}$ among tissues within a plant is usually small. For example, Tieszen and Bouton (54) found that variation among tissues was always less than 2% for six species of C3 grasses growing in an east African savanna (Fig. 3). In general, $\delta^{13}C_{\text{cell}}$ was greatest in roots and live leaves, and lowest in dead leaves and stems. Other studies on trees have shown that roots generally have $\delta^{13}C_{\text{cell}}$ values that are 1–3% greater than those of other tissues (65,66), and wood tissue appears to have $\delta^{13}C_{\text{cell}}$ values that are 1–2% greater than those of leaves for a variety of deciduous and evergreen species (67).

Variation in $\delta^{13}C_{\text{cell}}$ among biochemical fractions within a plant is relatively large. Biochemical fractions with the highest $\delta^{13}C_{\text{cell}}$ values can differ from those with the lowest values by 10–12% (Fig. 4; Refs. 67 and 68). Pectin, amino acids, hemi cellulose, and sugars tend to have $\delta^{13}C_{\text{cell}}$ values greater than the intact plant from which they are extracted, whereas $\delta^{13}C_{\text{cell}}$ values of cellulose, lignin, and lipids are usually lower. It should be noted that a broad range of values has been reported for each biochemical fraction (Fig. 4).

IV. CARBON ISOTOPE FRACTIONATION DURING DECOMPOSITION

In a steady-state system, $\delta^{13}C_{\text{cell}}$ of the soil organic matter should be nearly identical to that of the plant community from which the organic matter was derived, unless the isotopic composition of plant tissue is altered during decomposition. Because decomposition is a slow and lengthy process, there is little direct evidence for evaluating the potential for isotope fractionation as plant carbon is incorporated into the soil organic matter pool. However, there is a substantial body of indirect evidence suggesting that fractionation during decomposition is small. If fractionation were to occur during decomposition, it would most likely result from the metabolic activities of decomposer organisms and/or from differential decomposition/preservation of isotopically distinct biochemical fractions of plant material (Fig. 4).

A. Differential Decomposition/Preservation

Miner and M. (69) demonstrated that the major biochemical fractions of plant tissue all decompose at relatively rapid rates compared to the mean residence times of organic carbon in soils. However, lignin decomposes at a significantly slower rate (50% mass loss per year) than cellulose (75% mass loss per year), hemicellulose (90% mass loss per year), or simple sugars (99% mass loss per year) (69). Because lignin often comprises a significant proportion (5–40%) of the total mass of plant tissues and because it is 5–6% lower in $\delta^{13}C_{\text{cell}}$ than bulk plant tissue, its slower
rate of decomposition has the potential to alter δ13C of plant material as it is incorporated into the soil organic matter pool. Although several studies have shown that the relative proportion of lignin in plant tissue increases as decomposition proceeds [70–73], this differential preservation does not appear to lower δ13C values of either the residual litter or the associated soil organic matter pool in well-drained terrestrial environments [72–74]. However, it should be noted that the δ13C of plant tissue decomposing beneath the surface of relatively anoxic salt marsh sediments has been shown to decrease over time [70,71].

Direct measurements indicate that the δ13C of plant tissue remains relatively constant during the early stages of decomposition (1–7 years). For example, δ13C of leaf litter from five dominant tree/shrub species in a subtropical thorn woodland in southern Texas changed by less than 0.5% in a non-directional manner during the first year of decomposition (Fig. 5), despite a 48–60% loss of litter mass. Wedin et al. [73] found small (0.6–1.5%) but significant shifts in the δ13C of litter from four grass species in an oak savanna in Minnesota. δ13C values of the C4 grass, Schizachyrium scoparium, decreased by 1.5%, whereas those of three C3 grass species increased by 0.6%. These shifts occurred over 2 years and were accompanied by a 70% loss of initial litter mass. They suggested that these differences were attributable to mixing of external soil organic carbon (which had a δ13C value intermediate between the C2 and C4 species) with residual litter carbon via fungal hyphal and/or microbial populations [73]. Melillo et al. [72] found that red pine needles lost 83% of

Figure 4. δ13C of plant biochemical fractions relative to the δ13C of the whole plant from which they were extracted. (Data from Ref. 73.)

Figure 5. δ13C values and mass loss rates during decomposition of leaves from five tree/shrub species in a subtropical thorn woodland in southern Texas. Leaves were placed in nylon-mesh litter bags and placed on the soil surface. Each point is the mean (± S.D.) of five replicate samples. (Data from Botton, Midwood, Archibald and Angerer [unpublished].)
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Thus, it is the case that soil organic matter is directly processed and transported by the detritus decomposition pathway. This is because the potential only exists for the residual organic carbon to undergo an increase in $\delta^{13}C$/CO$_2$ over time.

The greater the age of soil organic matter, the more time it has had to undergo isotopic fractionation by decomposition, and therefore the potential for the positive correlation between $\delta^{13}C$/CO$_2$ and soil $\delta^{13}C$/CO$_2$ is generally lower for the older organic carbon. However, it is possible to use soil $\delta^{13}C$/CO$_2$ values to reflect the cumulative effects of this activity.

C. Historic Change in $\delta^{13}C$/CO$_2$ of Atmospheric CO$_2$

It has been hypothesized that increasing $\delta^{13}C$/CO$_2$ values of soil organic matter with increasing depth in the soil profile could be a consequence of the decrease in the $\delta^{13}C$/CO$_2$ values of the atmosphere in the past 200 years. During this period, a number of factors have contributed to the decrease in the $\delta^{13}C$/CO$_2$ values of atmospheric CO$_2$, including increased burning of fossil fuels and increased industrial activity, which have led to an increase in the release of CO$_2$ to the atmosphere. Additionally, the burning of biomass and other organic materials has also contributed to the decrease in $\delta^{13}C$/CO$_2$ values.

In contrast, the $\delta^{13}C$/CO$_2$ values of soil organic matter have generally increased over time due to the incorporation of 13C-rich carbon from the atmosphere into the soil. This increase in $\delta^{13}C$/CO$_2$ values has been observed in a number of studies, and it is thought to reflect the cumulative effects of these processes.

B. Isotope Fractionation by Decomposing Organisms

One possible explanation for the isotope enrichment is the fractionation that occurs during the decomposition of soil organic matter. Studies have shown that decomposing organisms can enrich the 13C content of soil organic matter. This enrichment is thought to occur through the preferential decomposition of 12C-rich compounds, which results in a higher $\delta^{13}C$/CO$_2$ value for the remaining soil organic matter.

The extent of this enrichment can be influenced by a number of factors, including the age of the soil organic matter, the availability of 13C-rich compounds, and the activity of decomposing organisms. These factors can all influence the degree of enrichment, and therefore the $\delta^{13}C$/CO$_2$ values of soil organic matter can provide information about the processes that have occurred in the soil.

A. Determining Factors

The $\delta^{13}C$/CO$_2$ values of soil organic matter can be influenced by a variety of factors, including the age of the soil organic matter, the availability of 13C-rich compounds, and the activity of decomposing organisms. These factors can all influence the degree of enrichment, and therefore the $\delta^{13}C$/CO$_2$ values of soil organic matter can provide information about the processes that have occurred in the soil.

Soils that have been exposed to higher temperatures and/or lower moisture levels may have lower $\delta^{13}C$/CO$_2$ values due to increased decomposition and a higher rate of 13C enrichment. Similarly, soils that have been exposed to lower temperatures and/or higher moisture levels may have higher $\delta^{13}C$/CO$_2$ values due to reduced decomposition and a lower rate of 13C enrichment.

The $\delta^{13}C$/CO$_2$ values of soil organic matter can also be influenced by the presence of certain plant species. For example, plant species that have a high proportion of 13C-rich compounds in their biomass may enrich the 13C content of the soil organic matter, resulting in a higher $\delta^{13}C$/CO$_2$ value.

In general, the $\delta^{13}C$/CO$_2$ values of soil organic matter can provide useful information about the processes that have occurred in the soil, and they can be used to help understand the dynamics of the soil organic carbon cycle.
of the soil organic matter pool, it should be possible to accurately estimate the long-term, integrated \( ^{13}C_{\text{soil}} \) of the plant community that contributed to the soil organic matter at a given site. In fact, Baleshwar et al. [60] were able to predict \( ^{13}C_{\text{soil}} \) of plant communities with a 95% confidence of 9% by using a simple equation containing only the \( ^{13}C_{\text{soil}} \) value and the concentration of organic carbon in the associated soil. Thus, the strong relationship between the \( ^{13}C_{\text{soil}} \) of the soil organic matter and that of the plant community indicates that the \( ^{13}C_{\text{soil}} \) of soil organic matter should reflect the relative productivity of C3 versus C4 sources and can be used to evaluate plant-community history at a particular site.

### 8. Relationships with Time

A site-specific history or chronology of relative C3-C4 productivity can be developed by measuring the \( ^{13}C_{\text{soil}} \) of soil organic matter at different depths in the soil profile and/or in different soil organic matter pools or components that differ in turnover rates. Radiocarbon measurements indicate that the mean age of soil organic matter increases with depth in the profile and suggest that organic carbon in surface soils has a mean age of less than a few hundred years, whereas organic carbon in a depth of 1 m may have a mean age of approximately 1000–4000 years [83]. Shifts in relative C3-C4 productivity within the past 100 years or less should be the most evident in the \( ^{13}C_{\text{soil}} \) of organic carbon in the surface soil; in contrast, C3-C4 shifts that occurred several hundred years ago might still be evident in the \( ^{13}C_{\text{soil}} \) of organic carbon deep in the profile, but not in the surface soil. Thus, a temporal record of relative C3-C4 productivity may be established by measuring \( ^{13}C_{\text{soil}} \) of soil organic carbon at different depths throughout the soil profile.

Another approach for developing a temporal record of relative C3-C4 productivity involves the measurement of \( ^{13}C_{\text{soil}} \) on organic carbon in soil organic matter pools that differ in turnover rate, such as different particle size fractions [12, 13, 89–91; Chapter 3, this book] or aggregate size fractions [82–95]. Organic matter in clay and silt size fractions generally has a slower rate of turnover than organic matter in sand size fractions [12, 13, 89–91]. Similarly, organic matter turnover rates are significantly greater in macroaggregates (>212 μm) than in micro-aggregates (<212 μm) [92–95]. Thus, if a change in relative C3-C4 productivity has occurred, the \( ^{13}C_{\text{soil}} \) of the current organic matter inputs should be most evident in coarse particle-size fractions and in macroaggregates, whereas the \( ^{13}C_{\text{soil}} \) of the previous plant community should be most evident in the organic matter in silt/clay fractions and microaggregates. \( ^{13}C_{\text{soil}} \) measurements of organic carbon in particle-size fractions from different depths throughout the profile would, therefore, provide the most detailed chronological account of relative C3-C4 productivity at a particular site. Radiocarbon measurements on these same organic carbon fractions (particle- or aggregate-size fractions) would further clarify the temporal aspect of relative C3-C4 productivity.
Finally, plant community dynamics can be reconstructed over paleoecological time scales by measuring δ13C values of organic carbon preserved in paleosols, which may range in age from thousands to millions of years. Cerning et al. [22] have shown that, at least in some cases, δ13C values of soil organic carbon can persist without diagenetic alteration for at least 5 million years.

VI. δ13C of SOIL ORGANIC MATTER AS AN INDICATOR OF VEGETATION CHANGE: APPLICATIONS

A. Need for Methodology to Document Vegetation Change

A major limitation in the study of vegetation dynamics is the lack of direct and reliable methods for detecting and documenting vegetation change at appropriate spatial and temporal scales [96]. Traditional methods include the use of anecdotal records of early settlers of a region, historical records of land ownership and use, aerial photographs, remote sensing imagery, analysis of pollen and peatbogs, and population characteristics of the present plant community. Each of these methods has limitations, and, in some regions, it is possible that none of these methods will be available or feasible. As a result, the development of new methods for detecting vegetation change is essential to improve understanding of vegetation dynamics in natural and managed ecosystems.

In plant communities where C3 and C4 plant species coexist at present, or where they may have coexisted at one time, δ13C of soil organic matter appears to be a useful tool for reconstructing the relative productivity of C3 versus C4 species. Although this knowledge of relative C3-C4 productivity derived from δ13C does not provide any information regarding actual species composition or the relative importance of individual species, C3 and C4 species generally segregate into structurally (e.g., C3 trees/shrubs versus C4 grasses) and/or functionally (C3 warm season grasses versus C4 season grasses) distinct groupings of plants. As a result, documentation of changes in the relative productivity of these structural/functions groups often provides ecologically meaningful information regarding vegetation dynamics in natural or managed ecosystems. The remainder of this review will focus on specific implications of δ13C of soil methodology to the study of vegetation change:

B. Grazing Effects in North American Grassland

Livestock grazing occurs on approximately 50% of global land area and is perhaps the most widespread form of human land use. The ecological consequences of grazed activity often include changes in the composition and relative importance of plant species [97]. Because vast and often remote grazed areas are affected, there has been relatively little direct assessment, documentation, or long-term monitoring of plant community changes resulting from grazing. We evaluated the use of δ13C of soil organic carbon as a tool for assessing vegetation changes resulting from livestock grazing in an area where grazing history was well known and where an ungrazed control area was available to serve as an indicator of the original vegetation.

This study was conducted in east-central Texas (30°50'N, 90°10'W) on a well-drained upland in the San Antonio Prairie, a southern component of the tallgrass prairie biome of North America. The vegetation was a Schizachyrium scoparium-Critesbonerum mature community dominated by C4 grasses. Soils in the study area were Borden series (fine, montmorillonitic, thermic, Vertic Argiustolls) and Normangee series (fine, montmorillonitic, thermic, Vertic Haplustalfs).

We compared a 10-ha stand grazed heavily and continuously by cattle since 1969 with an adjacent stand with no record of grazing since 1832 when the site was first surveyed. In 1987, δ13C values of aboveground vegetation, roots, and soil organic carbon were determined in each stand. Inorganic carbon was removed from soil samples prior to isotopic analysis by precipitation with HCl. The proportion of organic carbon derived from C4 sources in plant and soil samples was estimated by the mass balance equation:

\[ \delta^{13}C_{\text{org or plant}} = (\delta^{13}C_{\text{C2}}(x) + \delta^{13}C_{\text{C2}}(1 - x)) \]

where \( \delta^{13}C_{\text{org or plant}} \) was the δ13C of plant or soil organic matter samples, \( \delta^{13}C_{\text{C2}} \) was the average δ13C value of C2 plants (−13%), \( x \) was the proportion of carbon from C4 plants sources, \( \delta^{13}C_{\text{C2}} \) was the average δ13C value of C2 plants (−27%), and 1 − x was the proportion of carbon derived from C3 plant sources.

Eighteen years of livestock grazing caused a significant increase in C4 forbs and C4 grasses and a decrease in the importance of C3 grasses relative to the ungrazed stand. Estimates of vegetative cover indicated that several C3 forbs and one C3 grass (Spiota flexuosa) increased in relative importance in the grazed stand relative to the ungrazed stand. This shift was reflected in the isotopic composition of the aboveground biomass, roots, and soil organic matter (Fig. 7A). The average δ13C value of bulk vegetation from plots (n = 15) clipped to ground level was −22.4 ± 1.1%, indicating that approximately 30% of standing crop biomass was C4 in origin. By contrast, δ13C of vegetation in the ungrazed area was −16.7 ± 0.6%, indicating that approximately 70% of the biomass was C3.

A similar isotopic signal was present belowground in the root biomass (Fig. 7A). In the grazed stand, δ13C of roots ranged from −24% near the soil surface to −18% deeper in the soil profile, with an average of −21.6 ± 1.1% (approximately 30% C4 biomass) for all samples throughout the profile. The increase in root δ13C values with soil depth in the grazed stand suggested that the C3 component which increased in response to grazing was not rooted as deeply in the coexisting C4 vegetation and contributed less to root production below 40 cm in the soil profile. δ13C values of roots in the ungrazed area varied little with depth (Fig. 7A) and averaged −16.1 ± 0.8% (approximately 70% C3 biomass) for all samples. Therefore, current aboveground and belowground organic matter inputs
in the grazed area consisted of approximately 39% C₄ biomass, whereas those in the ungrazed area consisted of approximately 76% C₃ biomass. The carbon isotopic composition of soil organic matter in the ungrazed area ranged from −1% at the top of the profile to −14% deeper in the profile (Fig. 7B) and was similar to the isotopic composition of the current organic matter inputs in that area. The increase in δ¹³Corg with increasing soil depth may reflect a gradual decrease in C₄ relative productivity in the plant community through time. However, as discussed earlier (Sections IV.C–IV.D), this increase is more likely due to isotopic fractionation during decomposition and/or the decrease in

the δ¹³Corg of atmospheric CO₂ during the past 200 years. In the grazed stand, δ¹³Corg values of soil organic matter were significantly lower than those in the ungrazed stand at all but the 75–90-cm-depth increment, reflecting the lower δ¹³Corg values of the current organic matter inputs in the grazed community (Fig. 7B). The soil organic matter in the 0–5-cm-depth increment (−21%o) was isotopically similar to both aboveground and belowground biomass (−22%), indicating rapid organic carbon turnover at that depth.

The current plant community in the grazed area has left a significant isotopic imprint on the soil organic matter and reflected the increase in relative C₄ productivity resulting from the initiation of livestock grazing in 1999. In fact, organic matter in the surface soil of the grazed site accurately reflected the relative C₄–C₃ productivity of the plant communities in place when the soil was sampled in 1987. However, the isotopic composition of the soil organic matter deeper in the profile (>15 cm) reveals that approximately 70–85% of the soil carbon was derived from C₃ plants and that the grazed site was occupied at one time by a C₃-dominated plant community. Therefore, at this site with well-known land use and vegetation histories, the δ¹³Corg method accurately reflected the vegetation changes known to have occurred there.

C. Tree–Grass Dynamics in Savanna Ecosystems

The coexistence of woody plants and grasses in subtropical and tropical savanna ecosystems is currently of great interest due to the rapidity with which the relative abundances of these components are modified by human influence, especially through manipulation of fire frequency and livestock grazing. In recent history, woody plants have increased in many grasslands and savannas in Africa, Asia, Australia, South America, and North America [98]. In these savannas, the woody plant component is comprised exclusively of C₃ species and the grass component consists of only C₄ species [12,16,17,53,99,100]; therefore, δ¹³Corg values of soil organic matter can be utilized to document shifts in the relative productivities of these two structurally and functionally distinct components of savanna ecosystems in response to human use, or natural environmental variation. Many studies have utilized δ¹³Corg of soil organic carbon to document grass–woody plant dynamics in tropical and subtropical savannas [12,16,98–102].

In the subtropical Rio Grande Plains of southern Texas, several lines of evidence suggest that relatively open savannas dominated by C₃ grasses have been largely replaced by thorn woodland dominated by C₄ woody plants during the past 100–200 years [98]. To further substantiate these observations, δ¹³Corg was measured on soil organic matter from areas now dominated by woodlands. If these areas were once relatively open C₃ grassland, then δ¹³Corg values of soil organic matter from areas now dominated by C₄ woodland should reveal the presence of organic carbon derived from C₃ sources. This study was conducted at the Texas
Agricultural Experiment Station LaCopita Research Area approximately 15 km from Alice (27°40'4 N, 58°12' W) in southern Texas. Six soil cores (5 cm x 150 cm) were taken in May 1990 in each of two closed-canopy woodland stands dominated by the woody legume Prosopis glandulosa and subdivided into six depth intervals. Soils in these stands were fine loamy Pach arbitrarily. δ13C was measured on aboveground litter, roots, bulk soil organic matter, and organic material associated with different particle size classes. Inorganic carbon was removed from soil samples prior to isotopic analysis by pretreatment with HCl. The 14C age of bulk soil organic matter was determined on soil samples from these woodlands.

Both woodlands had similar species composition and canopy cover. δ13C values of litter, roots, and soils were not significantly different between sites, so data were pooled. δ13C of litter (~26‰) and roots (~25‰) substantiated that both sites were dominated by C3 trees and shrubs (Fig. 8A). δ13C values of roots varied little throughout the soil profile (Fig. 8A).

By contrast, δ13C values of soil organic matter were all greater than ~19‰ (Fig. 8A), indicating that a significant proportion of soil organic carbon beneath woodlands was derived from C4 grasses. At 0–15 cm, δ13C of soil organic carbon was ~19‰, indicating that approximately 60% of the organic carbon in that depth interval was derived from C4 sources. From 15 to 90 cm, δ13C values ranged from ~16‰ to ~14‰, indicating that approximately 80–90% of soil organic carbon in that interval was derived from C4 plants. Below 90 cm, δ13C values became more negative and approached a value of ~19‰. The 14C ages of soil organic carbon increased with depth in the profile, and ranged from 115.8 ± 0.766 modern at 0–15 cm to 2142 ± 95 years at 90–120 cm (Fig. 9). Because the present C4 plant community is isotopically evident only in organic carbon above 15 cm in the profile (Fig. 8A), and because this carbon above 15 cm has a mean residence time of less than ~200 years (Fig. 9), it appears that the change from C3 grassland/savanna to C4 woodland occurred during the past 200 years.

Knowledge of soil organic carbon turnover rates (see Chapter 3 of this book) would enable more accurate estimates of the chronology of this vegetation change. The δ13C values of particle-size separates (Fig. 8B) revealed that the sand fraction consisted exclusively of new carbon derived from the current C4 woodland vegetation. Conversely, fine and coarse clay fractions were both slightly more enriched in 13C than the whole soil and appeared to contain organic carbon derived largely from the previous C3 grassland. Although fine and coarse silt were both more 13C-enriched than the sand fraction, both clearly contained some carbon derived from the present community. Because the carbon from the present C4 woodland is younger than the residual C3 carbon in these soils, organic carbon turnover rates in this system appear to decrease in the sequence sand > silt > clay. Because most organic carbon in this system resides in the "old" clay fractions, the isotopic composition of the whole soil is dominated largely by the signal in these fractions.

![Figure 8](image-url)  
**Figure 8** δ13C values of (A) litter, roots, and bulk soil organic matter, and (B) organic matter associated with soil particle size fractions in a sub-tropical woodland ecosystem in southern Texas. In (A), each point is the mean (= ± SE) of 12 soil cores and is plotted as the midpoint for each of the following depth intervals: 0–15, 15–30, 30–60, 60–90, 90–120, and 120–150 cm. Data in (B) are derived from analysis of a single soil core. (From Ref. 38, with permission from American Geophysical Union.)

As a consequence of variation in their carbon turnover rates, δ13C values of particle-size separates provide an enhanced view of plant community history.

Previous studies based on sequential aerial photography, present vegetation patterns, and tree age determinations using 14C dating and annual ring counts all suggest that the present subtropical woodland replaced relatively open grassland or savanna during the past 100–200 years [98]. The large discrepancy between δ13C of current organic matter inputs and that of soil organic carbon confirms these findings and indicates that ~60–90% of the organic carbon now residing in
palaeoecological data as far back as the late Miocene [2]. δ13C values of palaeo-organic carbon, a proxy for vegetation productivity, can be used to assess the impact of climate change on various spatial and temporal scales. However, δ13C values of C3 and C4 species are often correlated and it is difficult to distinguish between these two types of plants using this proxy alone.

The δ13C record from the late Miocene to the present provides insights into the effects of climate change on vegetation. For example, the late Miocene period was characterized by high temperatures and lower precipitation, leading to a dominance of C3 plants. In contrast, the current climate, with cooler temperatures and higher precipitation, favors C4 plants. The δ13C record can thus be used to infer past climate conditions and understand the response of vegetation to climate change. For instance, the transition from C3 to C4 vegetation during the late Miocene suggests a shift from a warmer, drier climate to a cooler, wetter one.

Despite these challenges, the δ13C record is a valuable proxy for understanding past climate and vegetation changes. It provides a valuable tool for paleoclimatologists and paleoecologists, allowing them to reconstruct past environments and gain insights into the dynamics of past ecosystems. However, the interpretation of δ13C records requires careful consideration of the factors that influence δ13C values, such as the isotopic composition of atmospheric carbon dioxide, the fractionation during photosynthesis, and the isotopic composition of the underlying geology.

The δ13C record from the late Miocene to the present shows that vegetation has responded to climate change in a manner consistent with modern climate patterns. For example, the late Miocene period was characterized by high temperatures and lower precipitation, leading to a dominance of C3 plants. In contrast, the current climate, with cooler temperatures and higher precipitation, favors C4 plants. The δ13C record can thus be used to infer past climate conditions and understand the response of vegetation to climate change. For instance, the transition from C3 to C4 vegetation during the late Miocene suggests a shift from a warmer, drier climate to a cooler, wetter one.
are also native to this region. However, because their contribution to the overall carbon content is relatively small, they probably had little effect on the net 14C output from the atmosphere.

From 9000 to 8000 YBP, 8C/12C values were about 1.4%, lower than at any other time during the Holocene. This appears to be the result of Holocene carbonate production.

The 8C/12C values were relatively low due to the cooler conditions at that time. The 8C/12C values ranged from -11.5 to -13.5% at 9000 YBP, followed by a return to earlier levels at 8000 YBP. The temperatures are in agreement with the Holocene climate pattern described by Moore et al. (1997). The results are also consistent with the 8C/12C values reported by Moore et al. (1997) for the Holocene period.

The 8C/12C values at 9000 YBP were about 0.5%, lower than at any other time during the Holocene. This appears to be the result of Holocene carbonate production. The 8C/12C values were relatively low due to the cooler conditions at that time. The 8C/12C values ranged from -11.5 to -13.5% at 9000 YBP, followed by a return to earlier levels at 8000 YBP. The temperatures are in agreement with the Holocene climate pattern described by Moore et al. (1997). The results are also consistent with the 8C/12C values reported by Moore et al. (1997) for the Holocene period.

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B/C_C on the Holocene (Fig. 10) are a consequence of changes in biomass grazing,
intensity, or manipulation of fire frequency by prehistoric humans in this re-
gion. In addition, it has been suggested that changes in atmospheric CO2
concentration during the late Quaternary could be responsible for changes in
C3–C4 relative productivity [113–115], with lower CO2 concentrations favoring C3
plants. However, because temporal changes in relative C4 productivity at Pt.
Hood (Fig. 10) are contrary to predictions based on changes in atmospheric CO2
concentration [19] over the same period, and because these results correlate strongly
with other climatic reconstructions not only from this same region but also from
other parts of the Great Plains, it seems likely that climate is the driving force be-
hind the vegetation changes documented here (Fig. 10).

Plant species composition has a strong impact on hydrology and biogeo-
chemistry at the ecosystem, landscape, and global levels of organization [116–118].
Consequently, the dramatic shifts in relative C3–C4 productivity that occurred
throughout the Great Plains region during the late Quaternary [18,104,105,108,
119] could be important in this regard. For example, C3 plants initiate growth later
in the growing season and are more water-use efficient than C4 plants [35,40].
These differences in plant water-use characteristics could potentially impact soil
water storage, groundwater recharge, humidity, rainfall, and cloud cover. In addi-
tion, C4 grasses have lower nitrogen concentrations and more structural compounds
than C3 grasses, which could influence the storage and dynamics of carbon dioxide,
and other nutrients in the soil [120,121]. In fact, simulation models have shown
that C3–C4 composition is an important determinant of soil carbon storage
in grasslands [122]. Therefore, in addition to enhancing our understanding of ve-
etation and climate change, knowledge of the C3–C4 composition of past plant com-
unities should also be useful for reconstructing functional characteristics of
paleoecosystems [118,123].

VII. SUMMARY AND CONCLUSIONS

Over the past 50 years, isotope ratio mass spectrometry has been utilized to doc-
ument variation in the natural abundance of stable carbon isotopes in the earth-
atmosphere–biosphere system. One of the most important and useful isotopic dif-
fferences from the standpoint of the plant–soil subsystem is the one that exists be-
tween C3 (low 13C) and high 13C) plants. A substantial body of evidence
indicates that the 13C/C4 of soil organic carbon integrates and records the rela-
tive contribution of C3 versus C4 plants to community net primary productivity in
a reasonably quantitative manner. Estimates of relative C3–C4 productivity from
13C/C4 of soil organic carbon will become increasingly more accurate and pre-
cise as we improve our understanding of environmental and biological effects on
carbon isotope discrimination by plants, carbon isotope discrimination during de-
composition, and the isotopic history of the atmosphere.

The 13C/C4 of soil organic matter has accurately documented vegetation
changes at numerous sites with well-known vegetation and land use histories,
indicating that this technique is useful for both short-term and long-term monitoring
of vegetation dynamics in natural or managed ecosystems. This tool will obviously
have its greatest value in those ecosystems where C3 and C4 plants coexist, such
as grasslands, savannas, deserts, marshes, and tropical/subtropical pastures. In ad-
dition, ecosystems that include plants with Crassulacean acid metabolism should be
amenable to this approach. It should also be pointed out that small differences in
13C/C4 between plants with different growth forms have been exploited to doc-
ument vegetation dynamics in plant communities comprised exclusively of C3
species [124].

The 13C/C4 values of organic carbon appear to persist unmodified in pa-
leo soils for several million years and, therefore, contain a wealth of paleoenvi-
ronmental information. Because the geographic distribution and the relative
productivity of C3 species are strongly correlated with temperature, 13C/C4 of
soil organic matter preserved in paleosols can enhance our understanding not only
of long-term vegetation dynamics but also of climatic history. The re-
cent development of models that accurately predict the geographic distribution of
relative C3–C4 productivity from climate variables [42,43] suggests that it
might be possible to extract quantitative climatic information from isotopic re-
constructions of C3–C4 productivity. 13C/C4 values of organic carbon in paleo-
soils appear to be useful as proxy indicators of climate to complement other
paleoecological methods (e.g., phyoliths, pollen, geomorphology, etc.) or in
situations where more traditional techniques are simply not feasible. In addi-
tion to enhancing our understanding of vegetation and climate change, knowl-
edge of the C3–C4 composition of past plant communities should also be useful
for reconstructing functional attributes (e.g., hydrology, biogeochemistry) of
paleoecosystems.

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