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Rapid report

Leaf respiratory CO₂ is ¹³C-enriched relative to leaf organic components in five species of C₃ plants

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Summary

Key words: C₃ plants, carbon isotope, dark respiration, fractionation, Keeling plot.

- Here, we compared the carbon isotope ratios of leaf respiratory CO₂ (δ¹³Cᵣ) and leaf organic components (soluble sugar, water soluble fraction, starch, protein and bulk organic matter) in five C₃ plants grown in a glasshouse and inside Biosphere 2. One species, Populus deltoides, was grown under three different CO₂ concentrations.
- The Keeling plot approach was applied to the leaf scale to measure leaf δ¹³Cᵣ and these results were compared with the δ¹³C of leaf organic components.
- In all cases, leaf respiratory CO₂ was more ¹³C-enriched than leaf organic components. The amount of ¹³C enrichment displayed a significant species-specific pattern, but the effect of CO₂ treatment was not significant on P. deltoides.
- In C₃ plant leaves, ¹³C-enriched respiratory CO₂ appears widespread. Among currently hypothesized mechanisms contributing to this phenomenon, non-statistical carbon isotope distribution within the sugar substrates seems most likely. However, caution should be taken when attempting to predict the δ¹³C of leaf respiratory CO₂ at the ecosystem scale by upscaling the relationship between leaf δ¹³Cᵣ and δ¹³C of leaf organic components.


Introduction

It is well known that carbon isotope discrimination takes place during plant photosynthetic CO₂ fixation, resulting in all higher plants being depleted in ¹³C in organic carbon relative to atmospheric CO₂. The models of ¹³C fractionation in photosynthesis have been well established (Farquhar et al., 1982). By contrast, studies on the carbon isotope ratio of CO₂ generated by dark respiration (δ¹³Cᵣ) are limited. Although a possible isotope effect during dark respiration might significantly influence the carbon isotope signature of plants and other components of an ecosystem, fewer studies have focused on determining the magnitude of this potential effect and the results appear contradictory (O’Leary, 1981; Lin & Ehleringer, 1997; Durand et al., 1999, 2001).

Studies on the carbon isotopic effects during respiration trace back half a century. Historically, carbon isotope discrimination during respiration was considered to be negligible
O’Leary, 1981; Farquhar et al., 1982, 1989; Flanagan &
Ehleringer, 1998). Early experimental studies observed that
$\delta^{13}C_{\text{R}}$ is very close (approx. ±1%) to bulk carbon in some
gerninating crop seedlings (Baertschi, 1953; Smith, 1971).
More recently (Lin & Ehleringer, 1997) cultured mesophyll
protoplasts of bean and corn leaves with carbohydrates of
known isotopic ratios as the only carbon source and found
no significant differences between $\delta^{13}C_{\text{R}}$ and $\delta^{13}C$ of the
substrate in either species.

Still, other studies suggest that respiratory CO$_2$ of plants
is remarkably $^{13}$C-enriched or $^{13}$C depleted (4–5% more
positive or –4–8% more negative) in comparison with
whole plant or leaf $\delta^{13}C$ (Smith, 1971; Troughton et al., 1974).
Recently, Duranceau et al. (1999, 2001) and Ghashghaie
et al. (2001) compared $\delta^{13}C$ of leaf respiration and leaf
organic components in beans, tobacco, and sunflower. They
report a 3–6% $^{13}$C-enrichment in respiratory CO$_2$ com-
pared to sucrose, the assumed substrate of dark respiration.
Although this hypothesis was not tested in other species, they
concluded that carbon isotope fractionation during dark
respiration was widespread in C$_3$ plants. In C$_4$ plants,
Henderson et al. (1992) found that the $\delta^{13}C$ of dry matter
was more negative than that predicted by the discrimination
occurring during CO$_2$ uptake and partly attribute the differ-
cence to an isotope effect during dark respiration. Further-
more, $\delta^{13}C_{\text{R}}$ can change daily or seasonally (Park & Epstein,
1961; Jacobson et al., 1970; Damesin & Lelarge, 2003) and
can be influenced by environmental or physiological factors
(temperature, respiratory quotient, etc., Tcherkez et al., 2003).

Despite the growing contradictory evidence, the assump-
tion that carbon fractionation in dark respiration is negligible
is widely applied in ecological and physiological studies
(Flanagan & Ehleringer, 1998; Yakir & Sternberg, 2000;
Ehleringer et al., 2002). At the ecosystem scale, the concept
of ecosystem $^{13}$C discrimination ($\Delta^{13}C_{\text{E}} = \delta^{13}C_{\text{R}} - \delta^{13}C_{\text{atm}}$
$(1 + \delta^{13}C_{\text{F}})$, or $\Delta^{13}C = \delta^{13}C_{\text{REE}} - \delta^{13}C_{\text{R}}$), has recently been
used to partition NEE (Net Ecosystem Exchange) into
photosynthetic and respiratory components (Bowling et al.,
2001), by assuming that the $\delta^{13}C_{\text{R}}$ should reflect the
$^{13}$C signature of total organic carbon in the ecosystem
(Buchmann et al., 1997; Yakir & Sternberg, 2000). Likewise,
the $\delta^{13}C$ of organic carbon in leaf, soil, and litter were used to
estimate the $\delta^{13}C_{\text{R}}$ generated by each components (Lin et al.,
1999, 2001). If the $\delta^{13}C_{\text{R}}$ does not correctly reflect the $\delta^{13}C$
of the respiration substrate pool, the conclusions of these
studies will need to be reconsidered and modified accordingly.

Additional uncertainties regarding the use of $\delta^{13}C_{\text{R}}$ as a
tool for understanding ecosystem scale processes arise from
several other factors. For example, initial studies focused on
seedlings or tubers and subsequent leaf scale studies were con-
ducted in only a few crop species. In addition, plant materials
were subjected to a CO$_2$-free environment in all previous
studies, which may itself influence leaf $\delta^{13}C_{\text{R}}$ (O’Leary, 1981).
Clearly, much more detailed information on the species

**Materials and Methods**

**Plant materials**

We studied five C$_3$ plants, which are among the most
abundant species in the Tropical Rain Forest (TRF) and
Intensive Forest Biome (IFB) of Biosphere 2 (a 1.29 hectare
glass enclosed research facility in Oracle, AZ, USA). This
leaf scale study was also designed to provide background
information for further investigation on the respiratory isotope
effect at mesocosm scale within Biosphere 2. Among 4
tropical species we studied, Musa paradisiaca (tree), Coffea
arabica (shrub), and Epipremnum pinnatum (vine) were grown
in a glasshouse (the demonstrate lab or DL), while
Clitoria racemosa (tree), was grown in the TRF biome. *Populus deltoides*
(IFB monoculture), a temperate tree species, was grown in
three CO$_2$ concentrations: close to ambient in the DL,
800 p.p.m. in the IFB mid bay (MB) and 1200 p.p.m. in
west bay (WB). In the IFB, tank supplied CO$_2$ with a very
low $\delta^{13}C$ (c. –28%) was used to maintain elevated CO$_2$
concentrations. All plants grew under a natural photoperiod
and night time temperatures of 23–28°C depending on the
location.

**Air sampling**

All air samples were collected between 20:00 h to 00:30 h,
when plants were in natural darkness. One to several healthy,
intact, visually mature (well expanded and with developed
cuticle) leaves were sealed in an opaque respiration chamber
(modified from a mylar balloon) with a small fan to ensure
air mixing. The chamber was connected to a closed loop gas
device including a pump, a CO$_2$ infrared gas analyzer
(LI-6200, Licor, Inc., Lincoln, NE, USA), a desiccant tube
containing magnesium perchlorate and six 100 mL flasks
(Fig. 1a). The entire system was 10–15 L to hold the largest
leaf (Epipremnum pinnatum) in our study and was checked for
leakage before each sampling by exhaling on all connections.
The airflow rate was approx. 1 L$^{-1}$ per min. Ambient air was
pumped through the entire system before closure and then allowed to circulate for 10–15 min to ensure adequate mixing before sampling. The air samples were collected in sequence by closing both stopcocks on a flask for each 15–20 ppm CO₂ increment. Humidity was not strictly controlled in our study.

We compared our leaf-level Keeling plot method with a traditional CO₂ free chamber connected to the Li-6200 photosynthesis system and found that two methods yielded similar results in leaf CR (±0.5%) when the incubation chamber was well sealed. However, the incubation method often gave much more scatter results with same plant leaf than leaf-level Keeling plot approach.

Leaf sampling and chemical extraction

After air sampling, half of the leaf material contained within the cuvette was immediately frozen in liquid nitrogen and then stored in −20°C freezer for subsequent extraction of carbohydrate and protein. The remaining leaf material was dried in a 60°C oven for carbon isotope analysis of bulk leaf organic matter.

A subsample of 0.1–1 g of leaf material was used for soluble sugar and starch extraction. For each 0.1 g of sample, 1 mL of deionized water was added and the mixture was ground in a chilled mortar and pestle. The resulting extract was kept at 0°C for 20 min before centrifugation at 12 000 g for 10 min. The supernatant containing the soluble fraction was then boiled for 3 min and centrifuged again as already described (Duranceau et al., 1999). The water soluble fraction was then mixed with Dowex-50 (H⁺) and Dowex-1 (Cl⁻) resins in sequence to remove amino acids and organic acids, respectively. The eluate has been shown to have a carbon isotope composition representative of leaf soluble sugars (Brugnoli et al., 1988). The pellets were washed in ethanol (80% v/v) at 80°C to eliminate chlorophyll and then suspended twice in 6 mol/L HCl at 5°C (1 h each) to solubilize the starch. After adding methanol (4× by volume), the supernatant was kept at 5°C overnight and starch precipitated was desiccated in a freeze dryer (Damesin & Lelarge, 2003, with a few modifications).

Proteins were extracted by boiling the supernatant of grounded leaf tissue (in 2% NaCl; 10 000 g 15 min) for 30 min (Jacobson et al., 1970). The precipitant was dried overnight in a desiccator at room temperature. All products from these extractions were kept at −20°C until carbon isotope analyses were performed. According to the references mentioned above, fractionation of carbon isotopes did not occur during the extraction processes.

Carbon isotope analysis

The carbon isotope ratios in delta notation were expressed as δ¹³C (%o) = \[\frac{R_{\text{sample}} - R_{\text{standard}}}{R_{\text{standard}}} \times 1000\] where R is the molar ratio of ¹³C/¹²C. δ¹³C was measured in an Isochrom isotope-ratio mass spectrometer (Fison Instrument Inc., Manchester, UK) at Biosphere 2 Center (B2C). δ¹³C of the leaf organic components was analyzed either at B2C or with an Europa 20/20 continuous flow (CF) isotope ratio mass spectrometer (IRMS) coupled with an ANCA NT combustion system at Lamont-Doherty Earth Observatory (PDZ-Europa, Cheshire, UK). NIST sucrose was used as the standard for intermachine calibration. All δ¹³C values are expressed relative to Pee Dee Belemnite (PDB).

The mixing model of Keeling (1958, 1961) was used to calculate the isotope ratio of CO₂ respired by a leaf:

\[\delta^{13}C_{\text{cham}} = \left[\frac{\text{CO}_2_{\text{atm}}}{\delta^{13}C_{\text{atm}} - \delta^{13}C_R} + \delta^{13}C_R\right] \times \frac{\text{CO}_2_{\text{cham}}}{\text{CO}_2_{\text{atm}}}\]

where [CO₂] is the concentration of CO₂ and δ is the stable isotope ratio of CO₂. The subscripts cham, atm and R represent the air within the chamber, the air in experimental atmosphere and respiratory CO₂, respectively. Geometric mean regressions were used to establish the linear relationship between δ¹³C_{cham} and 1/[CO₂_{cham}] (Pataki et al., 2003) and the intercept at the Y axis is the δ¹³C value of leaf respiratory CO₂ (Fig. 1b).

Statistical analysis

A one-way analysis of variance (ANOVA) was used to test the species effects on the possible differences of δ¹³C between the respiratory CO₂ and leaf organic components. Effects were
considered to be significant at the 0.05 probability level. In addition, a student's t-test was used for multiple comparisons among *P. deltoides* grown in three CO₂ concentrations to evaluate the effects of CO₂ treatments.

Linear regressions were used to analyze the relationship between δ¹³Cₚ and δ¹³C of the leaf organic components of all samples or averages of each species/treatment combination. Data from similar leaf-level studies (Duranceau *et al.*, 1999, 2001; Ghashghaie *et al.*, 2001; Tcherkez *et al.*, 2003), which had three C₃ crop species in three sets of environmental or genetic treatments, were included in the regression. We assume that the average δ¹³C of all sugars and water soluble materials (soluble sugar and organic acids) analyzed in those studies are equivalent to the 'soluble sugar' and 'water soluble fraction' in our study.

### Results

The isotopic signatures of the measured pools exhibited a similar pattern for all five species and the three CO₂ treatments for *P. deltoides*. In each case, leaf δ¹³Cₚ was the most positive, followed by the δ¹³C of starch (except *M. paradisiacal*), while the δ¹³C of the bulk organic matter was the lightest (Fig. 2). The amount of ¹³C-enrichment in leaf δ¹³Cₚ was 3.5–5.9‰ relative to soluble sugar (Table 1), the assumed major substrate for dark respiration. Compared with the water soluble fraction, starch, and bulk organic matter, the amount of ¹³C enrichment in respiratory CO₂ was 2.7–5.2‰, 1.4–4.2‰, and 4.1–6.9‰, respectively, depending on species (Table 1). During the sampling period, leaves released < 0.001 g carbon, which should not
The amount of $^{13}$C enrichment in respiratory CO$_2$ (%o) in comparison with leaf organic components, shown by mean ± SEM (n = 3–6). The table below presents the data for different species.

<table>
<thead>
<tr>
<th>Species</th>
<th>Soluble Sugar</th>
<th>Water Soluble Fraction</th>
<th>Starch</th>
<th>Protein</th>
<th>Organic Matter</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. deltoides</em> Bartr. (Ambient CO$_2$)</td>
<td>3.83 ± 0.37</td>
<td>3.81 ± 0.52</td>
<td>2.32 ± 0.37</td>
<td>3.30 ± 0.98</td>
<td>4.27 ± 0.53</td>
</tr>
<tr>
<td><em>P. deltoides</em> Bartr. (800 p.p.m.)</td>
<td>5.24 ± 1.68</td>
<td>4.90 ± 1.73</td>
<td>4.39 ± 1.37</td>
<td>-</td>
<td>6.46 ± 1.73</td>
</tr>
<tr>
<td><em>P. deltoides</em> Bartr. (1200 p.p.m.)</td>
<td>4.62 ± 0.86</td>
<td>4.25 ± 1.24</td>
<td>3.70 ± 1.80</td>
<td>-</td>
<td>6.83 ± 1.80</td>
</tr>
<tr>
<td><em>M. paradisiaca</em></td>
<td>5.86 ± 0.16</td>
<td>3.71 ± 0.29</td>
<td>4.06 ± 0.46</td>
<td>-</td>
<td>5.55 ± 0.47</td>
</tr>
<tr>
<td><em>C. arabica</em></td>
<td>5.12 ± 0.67</td>
<td>5.11 ± 0.68</td>
<td>2.77 ± 0.52</td>
<td>-</td>
<td>6.14 ± 0.57</td>
</tr>
<tr>
<td><em>E. pinnatum</em></td>
<td>3.74 ± 0.51</td>
<td>2.67 ± 0.16</td>
<td>1.91 ± 0.83</td>
<td>2.88 ± 0.33</td>
<td>4.05 ± 0.55</td>
</tr>
<tr>
<td><em>C. racemosa</em></td>
<td>3.40 ± 0.39</td>
<td>3.49 ± 0.46</td>
<td>1.28 ± 0.24</td>
<td>-</td>
<td>5.16 ± 0.36</td>
</tr>
<tr>
<td>ANOVA of Species Effect</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.066</td>
</tr>
</tbody>
</table>

One way ANOVA results of the species effect of ambient grown plants are listed in the last row (P < 0.05 are considered significant). In *P. deltoides*, $^{13}$C enrichments in respiratory CO$_2$, relative to all organic components are not significantly influenced by the growth [CO$_2$] (multicomparison with student’s t-tests, P = 0.10–0.87).

The correlation between leaf $\delta^{13}$C$_{org}$ (y) and leaf $\delta^{13}$C of leaf organic components (a, soluble sugar; b, water soluble fraction; c, starch; d, organic matter). Data from previous similar studies at leaf scale are also included. $R^2$ of the regression line (in dashes) and the mean of $^{13}$C-enrichment are shown. For all organic components compared, the slopes of linear regression are close to 1 (solid line, P = 0.08–0.96). Each point represents a data set from a single species or treatment, shown as mean ± SEM among leaves.

Fig. 3 Correlation between leaf $\delta^{13}$C$_{org}$ (y) and leaf $\delta^{13}$C of leaf organic components (a, soluble sugar; b, water soluble fraction; c, starch; d, organic matter). Data from previous similar studies at leaf scale are also included. $R^2$ of the regression line (in dashes) and the mean of $^{13}$C-enrichment are shown. For all organic components compared, the slopes of linear regression are close to 1 (solid line, P = 0.08–0.96). Each point represents a data set from a single species or treatment, shown as mean ± SEM among leaves.

The correlation between leaf $\delta^{13}$C$_{org}$ and $\delta^{13}$C of the leaf organic components was highly significant (P < 0.01). On average, the differences were not statistically significant (Table 1), partly due to the large variation of leaf $\delta^{13}$C$_{org}$ in the MB and WB of the IFB.
regression line was close to 1 (F-test to compare actual slope and 1, \( P = 0.08 \)–0.98).

**Discussion**

Our results obtained with the leaf scale Keeling plot method are comparable to previous studies using a CO\(_2\) free respiration chamber (Park & Epstein, 1961; Jacobson et al., 1970; Smith, 1971; Durancieau et al., 1999; Ghashghaie et al., 2001; Damesin & Lelarge, 2003; Tcherkez et al., 2003). O‘Leary (1981) pointed out that the CO\(_2\) free environment might influence stomatal conductance and the extent of anaplerotic respiratory CO\(_2\) fixation, but the effect on leaf \( \delta^{13}C_R \) has not been experimentally evaluated. The 'leaf Keeling plot' approach maintains the leaf under CO\(_2\) concentrations closer to their initial growth conditions (not more than 150 p.p.m. above background level, which is higher than the natural atmosphere in our study because of the plant and soil respiration in the glasshouse). In addition, the small air sampling flask (100cc) used is significantly more convenient for field measurement in remote areas. However, because leaf \( \delta^{13}C \text{of } C_4 \text{ plant} \) is similar to surrounding ambient CO\(_2\) (–7%o to –15%o vs. –8%o), the change in the \( \delta^{13}C \) within the respiration chamber CO\(_2\) is not large enough (Pataki et al., 2003) to use the leaf-scale Keeling plot method with \( C_4 \) plants directly (data not shown).

In all five \( C_3 \) species we studied, respiratory CO\(_2\) was more \( 13\text{C-enriched} \) than the leaf organic components. Compared with soluble sugar, leaf \( \delta^{13}C_R \) was 3.5%o to 5.9%o more positive, which is consistent with the observation of Ghashghaie et al. (2001) and Durancieau et al. (1999, 2001) in crop plants. Therefore, the results led us to reject hypothesis (1) and conclude that a 3%o to 6%o \( 13\text{C-enrichment} \) relative to major respiratory substrate is widespread in leaf respiratory CO\(_2\) from \( C_3 \) plants. The statistical significance found in the species is a basis for rejecting hypothesis (2) as well. Interestingly, in \( P. \text{ Detoides} \), the growth CO\(_2\) concentration did not influence the pattern of \( 13\text{C-enrichment} \) to a significant extent, supporting our hypothesis (3). We attribute the large variation in the amount of \( 13\text{C-enrichment} \) in 800 p.p.m. and variable tank CO\(_2\) injections. The variable background air CO\(_2\) isotope signature could increase the isotopic heterogeneity in the substrate pool and ultimately, the variation in leaf \( \delta^{13}C_R \).

Tcherkez et al. (2003) concluded that leaf \( \delta^{13}C \) in \( C_3 \) plants is determined by (1) the carbon source used for respiration (2) possible isotope effects of respiratory enzymes, and (3) non-statistical distribution of \( 13\text{C} \) in glucose. It is difficult to justify that an isotopically heavier respiratory substrate was used to any significant extent in addition to the pools measured here (particularly in light of the good correlations between putative substrates and \( \delta^{13}C \text{R} \); Fig. 3). Also, previous studies on mesophyll protoplasts (Lin & Ehleringer, 1997) indicated that fractionation likely does not occur in the main stream of respiratory enzyme reactions (glycolysis and TCA cycle). Instead, our synthesis of current results suggest that the non-statistical distribution of \( 13\text{C} \) in sugars (Rossmann et al., 1991) is the most reasonable explanation for \( 13\text{C-enriched} \) respiratory CO\(_2\). In dark respiration, the C-3 and C-4 of carbon atoms of glucose are \( 13\text{C-enriched} \) (20.9%o in average), and are released early in glycolysis. The other 4 carbon atoms are isotopically lighter (27.1%o in average) and can enter secondary metabolisms through the TCA cycle. Thus a greater contribution of the C-3 and C-4 carbon atoms to respired CO\(_2\) would result in the isotopically heavier \( \delta^{13}C_R \) we observed. Based on the results of Rossmann et al. (1991), we estimate that a 3%o enrichment in \( 13\text{C} \) of respiratory CO\(_2\) requires that 82% of the respired carbon be driven from C-3 and C-4. Although this number is possible, it is critical to conduct a more complete carbon budget for the entire leaf in future studies. Experiments similar to those of Lin and Ehleringer (1997), culturing protoplast in substrates labeled with \( 13\text{C} \) on certain carbon atoms, may directly illustrate the origin of respiratory CO\(_2\).

The concept of discrimination, defined as \( \Delta = (\delta_{\text{source}} - \delta_{\text{product}})/\delta_{\text{source}} - 1 \), applies to reactions with distinguishable source and product. However, in dark respiration, diverse substrates can be oxidized and a variety of compounds can be produced. Generation of CO\(_2\) is only one branch of the overall metabolic network and it is not clear how an enzyme isotope effect (e.g. pyruvate dehydrogenase, Deniro & Epstein, 1977) may influence the overall \( \delta^{13}C_R \). Therefore we suggest that more suitable terminology is needed the \( 13\text{C-enrichment of leaf respiratory CO}_2 \), \( C_3 \) plants.

The strong correlation between leaf \( \delta^{13}C_R \) and \( \delta^{13}C \) of leaf organic components suggest that in \( C_3 \) plants the amount of \( 13\text{C-enrichment} \) is limited within a narrow range based on the similar mechanism involved. Therefore, it is possible to scale up leaf level results to predict leaf \( \delta^{13}C_R \) at the ecosystem level. For example, the average \( 13\text{C-enrichment of leaf } \delta^{13}C \text{R relative to bulk organic matter (5.8%o, Table 1) can be used to approximate a whole } C_3 \text{ canopy. Obviously, a rigorous estimate of ecosystem scale effects would require more species specific data, as well as the effect of environmental factors (temperature, moisture, etc.) on the amount of } 13\text{C-enrichment in respiratory CO}_2 \).

Synthesizing the currently available data, we found that significant inconsistency occurs in the results between the leaf and ecosystem levels. In most cases, \( \delta^{13}C \) of soil respiration, a much larger component than foliar respiration (Law et al., 2001), is more positive than ecosystem respiration, indicating relatively \( 13\text{C-depleted vegetation } \delta^{13}C_R \). The scale difference may originate from the fact that, leaf respiration has fairly homogenous substrates and generates CO\(_2\) relatively 'instantaneously'; while at the ecosystem level, photo-assimilated carbon is released over much longer timescales as a 'lagged
and prolonged flux, which reflect more heterogeneous pools. Clearly, caution must be taken when predicting vegetation δ13C by scaling up leaf level results. At the leaf scale, further studies are required to understand (1) long-term patterns (ontogenetic, season and annual) of leaf δ13C, (2) the contribution and (3) representativeness of leaf respiration to total plant respiration. Alternatively, direct comparisons of δ13C of assimilated and respired CO2 at the ecosystem level could also provide critical information to create more robust models.

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References


