RAPID COMMUNICATIONS IN MASS SPECTROMETRY
*Rapid Commun. Mass Spectrom.* 2005; **19**: 1381–1391
Published online in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/rcm.1912

# Post-photosynthetic fractionation of stable carbon isotopes between plant organs—a widespread phenomenon†

**Franz-W. Badeck[1]*, Guillaume Tcherkez[2], Salvador Nogués[2‡], Clément Piel[3] and Jaleh Ghashghaie[2]**

[1]Potsdam Institute for Climate Impact Research (PIK), P.O. Box 601203, 14412 Potsdam, Germany
[2]Laboratoire d'Ecophysiologie Végétale, UMR 8079, Bât.362, Université de Paris XI, 91405-Orsay Cedex, France
[3]Chair of Grassland Science, Technical University of Munich (TUM), Am Hochanger 1, D-85350 Freising-Weihenstephan, Germany

Received 1 November 2004; Revised 6 March 2005; Accepted 6 March 2005

**Discrimination against $^{13}C$ during photosynthesis is a well-characterised phenomenon. It leaves behind distinct signatures in organic matter of plants and in the atmosphere. The former is depleted in $^{13}C$, the latter is enriched during periods of preponderant photosynthetic activity of terrestrial ecosystems. The intra-annual cycle and latitudinal gradient in atmospheric $^{13}C$ resulting from photosynthetic and respiratory activities of terrestrial plants have been exploited for the reconstruction of sources and sinks through deconvolution by inverse modelling. Here, we compile evidence for widespread post-photosynthetic fractionation that further modifies the isotopic signatures of individual plant organs and consequently leads to consistent differences in $\delta^{13}C$ between plant organs. Leaves were on average 0.96‰ and 1.91‰ more depleted than roots and woody stems, respectively. This phenomenon is relevant if the isotopic signature of $CO_2$-exchange fluxes at the ecosystem level is used for the reconstruction of individual sources and sinks. It may also modify the parameterisation of inverse modelling approaches if it leads to different isotopic signatures of organic matter with different residence times within the ecosystems and to a respiratory contribution to the average difference between the isotopic composition of plant organic matter and the atmosphere. We discuss the main hypotheses that can explain the observed inter-organ differences in $\delta^{13}C$.** 

Enzymatic and diffusional fractionation processes lead to discrimination against $^{13}C$ during photosynthesis that varies between $C_3$, $C_4$, and CAM plants.[1] In $C_3$ plants, $CO_2$ is fixed within the Calvin cycle yielding $C_3$ compounds as first products and an isotopic signature that varies between −21‰ and −35‰. In $C_4$ plants, $CO_2$ is first fixed in $C_4$ organic acids that transport the carbon to separate tissues where sugar synthesis within the Calvin cycle takes place. The isotopic composition ranges between −9‰ and −20‰. CAM plants have an intermediate isotopic signature between $C_3$ and $C_4$ with variation depending on the proportion of carbon fixed in the dark into $C_4$ organic acids or in the light via the Calvin cycle. For instance, under water-deficient conditions, CAM plants open their stomates and fix $CO_2$ mainly at night time via the $C_4$ pathway accumulating malic acid. During the day, they close their stomates avoiding dehydration, and then the malic acid is decarboxylated providing $CO_2$ to the Calvin cycle.

*Correspondence to: F.-W. Badeck, Potsdam Institute for Climate Impact Research (PIK), P.O. Box 601203, 14412 Potsdam, Germany.
E-mail: badeck@pik-potsdam.de
†Presented at the Joint European Stable Isotope Users Group Meeting, Vienna, 30 August–3 September, 2004.
‡Present address: Departament de Biologia Vegetal, Universitat de Barcelona, 645 Diagonal Av., Barcelona, Spain.

Photosynthesis leaves enriched $CO_2$ behind in the atmosphere. Carbon stored in plant materials is released to the atmosphere by respiratory processes. Therefore, the atmospheric $CO_2$ is relatively more enriched during summer when carbon fixation by photosynthesis is much greater than release by respiration. The degree of limitation of photosynthesis by resistances to diffusion of $CO_2$ to the sites of carboxylation and by photosynthetic capacities and the corresponding draw down of $CO_2$ is variable and hence also photosynthetic discrimination ($\Delta$) varies with the relative contribution of enzymatic and diffusional fractionation processes ($b$ and $a$, respectively). This variation has been shown to be a linear function of the ratio of $CO_2$ partial pressure in the leaf interior sub-stomatal cavities and air surrounding the leaf ($p_i$ and $p_a$, respectively) [i.e. $\Delta = a + (b-a)\ p_i/p_a$].[2] All the factors changing stomatal conductance and/or photosynthetic capacity (e.g. water deficit, light, vapour pressure deficit), thus changing $p_i/p_a$, will also change the photosynthetic discrimination. Stomata are apertures in the leaf surface that facilitate gas exchange and can be actively regulated by the plant.

The seasonal signal of $\delta^{13}C$ in atmospheric $CO_2$ and the variation of discrimination with the water relations of the plants open up a wide range of applications for isotopic studies. They can be used to partition fluxes in the

ecosystem,[3] to determine the fate of carbon of $C_3$ vegetation in $C_4$ soils, and vice versa,[4] to study resource use in parasite-host systems,[5,6] or to detect the time delay with which C is respired via root and soil respiration.[7] In applying these methods it needs to be known if the isotopic signature of plant materials derived from photosynthesis can further be altered by post-photosynthetic fractionation processes. Evidence for the operation of (photo)respiratory fractionation in leaves has recently been reviewed.[8] Results published subsequently have confirmed that leaves and whole shoots respire $CO_2$ enriched relative to the respiratory substrate for five woody species[9] and four cultivated herbaceous species,[10,11] respectively. The isotopic signatures of plant materials can be further modified if fractionation occurs during transport of metabolites or if respiratory fractionation is different in different organs. If this is the case, then the useful simplifying assumption that all plant organs built with substrates of the same photosynthetic origin will have an identical isotopic composition[12] cannot be verified.

Post-photosynthetic fractionation at the biochemical level is a well-documented phenomenon. Fractionation due to equilibrium and kinetic isotopic effects determines differences in isotopic signatures between metabolites and intramolecular positional isotopic effects.[13,14] Here, we only refer to these differences where they are relevant for the interpretation of isotopic signatures of whole plant organs. Remarkable differences between $\delta^{13}C$ of different plant organs have been documented for organic matter or specific biochemical substances in many cases. For example, with some exceptions, leaves are generally isotopically lighter than the other plant organs[15] and it has been postulated that this is due to a generic difference between autotrophic (gaining energy from photosynthesis) and heterotrophic (gaining energy from respiration of organic molecules) organs.[16]

However, at present no clear-cut conclusions can be drawn from the observation of differences in $\delta^{13}C$ of different organs. *A priori* it cannot be ruled out that these differences are an occasional product of the use of substrates produced at different times under different environmental and ontogenetic conditions (as, e.g., proposed by Francey and coworkers[17] to explain the differences between leaves and wood). For reasons of parsimony this is a hypothesis to be favoured because it can potentially explain the phenomenon on the grounds of already known sources of variation in photosynthetic discrimination in the course of time. However, a metabolic origin of the variation of $^{13}C$ abundance between organs cannot be ruled out *a priori*. In search of alternative hypotheses explaining the differences between C-isotopic compositions of organs, it can be proposed that: (1) discrimination occurs during the transport of assimilates; (2) the metabolites used for export are enriched in $^{13}C$ (e.g. sucrose) with respect to the photosynthetic products; (3) respiratory processes discriminate against $^{13}C$ at different degrees in different organs along with different types of metabolic pathways; (4) the biochemical composition varies from one organ to another along with characteristic signatures of metabolites; and (5) different rates of carboxylation for replenishment of Krebs cycle intermediates (via PEP-carboxylase, PEPc, EC 4.1.1.31) lead to varying rates of incorporation of relatively heavy carbon (some of these hypotheses were formulated earlier[18–21]).

The aim of the present work is to (a) check the consistency of the difference in $\delta^{13}C$ of autotrophic and heterotrophic organs with an analysis of published data and (b) to evaluate the alternative hypotheses that can potentially explain these differences. We also report some supplementary unpublished data on French bean plants.

## RESULTS

### Differences between $\delta^{13}C$ of organs and metabolites from published sources

Published results on $\delta^{13}C$ of organic matter or a specific substance class were compiled from studies that reported at least results for leaves and one other organ (see list in the Appendix). Data were not normally distributed. The hypothesis of zero difference between sample means was tested with the Wilcoxon signed rank test for paired samples. Statistical analyses were done with R.[22] For 410 reported results on isotopic composition of plant organs, leaves were on average 1.26‰ more depleted in $^{13}C$ than other plant organs (see Table 1 and Fig. 1). In only 38 cases were leaves found to be isotopically heavier; in 39 cases the difference was smaller than 0.2‰. A total of 333 comparisons resulted in leaves being more than 0.2‰ more depleted in $^{13}C$. Nine out of the 39 cases where leaves were $^{13}C$-enriched by more than 0.2‰ were measured on *Pinus edulis* branch vs. leaves including the most extreme value. For 38 reported results on isotopic composition of total belowground and aboveground plant

**Table 1.** Differences in $^{13}C$-isotopic signature of organic mass or classes of metabolites of different plant organs ($p$ = error level for rejection of null hypothesis of zero difference)

| | Belowground—aboveground | Other organs—leaves | Roots—leaves, $C_3$ | Roots—leaves, $C_4$ |
|---|---|---|---|---|
| Average | 1.23 | 1.26 | 1.08 | 0.09 |
| Minimum | −1.4 | −2.06 | −0.43 | −1.8 |
| Maximum | 5.64 | 8.4 | 3.5 | 1.5 |
| Standard error of the mean | 0.19 | 0.07 | 0.09 | 0.28 |
| $p$ | <0.001 | <0.001 | <0.001 | 0.68 |
| n | 38 | 410 | 78 | 10 |
| n with diff. < −0.2 | 1 | 38 | 1 | 2 |
| n with diff. > +0.2 | 33 | 333 | 71 | 4 |
| n with −0.2 < diff. < 0.2 | 4 | 39 | 6 | 4 |

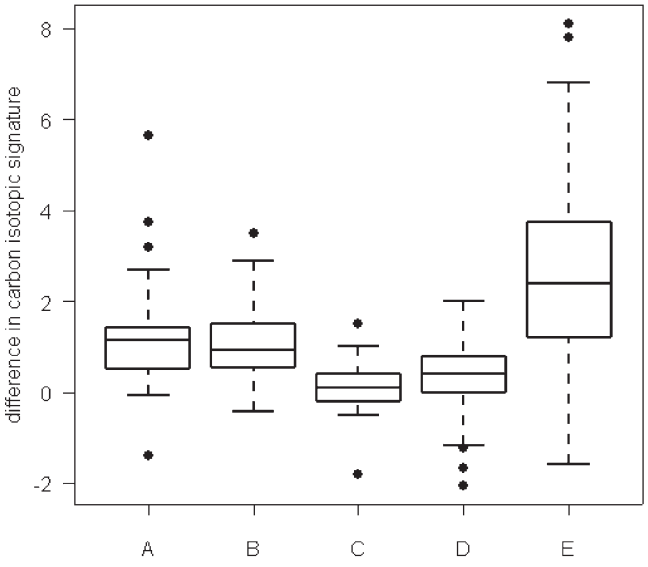


**Figure 1.** Differences between isotopic carbon signatures of plant organs: A) belowground—aboveground; B) roots—leaves, $C_3$ plants; C) roots—leaves, $C_4$ plants; D) non-woody stems—leaves; E) woody stems—leaves. The line in the middle of the box represents the median. The box spans from first to third quartile. The whiskers are at the level of the most distant data point within 1.5 times the interquartile distance from the box.

organs, the aboveground fraction was on average 1.2‰ more depleted in $^{13}C$ than the belowground fraction. These overall figures include classes of comparison with contrasting results. The 88 comparisons of root and leaf organic mass resulted in an average difference of 0.96‰. This is the only case for which sufficient data ($n > 9$ for individual groups) were available to analyse $C_4$ and $C_3$ vegetation separately. Roots of $C_3$ plants were always $^{13}C$-enriched (average 1.08‰) compared with leaves or had a statistically non-distinguishable signature, with one exception of wheat plants at ripeness where the difference was −0.43‰. The differences of the means mentioned up to here were all significantly different from zero at $p < 0.001$. As opposed to roots of $C_3$ plants, $C_4$ roots did not show a statistically significant difference compared with leaves ($n = 10$). In four cases the difference was less than 0.2‰. In two cases roots were isotopically lighter, in four cases heavier than leaves. In some cases also stems were found to be $^{13}C$-depleted compared with leaves. This was the case for woody stems (average difference 1.91, minimum −2.0, maximum 8.1, $n = 116$) as well as non-woody stems (average difference 0.32, minimum −2.1, maximum 2.0, $n = 90$). Thus, especially non-woody stems had isotopic signatures close to leaf tissues.

Cellulose was on average 1.29‰ $^{13}C$-enriched compared with the organic mass of the respective organ while lipids and lignins were 4.67‰ and 3.17‰ depleted, respectively (Table 2). Sugars and starch as cellulose were $^{13}C$-enriched compared with the organic mass of the organ from which they were extracted.

## DISCUSSION

### Differences between heterotrophic and autotrophic organs

Our analysis of published data of plant organ isotopic composition shows a consistent difference between leaves and other organs. Roots and woody stems were isotopically heavier than leaves, while non-woody stems showed only a slight similar trend for higher $\delta^{13}C$ than leaves. In 12% of the comparisons for non-woody stems a difference more negative than −0.2‰ was found, while in 67% of the cases the differences were above 0.2‰. The experimental data obtained on *Phaseolus vulgaris* (Table 3) fit into this pattern.

These results add to earlier evidence in favour of post-photosynthetic fractionation processes[15,16,19,20,23] that lead to consistent inter-organ differences in $\delta^{13}C$. The special case of herbaceous stems that bear isotopic signatures closer to those measured in leaves would fit into a general pattern if the observed differences are related to the degree of heterotrophy. Herbaceous stems are mostly green photosynthesising organs and in this respect metabolically closer to leaves than to roots or woody stems.

It is unlikely that changes in photosynthetic discrimination in the course of time can explain the general trends in organ $\delta^{13}C$ differences we observed in the current study. Intrinsic ontogenetic changes generally lead to a closure of stomates and decrease in photosynthetic discrimination in the course of time. Therefore, it can probably not explain differences between leaves and roots.

In a study on rice,[24] Scartazza and coworkers investigated whether differences in the isotopic composition of organs

**Table 2.** Differences in $^{13}C$-isotopic signature for the chemical components cellulose, lipids and lignin as compared with total organic matter

| | Cellulose—organic mass | Starch—organic mass | Sugars—organic mass | Lipids—organic mass | Lignin—organic mass |
|---|---|---|---|---|---|
| Average | 1.29 | 2.02 | 1.71 | −4.68 | −3.17 |
| Minimum | −1.3 | 0.0 | −3.8 | −8.8 | −6.7 |
| Maximum | 4.2 | 4.1 | 7.7 | −0.6 | 1.6 |
| Standard error of the mean | 0.08 | 0.21 | 0.3 | 0.23 | 0.30 |
| $p$ | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| n | 107 | 34 | 46 | 84 | 37 |
| n with diff. < −0.2 | 5 | 0 | 6 | 84 | 33 |
| n with diff. > +0.2 | 98 | 32 | 36 | 0 | 2 |
| n with −0.2 < diff. < 0.2 | 4 | 2 | 4 | 0 | 2 |

**Table 3.** Isotopic signature of carbon in total organic matter (TOM), starch, heat precipitated proteins (HPP) and sucrose of different organs of *Phaseolus vulgaris* L. cv. Contender. Culture conditions and analyses methods as in previous experiments[293346] (mean ± SD, n = 3, and n = 1 for data without SD)

| Organ | TOM | Starch | HPP | Sucrose |
|---|---|---|---|---|
| Bud | −29.11 ± 0.46 | −27.64 ± 0.41 | −28.61 ± 0.34 | −28.61 ± 0.03 |
| Cotyledon, leaf blade | −28.49 ± 0.16 | −27.87 ± 0.49 | −27.25 ± 0.36 | −28.30 ± 0.62 |
| Cotyledon, leaf veins | — | −26.37 ± 0.28 | −27.19 ± 0.43 | −28.46 ± 0.10 |
| Cotyledon, leaf petiole | — | −26.41 ± 0.73 | −25.88 ± 0.36 | −28.40 ± 0.17 |
| First leaf blade | −29.1 ± 0.42 | −28.28 ± 0.39 | −28.11 ± 0.24 | −28.45 ± 1.04 |
| First leaf veins | — | −27.94 ± 0.58 | −27.58 ± 0.76 | −28.32 ± 0.44 |
| First leaf petiole | — | −27.69 ± 1.11 | −28.54 | −28.22 ± 0.48 |
| Second leaf blade | −29.01 ± 0.69 | −27.51 ± 0.08 | −28.02 ± 0.26 | −28.56 ± 0.52 |
| Second leaf veins | — | −27.62 ± 0.28 | −28.00 ± 0.48 | −27.45 ± 0.19 |
| Second leaf petiole | — | −28.92 | −27.81 | −27.23 ± 0.39 |
| Third leaf (whole) | −29.14 ± 0.51 | −27.68 ± 0.51 | −28.80 ± 0.35 | −28.53 ± 0.34 |
| Root | −27.63 ± 0.13 | −26.50 ± 0.51 | −27.98 ± 0.39 | −28.17 ± 0.70 |
| Stem | −28.97 ± 0.27 | −27.73 ± 0.28 | −26.90 ± 0.61 | −28.67 ± 0.14 |

could be explained as a consequence of the use of assimilates that had been produced at different times with different overall photosynthetic discrimination. They found that even plants that were well watered all the time showed differences in $\delta^{13}C$ between organs and concluded that different photosynthetic discrimination in the course of the time cannot explain all the observed variation in isotopic composition.

In addition, studies of heterotrophic metabolism have provided further evidence for post-photosynthetic fractionation processes. Heterotrophically growing plant organs (tomato and tobacco) exhibit a more positive $\delta^{13}C$ than the substrates used for growth, irrespective of the use of externally supplied sugars or sugars imported from other plant organs.[19] $^{13}C$ signatures in young expanding leaves of several tropical tree species were less negative than they were in adjacent older, mature leaves.[25] This difference could not be explained by differences in $p_i/p_a$. The young leaves had higher $p_i/p_a$ ratios; consequently, a higher discrimination can be expected in the younger leaves. Also, the anatomical changes in the course of early leaf ontogeny should lead to increases in the resistance to gas transport in the course of time and thus impose a trend of decreasing discrimination, which in turn cannot explain the observed differences between old and young leaves. The author could convincingly show that seasonal variations in the environmental conditions and air $\delta^{13}C$ can be ruled out as explanation for the observed phenomenon.

Also, the result that sugars and starch were on average less depleted in $^{13}C$ than organic mass (Table 2) can be taken as an indication of post-photosynthetic discrimination. Generally, sugars and starch will represent the isotopic signature of recent photosynthetic products.[1] The wide range of variation in the difference between sugars and organic mass $\delta^{13}C$ can be expected because photosynthetic discrimination varies with stomatal aperture. However, on average, organic mass was more negative than sugars, indicating an overall apparent discrimination between primary photosynthetic products and the bulk organic matter.

The simplest hypothesis to be favoured, as long as it cannot be falsified, states that consistent differences in isotopic composition of plant organs can be explained by varying isotopic signature of assimilates produced at different times. On the basis of the results discussed so far we conclude that this hypothesis does not have a high explanatory power. There are good reasons to look for alternative hypotheses because the simplest hypothesis (i.e. the one to be favoured according to Occam's razor) cannot explain the main trends in the observations.

## $\delta^{13}C$ of organic matter in organs and fractionation during biosynthesis

If the phenomenon we seek to understand is not due to a simple difference in time of carbon assimilation at different discrimination, then it must be due to additional fractionating processes during the building up of the considered organs. A corollary of this statement is that there exists a difference in the isotopic signature of incoming and outgoing fluxes. The exchange fluxes that can potentially carry different signatures are: respiration, carbon fixation via carboxylases, import and export (including phloem and xylem transport, ablation of surface waxes, exudation, losses due to foraging of selected components by parasites and predators, and root uptake of organic substances).

In addition, other potential causes have frequently been invoked for the explanation of differences in isotopic composition of organs, namely differences in the biochemical composition of the organs.[24,26–28] Given that classes of biochemical substances do not have the same isotopic signature (Table 2), a high depletion of an organ in $^{13}C$ can be correlated with high lignin or lipid contents, while high cellulose content is expected to correlate with a relatively low depletion. However, the chemical composition of an organ cannot be the cause of a difference in the isotopic composition of two organs because of mass balance, but only a consequence of another exchange process that induces the overall difference. For example, even a high production rate of, e.g., lipids, leading to a high fraction of the total mass that is strongly depleted in $^{13}C$, cannot lead to a depletion of $^{13}C$ in organic matter, because the heavy carbon atoms not used in lipid synthesis will reside in other organ components that are isotopically heavier than the average organic mass. A correlation of high concentration of depleted compounds and organic matter that is depleted relative to the source can

only result (a) if fractionation occurs during respiration and heavy carbon atoms are respired preferentially, (b) if carbon export of isotopically heavy compounds occurs, or (c) if carboxylation reactions add carbon to the organ with an isotopic signature different from the signature of respiratory substrates. Thus, differences in chemical composition cannot be the cause of correlated differences in isotopic signature but only a corollary of exchange fluxes that enrich or deplete the organ under consideration.

## Discrimination during transport

The comparison of sugars in leaf blades, petioles and major veins (Table 3) as well as sugars in blades and phloem (= conduits within plant stems that transport assimilates from source to sink organs) sap of *Phaseolus vulgaris* did not show a consistent isotopic difference so that fractionation during transport leading to preferential export of heavy carbon isotopes from photosynthesizing leaves cannot be proven with these data. Phloem sap was collected with the method reported by Groussol *et al*.[29] Leaf petioles were placed in 5 mmol $L^{-1}$ EDTA at pH 6 for 4 h in the dark. Sugars in second leaf blades and sugars collected from phloem sap just below the second leaves had only slightly different isotopic signatures (sucrose in blade −27.06 and −27.05, sucrose in phloem sap −27.18 and −27.85, fructose plus myo-inositol in phloem sap −27.79 and −26.62 in sample 1 and 2, respectively).

Accordingly, only a small and non-significant difference between total phloem exudates (−30.4‰) and sugars plus organic acids in leaf blades (−29.9‰) of *Triticum aestivum* plants in the ear-forming stage was reported.[15] In the grain-filling stage also only a small and non-significant difference between total phloem exudates (−29.5‰) and sugars plus organic acids in leaf blades (−29.4‰) was found. However, sugars and acids extracted from the ears had an isotopic carbon signature of −26.6‰.

Hobbie and coworkers[16] interpreted findings by Pate and Arthur[30] on differences between leaf organic matter and phloem sap sample signatures in eucalypt trees as an indication for fractionation during export. However, as Pate and Arthur pointed out the leaf dry matter signatures were expected to be more negative than phloem exudates in a dry period of the year, because the leaves had been produced during the less stressed winter/spring period prior to the stress conditions. Thus the measurements show the contrast of leaf signatures and phloem signatures of plant materials of different age and cannot be taken as evidence for fractionation during export.

The strongest indication of a possible discrimination associated with the export of carbohydrates from leaf tissues comes from measurements of isotopic signatures of sugars in leaves and current year stem sections of European beech, *Fagus sylvatica*, all throughout the growing season.[26] From the end of May to October the leaf sucrose had a 2–3‰ more negative $\delta^{13}C$ than the sucrose extracted from stems. Similar differences of 2–3‰ were reported for the same species under different environmental conditions when the canopy weighed average of leaf sugar $\delta^{13}C$ was compared with the sugars sampled from phloem at breast height.[27] In contrast to this, in another study on European beech,[31] a depletion of phloem exudates of twigs relative to organics in the water-soluble fraction of leaves in the shade crown of beech by 0.5‰ was found in July. For the sun crown a similar but non-significant trend was measured whereas phloem bleeding-sap collected at breast height was enriched by 1.1‰ relative to leaf water-soluble organics. In September no difference was found between all the leaves, twigs and base of stem.

However, these data should be interpreted with caution because stem photosynthesis can provide a fraction of the stem sugars and exchange with storage carbohydrates can occur. Depending on the balance between respiration, gas transport in the stem and resistances to the gas exchange with the air surrounding the stems, the source $CO_2$ isotopic signature for stem photosynthesis can vary between the signature of $CO_2$ released by respiration and the atmospheric $CO_2$ signature and thus the discrimination during stem photosynthesis can vary with the balance of the carbon fluxes.

While the results discussed so far were obtained on aboveground organs, we are aware of only one comparison of leaf and root sugars reported in the literature.[18] Leaf sugars were found to be 2.1‰ more depleted on average of measurements at different times of the day in *Beta vulgaris*. In *Phaseolus vulgaris* a difference with the same sign although non-significant and much smaller (0.1–0.4‰) was found (Fig. 2).

The evidence for differences between $\delta^{13}C$ of transport metabolites sampled in the source organ and the sink organ or along the transport pathway supports the notion of an apparent discrimination associated with export fluxes. However, it does not allow differentiation between two groups of potential mechanisms: Either a discrimination occurs at a step of the export process itself or the exported substrates come from a pool within the exporting cell that has a signature different from the average signature of this substrate within the cell. Hobbie and Werner[20] favoured the latter hypothesis. They proposed a fractionation of photosynthetic products in leaf cells. Carbon isotopically lighter than primary photosynthetic products would be incorporated into lipids and lignin. The remaining enriched

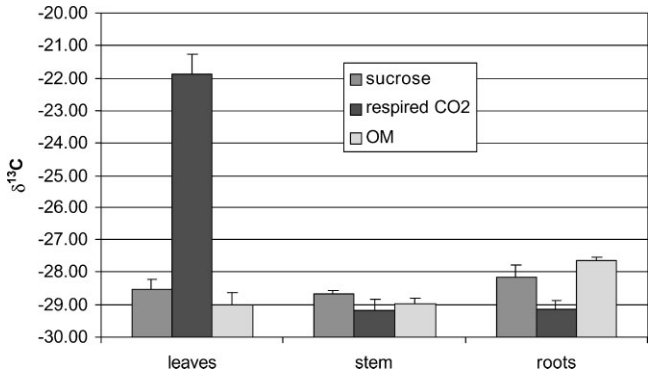


**Figure 2.** Isotopic signatures ($\delta^{13}C \pm SE$) of sucrose, respired $CO_2$ and dry organic matter in leaves, shoots and roots of *Phaseolus vulgaris*. $\delta^{13}C$ of root respired $CO_2$ was independent of the pH (varied between 2.8 and 7) used in the solution for washing the roots, indicating that it is not an artefact due to discrimination during solution/dissolution processes. The root respiration rate during these measurements was 2.11 ± 0.29 nmol $CO_2$ (g fresh weight)$^{-1}$ $s^{-1}$.

carbohydrates would be used for cellulose synthesis and export. Thus, exported carbohydrates would be less depleted than the primary photosynthetic products and so are sink organs like roots.

In summary, the hypothesis that fractionation during assimilate transport is the cause of the observed consistent differences between root and leaf carbon isotopic signatures currently cannot be rejected nor confirmed on the basis of the scarce experimental results available. More experiments with sampling of leaf source sugars and phloem sap sugars along the export paths are needed in order to further explore the potential role of fractionation during transport in post-photosynthetic fractionation processes.

## The effect of respiration

It has been shown for *Phaseolus vulgaris* leaves that $CO_2$ respired in the dark is constantly enriched by about 6‰ compared with sucrose,[32] a result confirmed by the 6.3‰ enrichment compared with sucrose as reported above for a more recent experiment on the same species. As opposed to this, the apparent discrimination in roots (Fig. 2) indicated net release of lighter carbon isotopes as compared with the root sugars. In both cases, the results on apparent discrimination are consistent with the difference found between sucrose, the potential substrate and bulk organic matter, the average product. These results suggest that release of heavy carbon in leaves leads to an organic matter that is isotopically lighter than the source molecules while in roots organic matter is relatively enriched in $^{13}C$ by preferential net release of lighter carbon isotopes.

A similar opposite apparent respiratory fractionation was found in a study[10] on whole shoots and roots of sunflower (*Helianthus annuus*), perennial ryegrass (*Lolium perenne*) and alfalfa (*Medicago sativa*). Respiratory $CO_2$ of whole shoots was enriched by 1.06–5.64‰ relative to organic mass, while the respiratory $CO_2$ of whole roots was depleted by 0.46–5.39‰ relative to organic mass (OM). At the same time roots had higher $\delta^{13}C$ than shoots, in accordance with the results reported with our review. The slope of a regression of the difference in OM signatures on the difference in respiratory discrimination (forced through the origin) indicates that, for every ‰ difference between shoot and root respiratory $CO_2$, the difference between OM signatures is about 0.26‰. This relationship is consistent with the view that respiratory discrimination is associated with biosynthesis, and the moles of carbon incorporated into biomass per moles of C consumed are approximately 0.75–0.8.

The isotopic signature of respired $CO_2$ of a given organ tended to be more negative when the respiratory quotient, RQ ($CO_2$ released per $O_2$ consumed), was lower (data not shown). The variation in $\delta^{13}C$ with RQ corresponds to results obtained on *Phaseolus vulgaris* leaves previously.[33] RQ varies with the oxidation state of the respiratory substrates. Less oxygen per mole of carbon released as $CO_2$ is required during respiration of highly oxidised substrates (e.g. organic acids) and more when highly reduced substrates (e.g. lipids) are used. At the same respiratory quotient, the $CO_2$ released by leaf respiration was always several ‰ less depleted in $^{13}C$ than the $CO_2$ released by respiration of roots and shoots (data not shown) suggesting that the signature of substrates used for respiration is not the only source of variation of the signature of respired $CO_2$.

If $^{13}C$ is preferentially released during leaf respiration[8] how can the opposite discrimination during root respiration be explained? The most promising candidate mechanism is dark-assimilation of carbon via PEPc. When apparent fractionation[8] during respiratory metabolism is measured, with the traditional gas-exchange methods currently available, the effects of carboxylation reactions cannot be factored out. In consequence, up to now the measured fractionation represents the net result of all processes exchanging $CO_2$ between organ carbon and the atmosphere. During dark respiration as well as during photosynthetic metabolism in the light, several carboxylating enzymes can potentially fix inorganic carbon and add carbon atoms to the organic matter.[34] PEPc carboxylates phosphoenolpyruvate and provides $C_4$ organic acids for the replenishment of the Krebs cycle. This function is required when organic acids are exported from the Krebs cycle either for regulation of cellular pH or as carbon skeletons for amino acid synthesis. The carbon fixed by PEPc is 5.7‰ enriched[34] in $^{13}C$ relative to source $CO_2$. Qualitatively, the incorporation of inorganic C *in vivo* by PEPc,[35] the release of some of the fixed carbon after short residence times,[36] and dilution of the label by transport of newly synthesised biomolecules from roots to other plant organs[37,38] have been demonstrated with $^{14}C$ pulse labelling. Use of organic acids in pH regulation and provisioning of carbon skeletons for amino acid synthesis both imply a high variability of PEPc activity[34] that is known to change with the type of nitrogen nutrition ($NO_3^-$ vs. $NH_4^+$),[38] availability of other nutrients in the soil solution,[39] and soil $CO_2$ partial pressure.[40,41] A general quantitative model for the rate of $CO_2$-fixation by PEPc is not yet available (however, see Raven and Farquhar[34] for a characterisation of the quantitative bounds at the whole plant level).

However, the observed rates and *in vitro* measurements of PEPc activity indicate that the PEP carboxylation can be sufficient to explain the differences between the signatures of respired $CO_2$, sugars and organic mass in roots. In *Phaseolus vulgaris*, for the results shown in Fig. 1, *in vitro* PEPc activity (measured as previously described[42,43]) was 5.3 and 29.3 nmol $s^{-1}$ (g fresh weight)$^{-1}$ in roots (comparable with the rates reported on the same species[44]) and leaves, respectively. Thus, in roots, the *in vitro* rate was higher than the net respiration rate of $2.11 \pm 0.29$ nmol $CO_2$ (g fresh weight)$^{-1}$ $s^{-1}$ suggesting that PEPc can potentially fix carbon at rates of several 10% of the gross respiration rate.

In the soil-root space most of the $CO_2$ is derived from respiration of organic molecules. The effect of variable rates of PEPc activity fixing $CO_2$ with signature of organic mass is illustrated in Fig. 3.

The model calculations show that the results obtained on the differences between the signatures of sugars compared with respired $CO_2$ and organic mass, respectively, in *Phaseolus vulgaris* roots can be explained by respiration rates and dark carboxylation rates in the range of commonly observed values. More experiments with pulse-chase labelling are needed to study the role of dark carboxylation and effects of export of amino acids and organic acids from the roots towards other organs.

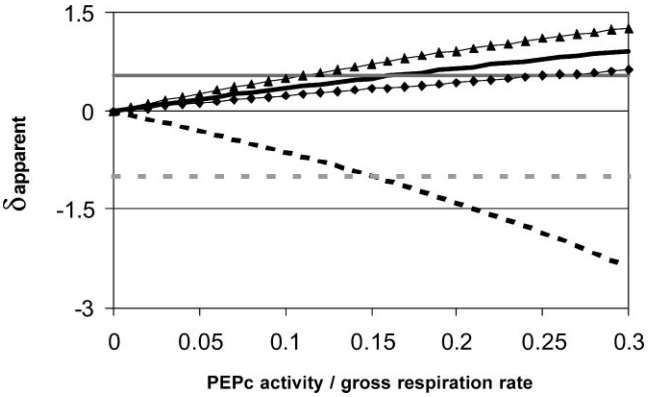


**Figure 3.** Modelled difference between signatures of respired $CO_2$ (dark broken line) and signature of organic mass (dark continuous line), respectively, and isotopic signature of source molecules for respiration and synthesis of organic mass for varying rates of PEPc activity and PEPc discriminating by −5.7‰ relative $CO_2$ in the gas phase. It was assumed that the source signature is constant in time, carbon fixed by PEPc is not subsequently respired within the Krebs cycle, and the use of respiratory substrates proceeds without discrimination. The signature in organic mass depends on the ratio of carbon used for syntheses to carbon respired, u. Three cases are shown for u = 1.5 (dark continuous line), u = 1.0 (dark continuous line with triangles), and u = 2.34 (dark continuous line with diamonds). The grey continuous line represents the difference between organic mass and sugars and the broken grey line the difference between respired $CO_2$ and sugars shown in Fig. 1 for *P. vulgaris*.

### Significance of the post-photosynthetic fractionation for ecological and physiological studies

For the application of inverse modelling the implicit or explicit[12] assumption is used that $\delta^{13}C_{plant} = \delta^{13}C_{leaf} = \delta^{13}C_{photosynthetic\ product}$, i.e. the respiratory flux always carries the signature of the photosynthetic products. We showed above that in general this is not the case in real plants.

However, if organic matter stemming from organs of different isotopic composition is respired during decomposition with an approximately constant mixture, the basic assumption could still be kept up in a modified way. This is the case if (a) the release of carbon on inter-annual time scales proceeds with virtually constant proportions from slowly and rapidly decaying fractions, (b) we are interested in averages over large areas that are relevant for inverse modelling. However, in managed ecosystems, large fractions of biomass are removed from the system (e.g. wood harvesting) and transported to distant places where they are used. A harvest flux that exports a fraction of biomass with an isotopic signature deviating from the average signature of primary photosynthetic products will bring about a horizontal isoflux. This isoflux changes the isotopic mass balance of the local and regional systems exchanging with the atmosphere. The relevance of this potential effect can be checked by an extensive comparison of tissues with low versus tissues with high residence times in the ecosystem.

Fung and coworkers[12] determined the overall discrimination during uptake of carbon by the biosphere ($\Delta_{bio}$) for the net primary production flux from estimates of photosynthetic discrimination. Thus it was assumed that autotrophic respiration releases carbon of the same signature as gross photosynthetic products. If the overall discrimination during autotrophic respiration was of the order of magnitude (0.7‰) as determined by Klumpp and coworkers,[10] this would increase the estimate of $\Delta_{bio}$ by 4–6% and change the estimated biospheric sink by about 5%.

When isoflux measurements are used to break down the different respiratory sources within an ecosystem, instantaneous differences between the signature of $CO_2$ respired by different organs as shown in Fig. 2 need to be known. These differences stem from differences in the isotopic signature of the respiratory substrate and apparent fractionation during respiration, which are both not yet well characterised, especially for roots. Therefore, further studies of the organ-specific respiratory isotopic signatures are required.

The difference between root and leaf isotopic composition can partly explain the observed profiles of $\delta^{13}C$ in soils.[45] Often the isotopic signature of organic mass in surface litter layers is close to isotopic composition of aboveground plant organs. Then, a rapid increase of $\delta^{13}C$ in soil organic matter with the first centimetres of increasing soil depth is observed, followed by a slower rise down to greater depth. Under $C_3$ vegetation the predominant input of more depleted leaf litter to the surface soil layers and less depleted dead roots to lower soil layers contributes to this observed profile.

Finally, a good understanding of post-photosynthetic fractionation in plants lays the basis for the use of measurements of isotopic signatures in gas-exchange fluxes as a non-invasive tool of studies of metabolism.

## CONCLUSIONS

When the variation of carbon isotopic signature of different plant organs due to the seasonal variation in photosynthetic discrimination is accounted for, there still remain differences between organ signatures that cannot be explained only on the grounds of the seasonal variation. Mass balance requires that these differences are associated with fractionation during exchange fluxes across the organ boundaries. The biochemical composition of the organs cannot be the cause of the observed inter-organ differences in isotopic signature but only a corollary of the exchange fluxes described above. The inter-organ differences can be caused either by fractionation associated with transport of metabolites across organ boundaries or by fractionation during heterotrophic metabolism. In the first case, either a selection of metabolites to be exported from a source organ may occur because of compartmentation within the source organ or fractionation at a metabolic branching point or the fractionation may occur during the export process itself. In the second case, fractionation along respiratory metabolic pathways may occur or dark fixation via carboxylases may introduce carbon with a signature distinct from the plant internal source carbon. Currently, there is evidence available in favour of both the two main groups of hypotheses. More studies are needed, especially on carbon import to roots and isotopic signatures of root

respired $CO_2$, in order to disprove any of the hypotheses. A good understanding of the nature of the post-photosynthetic fractionation between plant organs is required for the use of isoflux measurements in plant physiology, ecosystem studies and studies of the biogeochemistry of the terrestrial biosphere at the global scale.

## Acknowledgements

Salvador Nogués and Clément Piel acknowledge the financial support provided through the European Community's Human Potential Program under contract HPRN-CT-1999-00059, NETCARB. We thank many colleges who provided additional information to published papers and two anonymous reviewers for helpful comments and Max Hill for HPLC purification of sugars.

## REFERENCES

1. Brugnoli E, Farquhar GD. In *Photosynthesis: Physiology and Metabolism*, Leegood RC, Sharkey TD, v. Caemmerer S (ed). Kluwer: Dordrecht, 2000; 399–434.
2. Farquhar GD, O'Leary MH, Berry JA. *Aust. J. Plant Phys.* 1982; **9**: 121.
3. Yakir D, Wang XF. *Nature* 1996; **380**: 515.
4. Gleixner G, Bol R, Balesdent J. *Rapid Commun. Mass Spectrom.* 1999; **13**: 1278.
5. Pageau K, Simier P, Naulet N, Robins R, Fer A. *Aust. J. Plant Phys.* 1998; **25**: 695.
6. Richter A, Popp M, Mensen R, Stewart GR, Vonwillert DJ. *Aust. J. Plant Phys.* 1995; **22**: 537.
7. Ekblad A, Högberg P. *Oecologia* 2001; **127**: 305.
8. Ghashghaie J, Badeck F-W, Lanigan G, Nogués S, Tcherkez G, Deléens E, Cornic G, Griffiths H. *Phytochem. Rev.* 2003; **2**: 145.
9. Xu CY, Lin GH, Griffin KL, Sambrotto RN. *New Phytol.* 2004; **163**: 499.
10. Klumpp K, Schäufele R, Lötscher M, Lattanzi FA, Feneis W, Schnyder H. *Plant Cell Environ.* 2004; **28**: 241.
11. Ocheltree TW, Marshall JD. *J. Exp. Bot.* 2004; **55**: 2599.
12. Fung I, Field CB, Berry JA, Thompson MV, Randerson JT, Malmström CM, Vitousek PM, Collatz GJ, Sellers PJ, Randall DA, Denning AS, Badeck F, John J. *Global Biogeochem. Cycles* 1997; **11**: 507.
13. Schmidt HL, Gleixner G. In *Stable Isotopes, Integration of Biological, Ecological and Geochemical Processes*, Griffiths H (ed). Bios Scientific Publishers: Abingdon, 1998; 13–25.
14. Schmidt HL. *Naturwissenschaften* 2003; **90**: 537.
15. Yoneyama T, Handley LL, Scrimgeour CM, Fisher DB, Raven JA. *New Phytol.* 1997; **137**: 205.
16. Hobbie EA, Tingey DT, Rygiewicz PT, Johnson MG, Olszyk DM. *Plant Soil* 2002; **247**: 233.
17. Francey RJ, Gifford RM, Sharkey TD, Weir B. *Oecologia* 1985; **66**: 211.
18. Gleixner G, Danier HJ, Werner RA, Schmidt HL. *Plant Physiol.* 1993; **102**: 1287.
19. Terwilliger VJ, Huang J. *Phytochemistry* 1996; **43**: 1183.
20. Hobbie EA, Werner RA. *New Phytol.* 2004; **161**: 371.
21. Leavitt SW, Long A. *Nature* 1982; **298**: 742.
22. R Development Core Team, 2004. Available: http://www.R-project.org.
23. Arndt SK, Wanek W. *Plant Cell Environ.* 2002; **25**: 609.
24. Scartazza A, Lauteri M, Guido MC, Brugnoli E. *Aust. J. Plant Phys.* 1998; **25**: 489.
25. Terwilliger VJ. *Am. J. Bot.* 1997; **84**: 1693.
26. Damesin C, Lelarge C. *Plant Cell Environ.* 2003; **26**: 207.
27. Scartazza A, Mata C, Matteucci G, Yakir D, Moscatello S, Brugnoli E. *Oecologia* 2004; **140**: 340.
28. Staddon PL, Robinson D, Graves JD, Fitter AH. *Soil Biol. Biochem.* 1999; **31**: 1067.
29. Groussol J, Delrot S, Caruhel P, Bonnemain JL. *Physiol. Végétale* 1986; **24**: 123.
30. Pate J, Arthur D. *Oecologia* 1998; **117**: 301.
31. Gessler A, Rennenberg H, Keitel C. *Plant Biol.* 2004; **6**: 721.
32. Duranceau M, Ghashghaie J, Badeck F, Deleens E, Cornic G. *Plant Cell Environ.* 1999; **22**: 515.
33. Tcherkez G, Nogués S, Bleton J, Cornic G, Badeck F, Ghashghaie J. *Plant Physiol.* 2003; **131**: 237.
34. Raven JA, Farquhar GD. *New Phytol.* 1990; **116**: 505.
35. Nalborczyk E. *Acta Physiol. Plant.* 1978; **1**: 53.
36. Bryce JH, Rees TA. *Phytochemistry* 1985; **24**: 1635.
37. Hibberd JM, Quick WP. *Nature* 2002; **415**: 451.
38. Cramer MD, Lewis OAM, Lips SH. *Phys. Plant* 1993; **89**: 632.
39. Abadia J, Lopez-Millan AF, Rombola A, Abadia A. *Plant Soil* 2002; **241**: 75.
40. Viktor A, Cramer MD. *New Phytol.* 2003; **157**: 45.
41. Vuorinen AH, Kaiser WM. *J. Plant Physiol.* 1997; **151**: 405.
42. Uedan K, *Sugiyama Plant Physiol.* 1976; **57**: 906.
43. Watt M, Evans JR. *Plant Physiol.* 1999; **120**: 705.
44. Schweizer P, Erismann KH. *Plant Physiol.* 1985; **78**: 455.
45. Bird M, Kracht O, Derrien D, Zhou Y. *Aust. J. Soil Res.* 2003; **41**: 77.
46. Nogués S, Tcherkez G, Cornic G, Ghashghaie J. *Plant Physiol.* 2004; **136**: 1.

## APPENDIX

List of references from which data for the review of differences in organ-specific signatures were taken.

Abelson PH, Hoering TC. (1961) Carbon isotope fractionation of amino acids by photosynthetic organisms. Proc. Nat. Acad. Sci. USA 47: 623–632.

Arndt SK, Wanek W. (2002) Use of decreasing foliar carbon isotope discrimination during water limitation as a carbon tracer to study whole plant carbon allocation. Plant Cell and Environment 25: 609–616.

Araus JL, Santiveri P, Boschserra D, Royo C, Romagosa I. (1992) Carbon isotope ratios in ear parts of Triticale - influence of grain filling. Plant Physiology 100: 1033–1035.

Balesdent J, Girardin C, Mariotti A. (1993) Site related $\delta$13C of tree leaves and soil organic matter in a temperate forest. Ecology 74: 1713–1721

Ballentine DC, Macko SA, Turekian VC. (1998) Variability of stable carbon isotopic compositions in individual fatty acids from combustion of C4 and C3 plants: implications for biomass burning. Chemical Geology 152: 151–161.

Behboudian MH, Q. Ma, N.C. Turner, J.A. Palta (2000) Discrimination against (CO2)-C-13 in leaves, pod walls, and seeds of water-stressed chickpea. Photosynthetica. 38: 155–157.

Benner, R., M.R. Fogel, E.K. Sprague, R.E. Hodson (1987) Depletion of 13C in lignin and its implications for stable carbon isotope studies. Nature 329: 708–710.

Borland AM, Griffiths H, Broadmeadow MSJ, Fordham MC, Maxwell C. (1994) Carbon-isotope composition of biochemical fractions and the regulation of carbon balance in leaves of the C-3-crassulacean acid metabolism intermediate Clusia-Minor L growing in Trinidad. Plant Physiology 106: 493–501.

Brugnoli E, Farquhar GD. (2000) Photosynthetic fractionation of carbon isotopes. In Photosynthesis: Physiology and metabolism (ed. R. C. Leegood, T. D. Sharkey and S. v. Caemmerer), pp. 399–434. Kluwer Academic Publishers, Dordrecht.

Brugnoli, E., K.T. Hubick, S. von Caemmerer, S.C. Wong, and G.D. Farquhar (1988) Correlation between the carbon isotope discrimination in leaf starch and sugars of C-3 plants and the ratio of intercellular and atmospheric partial pressures of carbon dioxide. Plant Physiology 88: 1418–1424.

Brugnoli E, Lauteri M. (1991) Effects of salinity on stomal conductance, photosynthetic capacity, and carbon isotope discrimination of salt-tolerant (Gossypium hirsutum L.) and salt-sensitive (Phaseolus vulgaris L.) C3 non-halophytes. Plant Physiology 95: 628–635.

Brugnoli, E., M. Lauteri, and M.C. Guido (1994) Carbon isotope discrimination and photosynthesis: response and adaptation to environmental stress. In Plant Sciences 1994. Y. de Kouchkovsky and F. Larher, editors. Imprimerie Université Rennes I, Rennes. 269–272.

Cheng WX, Johnson DW (1998) Elevated CO2, rhizosphere processes, and soil organic matter decomposition. Plant and Soil 202: 167–174.

Chikaraishi Y, Naraoka H. (2001) Organic hydrogen-carbon isotope signatures of terrestrial higher plants during biosynthesis for distinctive photosynthetic pathways. Geochemical Journal 35: 451–458.

Craig H. (1953) The geochemistry of the stable carbon isotopes. Geochim Cosmochim Acta 3: 53–92.

Damesin C, Lelarge C. (2003) Carbon isotope composition of current-year shoots from Fagus sylvatica in relation to growth, respiration and use of reserves. Plant Cell and Environment 26: 207–219.

Deleens, E., N. Schwebel-Dugue, and A. Tremolieres (1984) Carbon isotope composition of lipidic classes isolated from tobacco leaves. FEBS Letters. 178: 55–58.

Desjardins T, Andreux F, Volkoff B, Cerri CC. (1994) Organic-carbon and C-13 contents in soils and soil size-fractions, and their changes due to deforestation and pasture installation in eastern Amazonia. Geoderma 61: 103–118.

Dignac MF, Bahri H, Rumpel C, Rasse DP, Bardoux G, Balesdent J, Girardin C, Chenu C, Mariotti A. (2005) Carbon-13 natural abundance as a tool to study the dynamics of lignin monomers in soil: an appraisal at the Closeaux experimental field (France). Geoderma, in press

Fotelli MN, Rennenberg H, Holst T, Mayer H, Gessler A. (2003) Carbon isotope composition of various tissues of beech (Fagus sylvatica) regeneration is indicative of recent environmental conditions within the forest understorey. New Phytologist 159: 229–244.

Francey, R.J., R.M. Gifford, T.D. Sharkey, and B. Weir (1985) Physiological influences on carbon isotope discrimination in huon pine (Lagarostrobos franklinii). Oecologia. 66: 211–218.

Gebauer G, Schulze E-D. (1991) Carbon and nitrogen ratios in different compartments of a healthy and a declining Picea abies forest in the Fichtelgebirge, NE Bavaria. Oecologia 87: 198–207.

Gebbing T, Schnyder H, Kuhbauch W. (1998) Carbon mobilization in shoot parts and roots of wheat during grain filling: assessment by C-13/C-12 steady-state labelling, growth analysis and balance sheets of reserves. Plant Cell and Environment 21: 301–313.

Gebbing T, Schnyder H. (2001) C-13 labeling kinetics of sucrose in glumes indicates significant refixation of respiratory CO2 in the wheat ear. Australian Journal of Plant Physiology 28: 1047–1053.

Gessler A, Schrempp S, Matzarakis A, Mayer H, Renneuberg H, Adams MA. (2001) Radiation modifies the effect of water availability on the carbon isotope composition of beach (Fagus sylvatica). New Phytologist 150: 653–664.

Ghashghaie J, Duranceau M, Badeck F-W, Adeline MT, Cornic G, Deléens E. (2001) d13C of CO2 respired in the dark in relation to d13C of leaf metabolites: comparison between Nicotiana sylvestris and Helianthus annuus under drought. Plant, Cell & Environment 24: 505–515.

Gleixner G, H.-J. Danier, W.R. A., and S. H.-L. (1993) Correlations between 13C content of primary and secondary plant products in different cell compartments and that in decomposing basidiomycetes. Plant Physiology. 102: 1287–1290.

Handley LL, Daft MJ, Wilson J, Scrimgeour CM, Ingleby K, Sattar MA. (1993) Effects of the ecto-mycorrhizal and vamycorrhizal fungi Hydnagium-Carneum and Glomus-Clarum on the delta-N-15 and delta-C-13 values of Eucalyptus-Globulus and Ricinus-Communis. Plant Cell and Environment 16: 375–382.

Hew, C.S., C.K.Y. Ng, S.S. Gouk, J.W.H. Yong, and S.C. Wong (1996) Variation in d13C Values For Different Plant Parts of an Oncidium Orchid. Photosynthetica. 32: 135–139.

Hobbie EA, Tingey DT, Rygiewicz PT, Johnson MG, Olszyk DM. (2002) Contributions of current year photosynthate to fine roots estimated using a C-13-depleted CO2 source. Plant and Soil 247: 233–242.

Huang Y, Eglinton G, et al. (1999) The effects of nitrogen fertilisation and elevated CO2 on the lipid biosynthesis and carbon isotopic discrimination in birch seedlings (Betula pendula). Plant and Soil 216: 35–45.

Hubick K, Farquhar G, Shorter R. (1986) Correlation between water-use efficiency and carbon isotope discrimination in diverse peanut (Arachis) germplasm. Australian Journal of Plant Physiology 13: 803–816.

Hubick K, Farquhar G. (1989) Carbon isotope discrimination and the ratio of carbon gained to water lost in barley cultivars. Plant Cell and Environment. 12: 795–804.

Ineson P, Cotrufo MF, Bol R, Harkness DD, Blum H. (1996) Quantification of soil carbon inputs under elevated CO2: C-3 plants in a C-4 soil. Plant and Soil 187: 345–350.

Ish-Shalom-Gordon N, Lin GH, Sternberg LDL. (1992) Isotopic fractionation during cellulose synthesis in 2 Mangrove species - Salinity effects. Phytochemistry 31: 2623–2626.

Ivlev AA, Pichuzhkin VI, Knyazev DA. (1995) Carbon-isotope distribution in wheat genotypes. Russian Journal of Plant Physiology 42: 345–349.

Jacobson BS, Laties GG, Smith BN, Epstein S, Laties B. (1970) Cyanide-induced transition from endogenous carbohydrate to lipid oxidation as indicated by the carbon-13 content of respiratory CO2. Biochimica et Biophysica Acta 216: 295–304.

Kennedy PG, Hausmann NJ, Wenk EH, Dawson TE. (2004) The importance of seed reserves for seedling performance: an integrated approach using morphological, physiological, and stable isotope techniques. Oecologia 141: 547–554.

Leavitt SW, Long A. (1982) Evidence for 13C/12C fractionation between tree leaves and wood. Nature 298: 742–744.

Leavitt SW, Long A. (1985) Stable-carbon isotopic composition of maple sap and foliage. Plant Physiology 78: 427–429.

Leavitt SW, Long A. (1986) Stable carbon isotope variability in tree foliage and wood. Ecology 67: 1002–1010.

Le Roux X, Bariac T, Sinoquet H, Genty B, Piel C, Mariotti A, Girardin C, Richard P. (2001) Spatial distribution of leaf water-use efficiency and carbon isotope discrimination within an isolated tree crown. Plant Cell and Environment 24: 1021–1032.

Lichtfouse E, Dou S, Girardin C, Grably M, Balesdent J, Behar F, Vandenbroucke M. (1995) Unexpected C-13-enrichment of organic components from wheat crop soils: Evidence for the in situ origin of soil organic matter. Organic Geochemistry 23: 865–868.

Lowdon JA, Dyck W. (1974) Seasonal variations in the isotope ratios of carbon in maple leaves and other plants. Canadian J. Earth Sci. 11: 79.

Macfarlane C, Warren CR, White DA, Adams MA. (1999) A rapid and simple method for processing wood to crude cellulose for analysis of stable carbon isotopes in tree rings. Tree Physiology 19: 831–835.

Macfarlane C, Adams MA, White DA. (2004) Productivity, carbon isotope discrimination and leaf traits of trees of Eucalyptus globulus Labill. in relation to water availability. Plant Cell and Environment 27: 1515–1524.

Martinelli LA, Almeida S, Brown IF, Moreira MZ, Victoria RL, et al. (1998) Stable carbon isotope ratio of tree leaves, boles and fine litter in a tropical forest in Rondonia, Brazil. Oecologia 114: 170–179.

Mary B, Mariotti A, Morel JL. (1992) Use of C-13 Variations at natural abundance for studying the biodegradation of root mucilage, roots and glucose in soil. Soil Biology & Biochemistry 24: 1065–1072.

Neilson R, L. L. Handley, D. Robinson, C. M. Scrimgeour, and D. J. F. Brown (1999) Natural abundances of N-15 and C-13 indicating physiological responses in Petunia hybrida to infection by longidorid nematodes and nepoviruses. Nematology 1: 315–320.

Nilsen ET, Sharifi MR. (1997) carbon isotopic composition of legumes with photosynthetic stems from mediterranean and desert habitats. American Journal of Botany 84: 1707–1713.

Northfelt DW, Deniro MJ, Epstein S. (1981) Hydrogen and carbon isotopic-ratios of the cellulose nitrate and saponifiable lipid fractions prepared from annual growth rings of a California redwood. Geochimica et Cosmochimica Acta 45: 1895–1898.

Ocheltree TW, Marshall JD. (2004) Apparent respiratory discrimination is correlated with growth rate in the shoot apex of sunflower (Helianthus annuus). Journal of Experimental Botany 55: 2599–2605.

Park R, Epstein S. (1960) Carbon isotope fractionation during photosynthesis. Geochim Cosmochim Acta 21: 110–126.

Park R, Epstein S. (1961) Metabolic fractionation of C13 & C12 in plants. Plant Physiology 36: 133–138.

Pate J, Arthur D. (1998) delta C-13 analysis of phloem sap carbon: novel means of evaluating seasonal water stress and interpreting carbon isotope signatures of foliage and trunk wood of Eucalyptus globulus. Oecologia 117: 301–311.

Qian JH, Doran JW. (1996) Available carbon released from crop roots during growth as determined by carbon-13 natural abundance. Soil Science Society of America Journal 60: 828–831.

Richter A, Popp M, Mensen R, Stewart GR, Vonwillert DJ. (1995) Heterotrophic carbon gain of the parasitic angiosperm Tapinanthus-Oleifolius. Australian Journal of Plant Physiology 22: 537–544.

Rieley G, Collister JW, Stern B, Eglinton G. (1993) Gas-Chromatography Isotope Ratio Mass-Spectrometry of Leaf Wax N-Alkanes from Plants of Differing Carbon-Dioxide Metabolisms. Rapid Communications in Mass Spectrometry 7: 488–491.

Robinson D, Handley LL, Scrimgeour CM, Gordon DC, Forster BP, Ellis RP. (2000) Using stable isotope natural abundances (delta N-15 and delta C-13) to integrate the stress responses of wild barley (Hordeum spontaneum C. Koch.) genotypes. Journal of Experimental Botany 51: 41–50.

Scartazza A, Lauteri M, Guido MC, Brugnoli E. (1998) Carbon isotope discrimination in leaf and stem sugars, water-use efficiency and mesophyll conductance during different developmental stages in rice subjected to drought. Australian Journal of Plant Physiology 25: 489–498.

Scartazza A, Mata C, Matteucci G, Yakir D, Moscatello S, Brugnoli E. (2004) Comparisons of delta C-13 of photosynthetic products and ecosystem respiratory CO2 and their response to seasonal climate variability. Oecologia 140: 340–351.

Schleser GH. (1990) Investigations of the d13C pattern in leaves of Fagus sylvatica L. Journal of Experimental Botany 41: 565–572.

Schleser GH. (1992) Delta-C-13 pattern in a forest tree as an indicator of carbon transfer in trees. Ecology 73: 1922–1925.

Schönwitz R, Stichler W, Ziegler H. (1986) d13C values of CO2 from soil respiration on sites with crops of C3 and C4 type of photosynthesis. Oecologia 69: 305–308.

Schweizer M, Fear J, Cadisch G. (1999) Isotopic (C-13) fractionation during plant residue decomposition and its implications for soil organic matter studies. Rapid Communications in Mass Spectrometry 13: 1284–1290.

Scott MP, J.L. Jane, and M. Soundararajan (1999) Carbon isotope ratios of amylose, amylopectin and mutant starches. Phytochemistry 52: 555–559.

Smith BN, Jacobson BS. (1976) H-2/H-1 and C-13/C-12 ratios for classes of compounds isolated from potato-tuber. Plant and Cell Physiology 17: 1089–1092.

Soe ARB, Giesemann A, Anderson TH, Weigel HJ, Buchmann N. (2004) Soil respiration under elevated CO2 and its partitioning into recently assimilated and older carbon sources. Plant and Soil 262: 85–94.

Spain A, LeFeuvre R. (1997) Stable C and N isotope values of selected components of a tropical Australian sugarcane ecosystem. Biology and Fertility of Soils 24: 118–122.

Staddon PL, Robinson D, Graves JD, Fitter AH. (1999) The delta C-13 signature of the external phase of a Glomus

mycorrhizal fungus: determination and implications. Soil Biology & Biochemistry 31: 1067–1070.

Syvertsen JP, M.L. Smith, J. Lloyd, and G.D. Farquhar (1997) Net carbon dioxide assimilation, carbon isotope discrimination, growth, and water-use efficiency of citrus trees in response to nitrogen status. Journal of the American Society for Horticultural Science 122: 226–232.

Tcherkez G, Nogués S, Bleton J, Cornic G, Badeck F, Ghashghaie J. (2003) Metabolic origin of carbon isotope composition of leaf dark-respired CO2 in French bean. Plant Physiology 131: 237–244.

Troughton JH, K.A. Card, and C.H. Hendy. (1974) Photosynthetic pathways and carbon isotope discrimination by plants. Carnegie Institution Washington Yearbook 73: 768–780.

Trouve C, Mariotti A, Schwartz D, Guillet B. (1994) Soil organic-carbon dynamics under Eucalyptus and Pinus planted on savannas in the Congo. Soil Biology & Biochemistry 26: 287–295.

Viktor A, Cramer MD. (2003) Variation in root-zone CO2 concentration modifies isotopic fractionation of carbon and nitrogen in tomato seedlings. New Phytologist 157: 45–54.

Wanek W, S. Heintel, and A. Richter (2001) Preparation of starch and other carbohydrates from higher plant leaves for stable carbon isotope analysis. Rapid Communications in Mass Spectrometry 15: 1136–1140.

Wedin DA, L.L. Tieszen, B. Dewey, and J. Pastor. (1995) Carbon isotope dynamics during grass decomposition and soil organic matter formation. Ecology 76: 1383–1392.

Willmer CM, Firth P. (1980) Carbon isotope discrimination of epidermal tissue and mesophyll tissue from the leaves of various plants. Journal of Experimental Botany 31: 1–5.

Willmer CM, Roksandic Z. (1980) Carbon dioxide discrimination by tissues and organs from oat and barley inflorescences. Journal of Experimental Botany 31: 1493–1495.

Wilson AT, Grinstead MJ. (1977) 12C/13C in cellulose and lignin as paleaothermometers. Nature 265: 133–135.

Winkler FJ, E. Wirth, E. Latzko, H.L. Schmidt, W. Hoppe, and P. Wimmer (1978) Einfluss von Wachstumsbedingungen und Entwicklung auf d13C-Werte in verschiedenen Organen und Inhaltsstoffen von Weizen, Hafer und Mais. Zeitschrift für Pflanzenphysiologie 87: 255–263.

Xu CY, Lin GH, Griffin KL, Sambrotto RN. (2004) Leaf respiratory CO2 is C-13-enriched relative to leaf organic components in five species of C-3 plants. New Phytologist 163: 499–505.

Yoneyama T, Ohtani T. (1983) Variations of natural 13C abundances in leguminous plants. Plant & Cell Physiology 24: 971–977.

Yoneyama T, Handley LL, Scrimgeour CM, Fisher DB, Raven JA. (1997) Variations of the natural abundances of nitrogen and carbon isotopes in Triticum Aestivum, with special reference to phloem and xylem exudates. New Phytologist 137: 205–213.

Yoneyama T, Fujiwara H, Engelaar W. (2000) Weather and nodule mediated variations in delta C-13 and delta N-15 values in field-grown soybean (Glycine max L.) with special interest in the analyses of xylem fluids. Journal of Experimental Botany 51: 559–566.