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Does bulk-needle $\delta^{13}\text{C}$ reflect short-term discrimination?

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Abstract – When bulk needle material is analysed for its carbon isotope signal, the net $\delta^{13}\text{C}$ underlies variation of the biochemical composition of the needle. Bulk needle material can be categorized into two different types of carbon pools, differing in the time period of assimilation: structural carbon and carbon with a rapid turnover. The bulk needle $\delta^{13}\text{C}$ is influenced by the relative amounts of the two pools. In the present study the $\delta^{13}\text{C}$ of rapid turnover carbon was estimated using gas exchange measurements. When these were compared to the respective bulk needle $\delta^{13}\text{C}$, a significant linear correlation was found (raw data: $p < 0.0005$; $R^2 = 0.25$ / daily means: $p < 0.05$; $R^2 = 0.39$), indicating that the rapid turnover carbon pool influences the overall $\delta^{13}\text{C}$. Therefore one has to be aware that bulk-needle $\delta^{13}\text{C}$ measurements can have the tendency either toward reflecting the $\delta^{13}\text{C}$ of structural carbon or toward reflecting the $\delta^{13}\text{C}$ of rapid turnover carbon.

needle / discrimination / carbon isotope / gas exchange / *Pinus sylvestris*

Résumé – Le $\delta^{13}\text{C}$ des aiguilles de pin sylvestre reflète-t-il la discrimination isotopique à court terme ? La composition isotopique en carbone des aiguilles entières dépend partiellement de leur composition biochimique. Le carbone d'une aiguille peut être représenté en deux réservoirs, incorporés à des périodes différentes dans la vie de l'aiguille : le carbone structurel et le carbone à rotation rapide. $\delta^{13}\text{C}$ de l'aiguille est influencé par les équilibres entre les deux pools. $\delta^{13}\text{C}$ du pool de carbone à rotation rapide a été estimé par des mesures d'échanges gazeux et comparée à celle du carbone total des aiguilles correspondantes. Une corrélation linéaire significative (données brutes: $p < 0,0005$; $R^2 = 0,25$ / moyenne journalière: $p < 0,05$; $R^2 = 0,49$) a été trouvée, montrant que le pool de carbone à rotation rapide influence le $\delta^{13}\text{C}$ total. Par conséquent, si on utilise des mesures de $\delta^{13}\text{C}$ d'une aiguille entière il faut être conscient que les résultats peuvent refléter la composition isotopique du carbone structurel ou à renouvellement rapide.

aiguille / discrimination / isotope du carbone / échange du gaz / *Pinus sylvestris*

1. INTRODUCTION

Discrimination against ^{13}C (Δ) on the physical and biochemical pathways from atmospheric CO_2 to organic plant carbon can be used to investigate a number of plant physiological processes. The largest proportion of isotopic discrimination takes place at the enzymatic CO_2

fixation step and is modulated by the CO_2 concentration in the substomatal cavity [7]. However post-photosynthetic isotope effects during different steps of the biochemical pathway of carbon in plants (e.g. pyruvate dehydrogenase complex) lead to distinct ranges of $\delta^{13}\text{C}$ of major biomolecule groups. Molecules following the lipid biochemical pathway are 5‰ to 10‰ more depleted

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in ^{13}C than whole leaf carbon. This depletion is associated with the oxidation of pyruvate to acetyl CoA [5]. The $\delta^{13}\text{C}$ of cellulose approximates the mean of total plant carbon, pectin is ^{13}C -enriched and lignin is ^{13}C -depleted [4]. Leaf starch from several species is ^{13}C -enriched [2] by 1‰ to nearly 3‰ relative to soluble sugars. Amino acids were found to be on average about 6‰ lighter than leaf carbon [22], but there are wide differences for $\delta^{13}\text{C}$ among amino acids. Bulk leaf material contains different biomolecule groups such as cellulose, lignin, starch, lipids, proteins, soluble sugars and other species-specific substances such as phenols and resin acids; consequently variations in biochemical composition can affect the bulk leaf $\delta^{13}\text{C}$.

Moreover, the carbon in bulk leaf material can be categorised into two different pools, which can differ in the time period of assimilation: 1) Structural components (cell walls, fibres), which are formed during the growth period of the leaf. Structural carbon originates from new assimilation during this period or from stored carbon. The length of the period during which the structural molecules are formed and the extent to which stored carbon is utilized are species dependent. 2) Sugars and, at time, starch are carbon pools with rapid turnover times and therefore mainly represent the $\delta^{13}\text{C}$ of recently assimilated carbon. Brugnoli et al. [2] showed that, on a daily basis, the $\delta^{13}\text{C}$ of leaf starch and soluble sugars closely reflect concurrently measured gas exchange parameters (c_i/c_a), i.e. the ratio of intercellular to atmospheric CO_2 partial pressures. Phenolic components show considerable seasonal variation in concentration [18] and are thought to function as part of the trees' defence systems, suggesting that turnover may be reasonably rapid [20]. However, the turnover of terpenes is discussed controversially in the literature [11].

The $\delta^{13}\text{C}$ of bulk leaf material is frequently used as an integrator of a range of plant physiological responses to environmental influences. It is often compared with other parameters such as the c_i/c_a ratio [7, 21] or related to climates associated with seed sources of common garden trees [10, 23, 24, 25, 26]. The $\delta^{13}\text{C}$ is also used in comparative, intra- or inter-species studies, relating it to leaf intrinsic water-use efficiency [8, 12, 13].

If the pool of leaf carbon with rapid turnover time represents a significant proportion of bulk leaf material and has an isotopic signature differing from that of structural carbon, it could influence the bulk needle $\delta^{13}\text{C}$. This would have to be considered in experimental designs to avoid a shift of the results either toward the influence of structural carbon or toward the rapid turnover carbon pool.

In the present study, I investigated the influence of rapid turnover (one day) leaf components on bulk needle $\delta^{13}\text{C}$ of *Pinus sylvestris* L. (Scots pine) in a natural Scottish environment. The $\delta^{13}\text{C}$ signal of the rapid turnover carbon pool originating from assimilation was estimated by averaging day-course gas exchange measurements on single pairs of needles. The gas-exchange measurements were related to environmental measures such as light intensity to qualify the sources of variation for carbon isotope discrimination in a natural Scottish environment. Subsequently, the measured needles were harvested in the evening and prepared for bulk needle $\delta^{13}\text{C}$ determination. These two parameters were used to estimate the influence of instantaneous on integrated discrimination. The results are put into a context with existing information in the literature about biochemical components in pine needles and their turnover.

2. MATERIALS AND METHODS

The *Pinus sylvestris* trees used for the gas exchange measurements were situated in the Newton Nursery of the Forestry Commission Research Division, Elgin, Scotland, UK. The trees were grafts from mother trees originating from different native pine forests in Scotland (Glen Tanar, Rannoch Moor, Loch Maree) and were planted in the common garden in 1976. Overall, six trees were chosen, and each tree was measured for one or two days in order to achieve a range of combinations of trees and climatic conditions. Over the measuring period from the 31/7/1996 to the 05/09/1996, 11 days of data were obtained.

A PP-SYSTEMS CIRAS-1 (Combined Infrared Gas Analysis System; PP-Systems, Unit 2, Glovers Court, Bury Mead Rd., Hitchin, Herts, SG5 1RT, UK) with manual CO_2 / H_2O air supply unit was used to measure the gas exchange parameters of single needle pairs. Pine needles have a complicated shape and are twisted to different degrees, therefore, in this case, complete surface area was calculated and used to standardise measurements. Based on a survey of the shape of pine needles ($N = 5$; resolution of 5 mm along the length of the needles), a mathematical model using a half elliptical cone stump and two measuring points on a needle of height and width at each [1] was preferred to the more often used half cylinder [17]. A PP-SYSTEMS narrow leaf cuvette was used with a single pair of needles suspended from end to end (45 mm). The positioning of the needle was definite, repeatable and no shading occurred.

To estimate instantaneous discrimination from in situ gas exchange measurements it was necessary to keep disturbance through measurements to a minimum, that is

keeping conditions in the cuvette as close to ambient as possible and the measuring time short (seconds). A constant CO_2 concentration (350 ppm) was supplied to the system via a CO_2 cartridge and the water vapour partial pressure ($p_{\text{H}_2\text{O}}$) in the air stream into the cuvette adjusted to about 70% of ambient $p_{\text{H}_2\text{O}}$.

Gas exchange parameters were calculated from the equations given in the CIRAS Operators Manual as derived from von Caemmerer and Farquhar [3]. Instantaneous discrimination against $\delta^{13}\text{C}$ (Δ) was calculated using *equation (1)*. Because the time intervals between the measurements were not regular, the estimation of the mean instantaneous discrimination of a given day (Δ_i), using a simple mean, would have emphasized measurements that were temporarily closer together. Therefore a weighted mean, using the lapses between measurements, was used in the data analysis. In comparison, the values were also standardized using assimilation rate. The bulk needle discrimination (integrated discrimination; Δ_{needle}) was calculated by assuming air $\delta^{13}\text{C}$ as source to be -8‰ and using *equation 2*.

All measurements were done on one-year old needles from exposed south-side branches (60 cm to 215 cm above ground). For any given day, five to six pairs of needles were chosen on one tree, preferably each pair from a different branch. The needles had to be exposed to the sunlight and not shaded by other needles, branches or trees. The selected needles were measured in the same sequence throughout the day, resulting in six to twelve measurements on each needle pair. Each measurement consisted of a rapid transfer of the needles to the leaf cuvette, and then usually 5, depending on the stability of environmental conditions up to 12 rapid (within seconds) recordings of values. These were averaged using a simple mean. All measured needles were harvested at the end of a day, the surface area was measured as described above and the gas exchange parameters recalculated using the measured surface area.

All needles used for gas exchange measurements were oven-dried (60 °C) and milled in a Glen Creston MM2000 ball mill. For the isotopic analysis 1 mg of the sample material was weighed into a 4×6 mm tin capsule (Elemental Microanalysis Ltd.). The samples were measured for $\delta^{13}\text{C}$ using a Europa Scientific™ ES 2020 ANCA-SL (automatic nitrogen carbon analyser - solid liquid).

3. RESULTS

Over the measuring period (summer 1996), the daily time-courses of discrimination and the corresponding bulk-needle $\delta^{13}\text{C}$ were measured for 52 pairs of needles.

For the measured maximum assimilation rates, c_i/c_a was estimated to be close to 0.5 (arrow in *figure 1*). This c_i/c_a ratio represents a Δ of about 16‰ (*equation 1*). However, maximum assimilation rates were only found for high PFD ($>1000\ \mu\text{mol m}^{-2}\ \text{s}^{-1}$; *figure 1*), and these high PFDs were only recorded for 17% of the measurements. Three-quarters of the measurements were done under PFDs below $600\ \mu\text{mol m}^{-2}\ \text{s}^{-1}$, and 50% of the measurements under $300\ \mu\text{mol m}^{-2}\ \text{s}^{-1}$. Therefore in the Scottish climate, a large part of carbon on an annual basis is assimilated under low light intensities (PFD) due to frequently cloudy days. At these low PFDs about 90% of measured ratios of c_i/c_a were between 0.7 to nearly 1 (*figure 1*), equivalent to Δ between 20‰ and 27‰ .

Using the assimilation rate frequency data presented in *figure 1* (number of assimilation rates measured within intervals of $1\ \mu\text{mol m}^{-2}\ \text{s}^{-1}$ divided by overall number of measurements), the amounts of carbon assimilated for intervals of assimilation rates were calculated (*figure 2*): time was approximated by the frequency values and therefore amount of carbon was calculated as product of mean assimilation rate within an interval multiplied by the frequency. The resulting values have no unit, but are proportional to $[\mu\text{mol m}^{-2}]$. This distribution gives an indication of the amount of carbon assimilated during time periods having low or high assimilation rates. For assimilation rates between 1 and $5\ \mu\text{mol m}^{-2}\ \text{s}^{-1}$ the amount of carbon assimilated remained at a constantly high value, whereas with higher assimilation rates the amount of carbon assimilated was increasingly lower.

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

Equation 1. Calculation of discrimination against ^{13}C from gas exchange parameters [7] where Δ is the total added discrimination, a is the discrimination against $^{13}\text{CO}_2$ during diffusion in free air (4.4‰), b is the discrimination during carboxylation (30‰ if all carboxylation is due to RuBisCO, here 27‰ was used [2] to allow for fixation by PEPC and mesophyll conductance), c_i is the partial pressure of CO_2 in the stomatal cavity, c_a the partial pressure in free air.

$$\Delta = \frac{\delta_{\text{source}} - \delta_{\text{product}}}{1 + \delta_{\text{product}}/1000}$$

Equation 2. Isotopic discrimination between a source and a product [8].

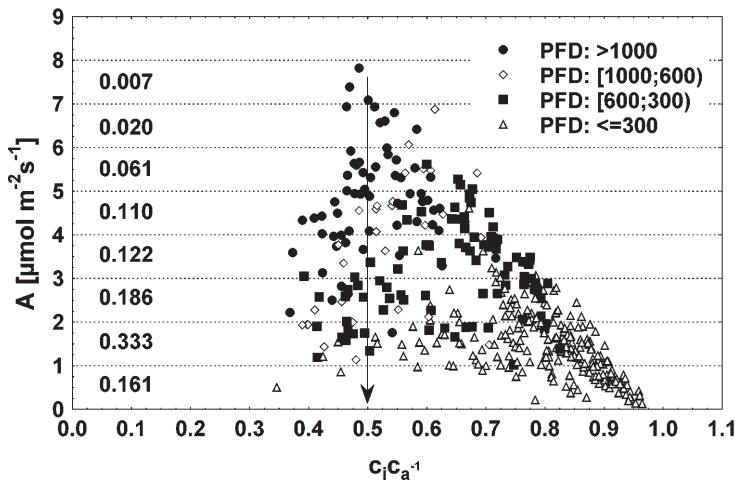


Figure 1. Relationship between the ratio of internal to external CO₂ partial pressure versus assimilation rate categorised by PFD for all values. Frequencies of assimilation rates in 1 $\mu\text{mol m}^{-2}\text{s}^{-1}$ intervals are shown. $N = 409$.

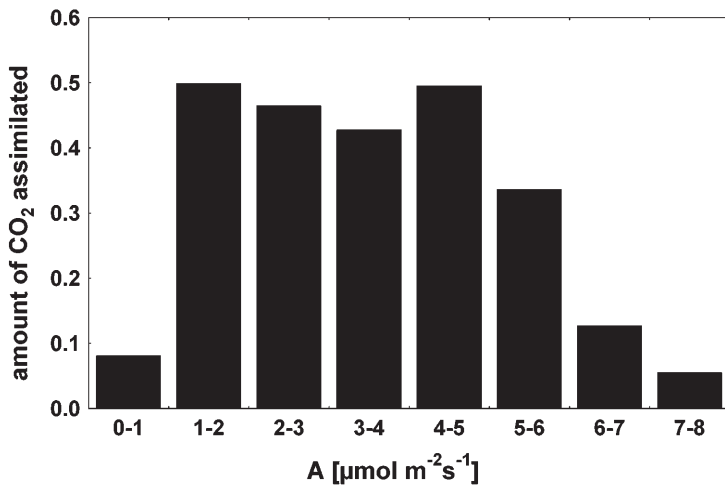


Figure 2. Calculated amounts of carbon assimilated (no unit but proportional to $[\mu\text{mol m}^{-2}]$), for intervals of assimilation rates ($1 \mu\text{mol m}^{-2}\text{s}^{-1}$): mean assimilation rate within an interval multiplied by the frequency of A.

The range of Δ_{needle} observed (17.8‰ to 21.9‰ equals a range of 4.1‰) was smaller than the range of Δ_i (9.0‰; *figure 3*), however the means were similar ($\Delta_{\text{needle}} = 20.0 \pm 1.0\%$; $\Delta_i = 20.7 \pm 2.3\%$). The linear regression of Δ_i against Δ_{needle} was found to be significant at $p < 0.0005$ with a R^2 of 25% (*figure 3*). The results using assimilation-rate-weighted means of Δ_i were similar: $p < 0.0005$; $R^2 = 0.26$; $y = 15.40 + 0.24x$. When the means for all measurements on each day of each tree were calculated, the regression was significant at $p < 0.05$ with a R^2 of 39% (*figure 3*; dashed line).

4. DISCUSSION

Under growing season conditions, rapid-turnover products of assimilates can be a considerable fraction of total needle carbon. Changes in environmental conditions in the course of a day will influence the carbon isotopic signal of the assimilatory products. With general environmental conditions changing in the course of the growing season, daily means of Δ will also vary. Under natural conditions in the field in Scotland, an important factor strongly influencing Δ proved to be PFD. Δ was

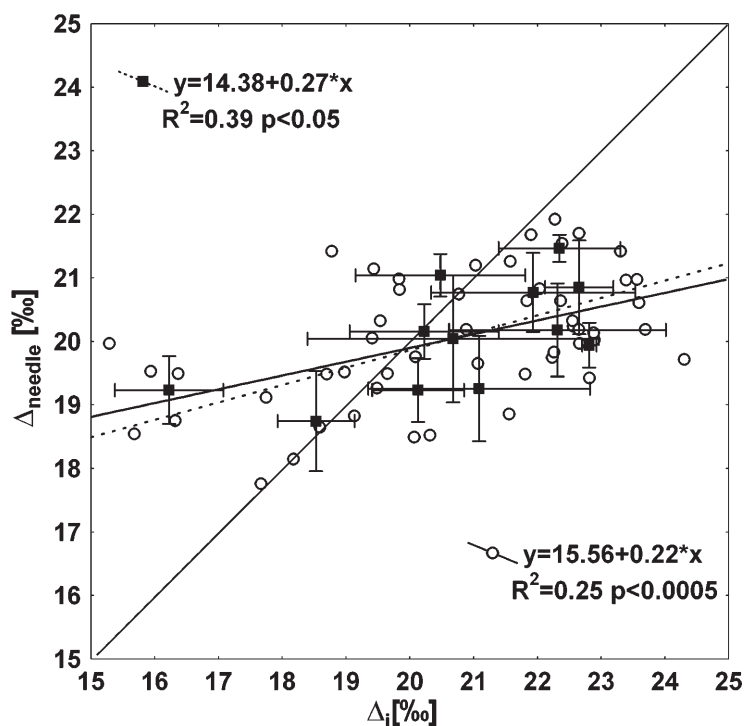


Figure 3. Open Circles: Linear regression between Δ_i and Δ_{needle} ($N = 52$). Δ_i was calculated from the average performance of the needle over one day, Δ_{needle} was calculated from $\delta^{13}\text{C}$ of bulk needle material harvested on the evening of that day. Filled Squares: means of all measurements for each day ($N = 11$; whiskers are standard deviations); the bisector is indicated.

calculated on different levels: instantaneous Δ by using gas exchange measurements (Δ_i) and a time-integrated Δ derived from $\delta^{13}\text{C}$ measurements of harvested needles (Δ_{needle}).

The instantaneous Δ against ^{13}C in C3 plants is mainly dependent on the c_i/c_a ratio (equation 1 [7]). From the relation of c_i/c_a to PFD and to Δ it was estimated that theoretical Δ at maximum photosynthetic rates was about 16‰, whereas for photosynthetic rates that were measured for 50% of the time Δ ranged from 20‰ to 27‰ (figure 1). Δ calculated for maximum assimilation rates of the measured pine trees was also at the extreme low end of Δ calculated from bulk needle material (17.8‰ to 21.9‰ for Δ_{needle} ; figure 3). These results might suggest that higher Δ dominated the $\delta^{13}\text{C}$ of fixed carbon, as more assimilates were produced by lower assimilation rates but at longer time intervals. This is supported by the estimates of the amount of carbon fixed during the measuring period (figure 2). Only for assimilation rates less than $5 \mu\text{mol m}^{-2} \text{s}^{-1}$, the amount of carbon fixed gave a constantly high value. Higher assimilation rates did not occur often enough to contribute significant amounts of carbon to the total of carbon fixed by the plant. This suggests that experimental set-ups that investigate photosynthesis under low light conditions could

contribute substantially to the understanding of functioning of trees in natural environments in the northern latitudes.

Discriminations as calculated from $\delta^{13}\text{C}_{\text{needle}}$ (Δ_{needle}) reflect the carbon assimilated into organic matter over a longer period of time. The material of a needle consists mainly of structural organic material (cellulose, lignin) laid down during the growth of the needle, of a storage pool of sugars, starch and fats and of resin (monoterpenes, resin acids). If the storage pools are sufficiently large, then they can influence the bulk needle $\delta^{13}\text{C}$. Information available in the literature about the composition of Scots pine needles (see table 1) show that single sugars together with starch can amount to over one third of needle dry weight (DWT); storage lipids in needles can be up to 1.4% DWT and phenolics can be up to 10% DWT. No information was available on the protein content of Scots pine needles. However, the protein pool (a large percentage of which is represented by Rubisco), assuming a fast turnover, would only further enlarge the percentage of rapid turnover assimilatory products in needles.

Turnover rates of sugars and starch in needles during the summer are high [14], therefore their $\delta^{13}\text{C}$ values reflect current photosynthetic responses [2]. This is

Table I. Percentage of Dry Weight (DWT) of chemical compounds commonly found in pine needles with a rapid turnover during the growing season.

Compound	%DWT **	Reference
Total monoterpenes	0.06–0.22%	[15, 16]
Total resin acids	0.37–0.44%	[15, 16]
Total phenolics	3.8–9.8%	[15, 18]
Total* lipids	0.47–1.38%	[9]
Total* Carbohydrates	3.9–38.3%	
Starch	2.3–28%	[7, 9, 19]
Glucose	0.5–3.0%	[7, 9, 16]
Fructose	1.0–5.0%	[7, 9, 16]
Sucrose	0–2.1%	[7, 16]
Galactose/Arabinose	0.05–0.09%	[9]
Raffinose/Melibiose	0.05–0.07%	[9]

* Marked totals are sum of maxima and minima of single compounds regardless of seasonal changes.

** % DWT recalculated from the original values; the used ratio of FWT/DWT is 2.7 ± 0.2 (N = 50).

reflected in the correlation found between Δ_i and Δ_{needle} for means based on measurements of single needles for one day and for daily means of the measured trees (*figure 3*). The correlation coefficients indicate that 25% / 39% of variation of $\delta^{13}\text{C}$ as measured in bulk needle (Δ_{needle}) can be explained by the $\delta^{13}\text{C}$ of recently assimilated CO_2 . This is about the same order of magnitude as the percentage of bulk needle dry weight represented by rapid turnover assimilatory products. The similar linear regression of daily means compared to the single-needle data (*figure 3*) shows that the environmental conditions on the day of measurement strongly influence the relationship between Δ_i and Δ_{needle} and provide a large amount of the measured variation. To facilitate the statistical analysis of the data, a sufficiently large range of gas exchange parameters and carbon isotope ratios measured was needed. To this end the measurements were done on different ecotypes of native Scots pine trees and during a range of environmental conditions. However, with the sampling strategy of the present study it cannot be ruled out that the variation could be also due to a “tree-effect”. Instances where the same tree was measured on different days indicated the possibility of a tree / environment interaction. Therefore it would be interesting for a future study to analyse more in detail the source of variation for $\delta^{13}\text{C}$, and especially the tree / environment interaction, using a more complete sampling strategy of trees and measurement days and several gas-exchange units.

The smaller range of Δ_{needle} compared to that of Δ_i (*figure 3*) indicates a dampening effect of structural car-

bon on the bulk needle $\delta^{13}\text{C}$. The instantaneous discrimination (Δ_i) reflects the maximum range of Δ occurring during the measurement period. This variation is only part of the $\delta^{13}\text{C}_{\text{needle}}$ against a background of structural carbon with a constant $\delta^{13}\text{C}$ value that will reduce any variation. The $\delta^{13}\text{C}$ of structural carbon reflects the $\delta^{13}\text{C}$ of assimilates during the period of needle expansion (in the case of one-year-old needles the spring of the previous year). This is especially clear with needles of Scots pine, because the carbohydrates used for the synthesis of the structural molecules originates from assimilation of older needles during spring and not from reserve material from the stem or the roots [14].

In conclusion, the present study suggests, that when using bulk $\delta^{13}\text{C}$ measurements one has to be aware that, depending on the sampling strategy, the data could either be confounded by the fractionation during recent assimilation (for example for comparative studies of trees in different environments or for temporarily different sampling times) or by the background $\delta^{13}\text{C}$ of structural carbon (for example for the estimation of short term water use efficiency). This is probably also true for other conifers and perhaps also for some broad-leaf species. Sampling strategies need to be adapted accordingly. The variation added by the influence of recent assimilation could be minimised by restricting the harvests of needle samples to short time periods (e.g. evening of one day) or to less assimilative active time periods (autumn/winter). Future research could aim at investigating the source of variation of $\delta^{13}\text{C}$ in more detail and use more direct techniques by investigating different groups of molecules separately. To render this feasible, the limitation of sample size gained from single pairs of needles needs to be overcome.

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