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Original article

The $\delta^{13}C$ of Scots pine (*Pinus sylvestris* L.) needles: spatial and temporal variations

Oliver Brendel*, Linda Handley and Howard Griffiths

Scottish Crop Research Institute, Invergowrie, Dundee, DD2 5DA, Scotland, UK

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Abstract – Spatial variation in carbon isotope composition ($\delta^{13}C$) within the crown of an individual tree complicates sampling strategies, but a systematic study has allowed constraining factors to be determined. Spatial and temporal variations of the $\delta^{13}C$ of *Pinus sylvestris* L. needles were investigated on three exposed, south-facing, branches over 17 months (two growing seasons). A positive relationship (about 2‰ m^{-1}) was found between needle $\delta^{13}C$ and trunk-needle distance on the branch. Abaxial needles were characterized by less negative $\delta^{13}C$ values (0.5‰) compared with adaxial needles. Both effects were interpreted in terms of branch hydraulic conductivity including the presence of compression wood. A close relationship was found between $\delta^{13}C$ measured in needles and in adjoining branch wood. Correcting the monthly data for spatial variation, a transient increase in needle $\delta^{13}C$ during spring was detected (about 0.6‰), whereas changes in needle $\delta^{13}C$ during summer, autumn and winter were minor and positively related to solar radiation.

carbon isotope composition / *Pinus sylvestris* / needle / branch length effect / compression wood

Résumé – Le $\delta^{13}C$ des aiguilles du Pin sylvestre (*Pinus sylvestris* L.) : variations spatiales et temporelles. L'existence d'une variabilité en composition isotopique du carbone ($\delta^{13}C$) dans la couronne d'un arbre individuel complique les stratégies d'échantillonnage. Il est nécessaire de déterminer les facteurs qui sont à l'origine de cette variabilité. Les variations spatiales et temporelles de $\delta^{13}C_{aiguilles}$ de *Pinus sylvestris* L. ont été étudiées pendant 17 mois (deux périodes de végétation) sur trois branches, exposées au sud. Une relation positive (2 ‰ m^{-1}) a été trouvée entre $\delta^{13}C_{aiguilles}$ et la distance à partir du tronc sur la branche. Les aiguilles abaxiales étaient caractérisées par des valeurs de $\delta^{13}C$ moins négatives (0,5 ‰) que les aiguilles adaxiale. Ces deux effets ont été interprétés sur la base du fonctionnement hydraulique des branches et de l'existence de bois de compression. En corrigeant les données mensuelles par rapport aux variations spatiales, une augmentation transitoire (0,6 ‰ environ) de $\delta^{13}C$ a été détectée au printemps, alors que durant l'été, l'automne et l'hiver, les variations restaient faibles et positivement liées au rayonnement solaire.

composition isotopique du carbone / *Pinus sylvestris* / aiguille / effet de la longueur de la branche / bois de compression

1. INTRODUCTION

When photosynthesis is the main, direct source of carbon, stable carbon isotope composition ($\delta^{13}C$) of conifer needles is a useful integrator of the long-term balance between photosynthetic capacity and stomatal conductance [13, 26] and of intrinsic water use efficiency [16, 37].

The bulk needle $\delta^{13}C$ can be decomposed into different components. Early spring carbon forming the structural components during growth of new needles is not derived directly from the new needles photosynthesis. Instead, carbohydrates are translocated from assimilatory products of the previous year's needles to the newly forming needles [17]. Therefore the structural carbon of the new needles should retain to a large degree the $\delta^{13}C$ signature of the spring assimilates of older needles. The relative amounts of non-structural carbon in leaves can fluctuate greatly over the annual growth cycle. The $\delta^{13}C$ signatures of these non-structural components can exhibit substantial annual variation either due to a variation in the $\delta^{13}C$ signature of primary assimilatory products or due to post-assimilation fractionations. Post-assimilation fractionations result in secondary carbon compounds having $\delta^{13}C$ values substantially different from the first products of photosynthesis [15]. Polar compounds, such as lipids, have a significantly more negative $\delta^{13}C$ than bulk plant $\delta^{13}C$ [8]; starch and sugars tend to be heavier. Furthermore the $\delta^{13}C$ of the bulk of non-structural components varies with changing relative concentrations of components with differing $\delta^{13}C$ signatures. This also includes the variation in the amount of relocated storage components in pine needles [12].

* Correspondence and reprints
Tel.: +33.383.39.40.41-41.95; fax: +33.383.39.40.69; e-mail: brendel@nancy.inra.fr
Current address: UMR INRA-UHP Écologie et Écophysiologie Forestière, INRA Centre de Nancy, 54280 Champenoux, France

The Farquhar model of photosynthesis [10, 11] predicts a linear correlation between $\delta^{13}C$ of primary assimilatory products and the ratio of assimilation rate to stomatal conductance. It has been shown for Scots pine needles that $\delta^{13}C$ calculated from a daily integration of gas exchange parameters using the Farquhar model is reflected to a certain degree by $\delta^{13}C$ of bulk needle material harvested the evening of the same day [3]. The environmental conditions of a needle (e.g. temperature, humidity, precipitation or irradiance) change with its position in the canopy and with temporal changes in climate, thereby influencing the $\delta^{13}C$ of primary assimilatory products via carbon assimilation rate and stomatal conductance. Needle stomatal conductance is also affected by branch hydraulic conductance. According to the catenary series model after van den Honert [31], the water potential at a specific point on a branch is dependent on the ratio of evapotranspiration to hydraulic conductance relative to a reference water potential (trunk, soil, etc.). A reduction of evapotranspiration by stomatal closure to maintain water potential would lead to less negative $\delta^{13}C$ of primary assimilatory products. It was found in several studies that increased total branch-length, which is reducing hydraulic conductance, was correlated with less negative $\delta^{13}C$ of apical needles [23, 24, 32, 33].

A part of the carbohydrates that are exported from needles will be used for branch growth [17]. Assuming that branch growth is mainly sustained by carbohydrates from assimilation and less by storage carbohydrates, wood and cellulose $\delta^{13}C$ at a needles position should reflect to a certain degree the $\delta^{13}C$ of the needle. Hence annual differences in climatic conditions can be reflected in the $\delta^{13}C$ of growth rings of the branch and of the trunk [9].

To study the temporal changes in bulk needle $\delta^{13}C$ by sampling needles in regular temporal intervals, the variability due to spatial effects needs to be estimated. Statistical models can be used to correct each sampling in time for spatial variability in bulk needle $\delta^{13}C$. We studied the $\delta^{13}C$ variations of three south-facing, fully exposed branches over 17 months, including two growing seasons. We characterised: (1) spatial effects, taking into account the response of bulk needle $\delta^{13}C$ to distance from the main stem or to position on the branch (abaxial / adaxial); (2) the $\delta^{13}C$ variations of branch wood and cellulose as related to distance from the main stem, among annual growth rings, and between abaxial and adaxial sides of branches; (3) the relationship between needle $\delta^{13}C$ and that of wood and cellulose taken from the same position on the branch; and (4) the temporal patterns of the different needle generations for bulk needle $\delta^{13}C$ corrected for spatial effects and the influence of environmental factors on the detected temporal variations.

2. MATERIALS AND METHODS

A Scots pine (*Pinus sylvestris* L.) tree (about 8 m height) growing in the University of Dundee Botanical Garden (56° 29' N, 3° 2' W) was sampled; it had been grown from seed taken from the Glen Falloch area of southern Scotland. Three branches having the distal ends approximately the same height above ground were chosen (2.60 m, 2.90 m and 2.50 m above ground, for branches 1, 2 and 3, respectively). All were on the south side of the tree, and the most proximal sampling point (1996 internode) was 3 m to 4 m away from the main stem so that all sampling points were outside the crown and out of its shade (3.28 m, 3.40 m and 4.06 m, for branches 1, 2 and 3, respectively). Branches 2 and 3 had unrestricted illumination, however sections of branch 1 were partially shaded during the summer of the second growing season (1998) by the leaves of a deciduous bush. Needles were sampled according to a pre-determined scheme (*figure 1a*) at monthly intervals from June 1997 to October 1998.

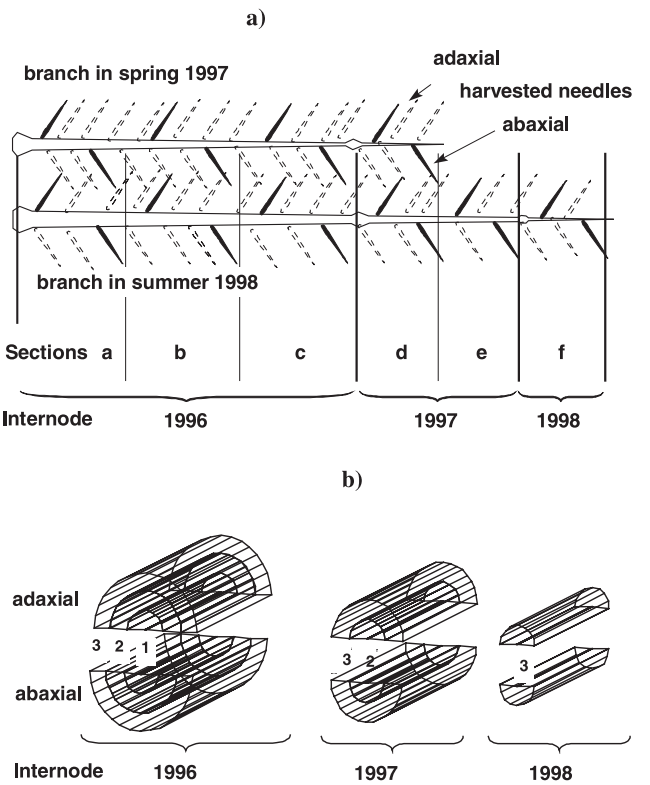

Figure 1. (**a**) Sampling scheme for monthly sampling, showing one branch in spring 1997 and in summer 1998. Each branch internode was divided in sections to assure equal sampling along the branch. Two distances from the beginning of each section were determined randomly for adaxial and abaxial needles (needles harvested are in black), respectively. These distances were the same for all sections during each harvest. The needles closest to the distance chosen were collected and the absolute distance from the base of the branch was determined; (**b**) graphical representation of a transect for each growth year at the end of the experiment where 1, 2 and 3 are the growth rings of 1996, 1997 and 1998, respectively.

2.1. Needles

In Scots pine new needles are formed at the same time as a new internode is formed. No other needles are formed on that internode, and the original needles live for three or more years. Scots pine needles grow in pairs on the same fascicle and in the following text a "pair of needles" will be referred to as "needle". Each internode was conceptually divided into sections (*figure 1a*). On each sampling date, the position for sampling one abaxial and one adaxial needle within a section was chosen using a random number generator.

The adaxial and abaxial needles closest to the calculated position within the section were sampled, and then the actual positions of the needles were determined relative to the base of the branch. Sampled needles were chosen with a maximum deviation of about 60° to the vertical. If no needle was available within this range, a needle out of this range was chosen (horizontal). For the harvests in early spring (June to July 1997 and June 1998), four young needles of each year were additionally sampled at the same position around the twig and pooled in order to provide enough material for analysis. Horizontal or young needles were excluded from positional analyses. The needles were dried overnight at 50 °C and milled in a Retsch MM2000 ball mill (Haan, Germany).

2.2. Wood

At the end of the experiment (October 1998) branches 2 and 3 were harvested. Branch 1 was accidentally lost to Botanical Garden maintenance just before the end of the experiment. When branches were sampled for analysis, traits typical for compression wood ([27] in [29]) were observed in the abaxial half of the transects: abaxial half-moon shaped red-brown latewood and greater ring-width in abaxial than adaxial wood.

From each section three one cm-long subsamples were cut at regular intervals, divided into abaxial and adaxial halves and the annual growth rings (*figure 1b*). Within each section, rings from each half were pooled. The wood was milled as described for needles, 1 mg subsamples retained for isotope analysis and from the remaining wood, cellulose was extracted as described in [4]. Briefly, the method uses a solution of concentrated nitric acid / 80% acetic acid in 1:10 dilution (0.2 mL in 2 mL) to digest the lignin, proteins and hemicelluloses in 50 mg of powdered wood sample. The digest is then washed out using ethanol followed by water. The samples are dried chemically with a pure ethanol-to-acetone progression and physically in a vacuum centrifugal evaporator (speed vac) at 100 mbar for 2 h. The original protocol [4] was modified to include two extraction cycles, and prolonging the ethanol washes to 5 min at 60 °C.

For the $^{13}C/^{12}C$ analysis, 1 mg of the sample material (needle, wood or cellulose) was weighed into a 4 × 6 mm tin capsule (Elemental Microanalysis Ltd.). The needle samples were measured using a Europa Scientific™ ES 2020 ANCA-SL (automatic nitrogen carbon analyser – solid liquid isotope – ratio continuous flow mass spectrometer); the wood and the cellulose samples were measured using a Finnegan continuous flow isotope ratio mass spectrometer (Delta S, Finnigan MAT, Bremen, Germany). Carbon isotope composition was calculated relative to the Pee Dee Belemnite standard [7] as:

$$\delta^{13}C = \frac{R_{sa} - R_{sd}}{R_{sd}} \times 1000\ [‰] \quad (1)$$

where R_{sa} and R_{sd} are the $^{13}C/^{12}C$ ratios of the sample and the standard, respectively. The analytical precision for repeated measures was about ±0.15‰ standard deviation.

2.3. Climate

Daily mean air temperature, precipitation and relative humidity measurements were obtained from the records of the University of Dundee Botanical Garden. Daily values of radiation and wind speed were obtained from SCRI, Dundee which is approximately 2 km from the Botanical Garden, both lying at the same altitude along the Tay River flood plain. Vapour pressure deficit (VPD) was calculated from the daily values of temperature and relative humidity. Potential evapotranspiration (PET) was calculated using mean air temperature, wind speed, radiation and VPD ([25] in [22]). Monthly (30 days) and five-day means of radiation, VPD and PET before each monthly sampling were calculated.

2.4. Statistical analyses

The difference between adaxial and abaxial needle $\delta^{13}C$ was tested for statistical significance using the pairwise Students t-test. The sources of variation for the $\delta^{13}C$ differences between adaxial and abaxial needles were modelled as:

$$(\delta^{13}C_{abaxial} - \delta^{13}C_{adaxial})_{jn} = \mu + d_j + \varepsilon_{jn} \quad (2)$$

where μ is the intercept, d is the date of the monthly sampling *j* (June 1997 to October 1998) and ε is the error term.

The variations for the ^{13}C of wood and cellulose were modelled as:

$$^{13}C_{cellulose,ilmn} = \mu + b_i + p_l + ry_m + \varepsilon_{ilmn}; \quad (3)$$

$$\delta^{13}C_{wood,imn} = \mu + b_i + ry_m + \varepsilon_{imn}; \quad (4)$$

where b are branches *i* (1 and 2), p is the position *l* (adaxial or abaxial) and ry is the year of ring development *m* (1996, 1997 or 1998).

For comparisons between needle $\delta^{13}C$ and wood or cellulose $\delta^{13}C$, the needle values need to be integrated over time. Furthermore the integrated needle $\delta^{13}C$ can then be compared with the different annual rings in each branch section (*figure 1b*). Comparisons pair always the $\delta^{13}C_{needle}$ of a section with the $\delta^{13}C_{wood}$ or $\delta^{13}C_{cellulose}$ of the same section. Three different comparisons were done:

(1) For each internode section (*figure 1a*), mean $\delta^{13}C$ for all needles was calculated over all harvests. This was compared with the mean $\delta^{13}C$ of all annual rings of each corresponding section. In the following text, this will be referred to as "overall mean comparison".

(2) Two means were calculated for needle $\delta^{13}C$ for each section: one for the needles harvested from July to October 1997 and a second mean was calculated for harvests from July to October 1998. These were compared with the respective annual rings (1997 and 1998) in each section (hence, ring 1996 is not included in this analysis). This compares the needle $\delta^{13}C$ of each of the two growing seasons with the annual ring that was formed during this season and stresses therefore the carbon isotope signal of carbon that was assimilated during the growing season. In the following text, this will be referred to as "growing season comparison".

(3) For each internode section (*figure 1a*), mean $\delta^{13}C$ for all needles was calculated over all harvests (as in comparison 1). This was compared with the $\delta^{13}C$ of the oldest, innermost annual ring of each section. This stresses the relationship of bulk needle $\delta^{13}C$ to the wood or cellulose $\delta^{13}C$ of the year during which the needle was formed. In the following text, this will be referred to as "year of formation comparison".

All comparisons were tested with either wood or cellulose $\delta^{13}C$ and either analysing adaxial and abaxial values separately or together. Correlation analysis [28] was used to correlate the branch against the needle $\delta^{13}C$ values.

To investigate the temporal changes in needle $\delta^{13}C$ of the three needle generations, we calculated:

$$\delta^{13}C_{ijkn} = \mu + b_i + d_j + a_k + \alpha\ \text{dist} + \varepsilon_{ijkn} \quad (5)$$

where b are branches *i* (1, 2 and 3), d is the date of the monthly sampling *j* (June 1997 to October 1998), a is the needle generation *k* (1996, 1997 or 1998), dist is the distance on the branch between harvested needle and the trunk and α the slope of the parameter distance. The same model was also calculated for each needle generation separately, excluding the parameter a from the model.

General linear models were analysed using the GLM module of SAS 8.1 (SAS Institute Inc., Cary, NC, USA), type III sum of squares were used for the parameter estimates. Students t-tests and linear

Table I. Means and standard deviations of $\delta^{13}C_{needle}$ for all needles sampled, categorized by (a) branch and (b) needle generation (NG). The categorization is highly significant for both cases (ANOVA, $P < 0.0005$), significant differences between categories are indicated by different lowercase letters, number of needles sampled are in parenthesis.

(a) branch	$\delta^{13}C_{needle}$	(b) NG	$\delta^{13}C_{needle}$
1	–26.52±0.50 (123)a	1996	–26.37±0.49 (288)a
2	–26.20±0.56 (159)b	1997	–26.44±0.53 (132)a
3	–26.59±0.44 (159)a	1998	–27.21±0.42 (21)b

Table II. Students t-test of needle $\delta^{13}C_{adaxial}$ versus $\delta^{13}C_{abaxial}$ showing means and standard errors, number of samples and the mean difference of adaxial-abaxial needle $\delta^{13}C$ ($Diff_{ad\text{-}ab}$); levels of significance are ***: $P < 0.00001$, *: $P < 0.05$.

Subset	$\delta^{13}C_{adaxial}$	$\delta^{13}C_{abaxial}$	N	$Diff_{ad\text{-}ab}$
All	–26.62±0.47	–26.18±0.50	167	–0.45***
96	–26.55±0.42	–26.10±0.41	109	–0.46***
97	–26.63±0.46	–26.21±0.56	51	–0.42***
98	–27.60±0.21	–27.11±0.20	7	–0.49*

regressions were done with STATISTICA 6 (StatSoft, Maisons-Alfort, France). Linear Correlation after [24] was programmed using STATISTICA 6 Basic.

3. RESULTS

3.1. Spatial variation of $\delta^{13}C_{needle}$

The needles on branch 2 were significantly less negative compared with those of the other two branches, whereas the needles on branch1 had a similar $\delta^{13}C$ as those of branch 3 (*table I*). The 1998 needle generation had significantly more negative $\delta^{13}C$ values compared with the 1996 and 1997 needle generations (*table I*). The ^{13}C of abaxial needles was about 0.42‰ to 0.49‰ less negative than the $\delta^{13}C$ of adaxial needles (*table II*) and the difference was stable among needle generations. A linear correlation analysis of adaxial needle $\delta^{13}C$ versus abaxial needle $\delta^{13}C$ was performed, taking into account the error term of both variables [24]. The slope of the principal axis was significant ($P < 0.00001$) with a coefficient of determination of $r^2 = 0.43$ ($r = 0.66$) and a slope close to one ($\delta^{13}C_{adaxial} = -2.39 + 0.93\ \delta^{13}C_{abaxial}$).

In order to investigate the influence of branch, needle age, distance from the main stem, harvest date and environment on the observed difference in $\delta^{13}C$ for ad- and abaxial needles, a statistical model was used. None of the tested factors or their interactions had a significant influence on the adaxial-abaxial $\delta^{13}C$ difference, with the exception of harvest date (model significant at $P < 0.0005$; $r^2 = 0.24$; Eq. (2)). However, of all harvest dates only the September 1998 sampling was significantly different from other harvests. None of the temporally adjacent harvest dates were significantly different, there was no evident development over time.

Table III. Correlation of averaged needle $\delta^{13}C$ values versus the respective wood or cellulose $\delta^{13}C$ values for comparisons 1 to 3; the data-sets include either all data or abaxial and adaxial values separately. Coefficients of determination are adjusted for small sample sizes: * < 0.05; ** < 0.005; *** < 0.0005. The number of data points for each comparison (N) is in parentheses. The correlations for comparison 3 versus $\delta^{13}C_{cellulose}$ are shown in *figure 2*. Mean differences of wood/cellulose $\delta^{13}C$ to respective needle $\delta^{13}C$ (MDN) were calculated for the centre of the principal axis for all data.

Hypothesis	Position	Wood $\delta^{13}C$	Cellulose $\delta^{13}C$
(1) overall mean comparison	All	41.1%** (20)	66.2%*** (20)
	Adaxial	34.6%NS (10)	63.8%* (10)
	Abaxial	75.3%** (10)	70.8%** (10)
	MDN	1.29‰	1.77‰
(2) growing season comparison	All	52.2%***(32)	54.1%*** (32)
	Adaxial	38.5%* (18)	32.3%* (15)
	Abaxial	78.7%***(18)	59.8%*** (18)
	MDN	1.44‰	1.82‰
(3) year of ring formation comparison	All	25.1%* (20)	74.6%*** (19)
	Adaxial	53.5%* (10)	71.2%** (10)
	Abaxial	54.4%* (10)	97.6%*** (9)
	MDN	1.33‰	2.02‰

3.2. $\delta^{13}C$ of wood and extracted cellulose

Whereas branch and ring year were significant factors in the model for wood $\delta^{13}C$ and cellulose $\delta^{13}C$ (Eqs. (3), (4); $P < 0.0001$), adaxial or abaxial position was only significant for cellulose $\delta^{13}C$ ($P < 0.005$), but not for wood ($P > 0.05$). There were no significant interactions among the tested parameters.

Several significant differences were detected between adaxial and abaxial branch material. Wood had a significantly more negative $\delta^{13}C$ (–25.1‰) than cellulose (–24.7‰; Students t-test: $P < 0.005$) and the difference between wood and cellulose $\delta^{13}C$ was significantly larger for abaxial (0.57±0.31‰) than for adaxial samples (0.40±0.30‰; Students t-test: $P < 0.05$). A pairwise Students t-test for adaxial versus abaxial $\delta^{13}C$ values was significant for cellulose $\delta^{13}C$ with less negative $\delta^{13}C$ values for the abaxial side ($P < 0.005$; N = 23; abaxial mean $\delta^{13}C$ = –24.42‰±0.53‰; adaxial mean $\delta^{13}C$ = –24.76‰±0.94‰; difference 0.29‰), however not for wood $\delta^{13}C$. A pair wise Students t-test for ring width showed significantly wider rings on the abaxial side ($P < 0.0001$; N = 23; abaxial mean ring width = 1.20 mm±0.20 mm; adaxial mean ring width = 1.32 mm±0.23 mm; difference 0.12 mm).

3.3. Comparisons between needle and branch $\delta^{13}C$

Needle $\delta^{13}C$ was found to be around 1.3‰ more negative than bulk wood $\delta^{13}C$, around 1.9‰ more negative than wood cellulose (*table III*) and was correlated significantly with wood and wood cellulose $\delta^{13}C$ for each of the three

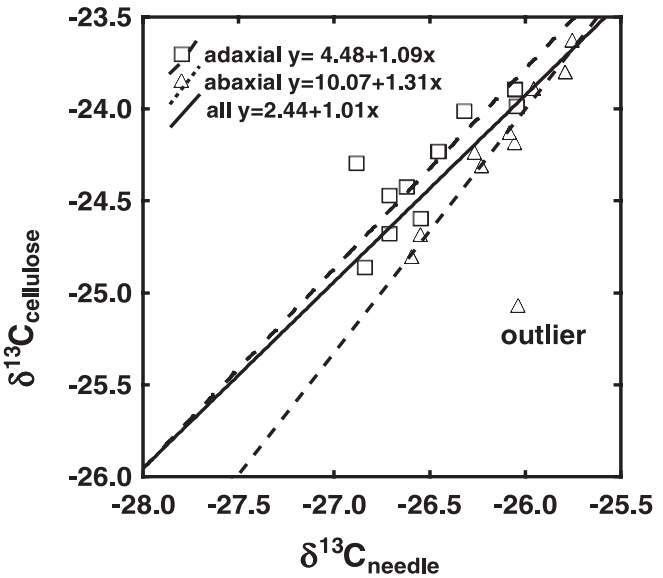

Figure 2. Correlations of the overall needle $\delta^{13}C$ mean for each section versus the $\delta^{13}C_{cellulose}$ of the oldest, innermost ring of each section (comparison 3, *table III*). The regression equations in the graph exclude the outlier.

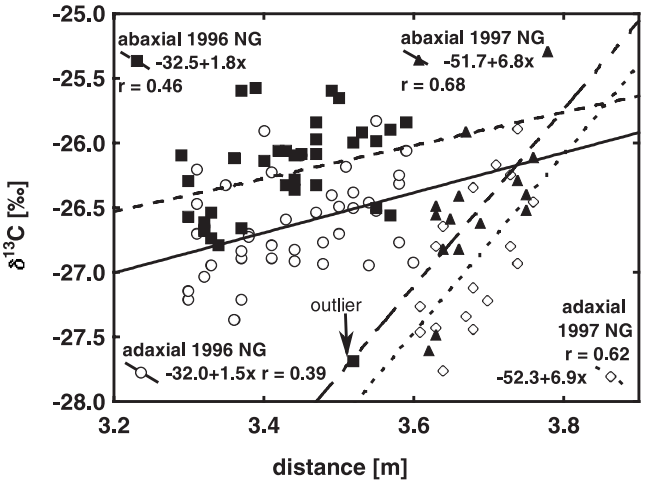

Figure 3. Linear correlations between trunk-needle distance and $\delta^{13}C_{needle}$, raw, single needle data for branch 1 and all harvests. The two needle generations 1996 and 1997 and adaxial versus abaxial needle $\delta^{13}C$ are represented by different symbols. Linear correlations were significant at $P < 0.05$.

comparisons made (*table III*, example of $\delta^{13}C_{needle}$ versus $\delta^{13}C_{cellulose}$ for comparison 3 shown in *figure 2*). Correlations were more significant when needle $\delta^{13}C$ was compared with $\delta^{13}C_{cellulose}$ rather than $\delta^{13}C_{wood}$, except for comparison 2, where values were similar. When correlations were done for adaxial and abaxial values separately, abaxial data yielded more significant correlations (*table III*). Therefore, variation in wood $\delta^{13}C$ was explained by variation in needle $\delta^{13}C$ from 35% to 53% for the adaxial and from 54% to 79% for the abaxial half, with highest values for cellulose $\delta^{13}C$ from the abaxial half (60% to 98%; *table III*).

3.4. Spatial and temporal model for needle $\delta^{13}C$

Different levels of needle $\delta^{13}C$ were found for branches, needle generations and adaxial/abaxial needle positioning (*figure 3*). These different levels and effects would confound an arithmetic mean of all harvested needles for a harvest date. Consequently it would not be adequate to use simple means for each harvest date to investigate the temporal changes in needle $\delta^{13}C$. A statistical model (Eq. (5)) was used to take account of the different effects and to calculate a least square mean (lsmean, *figure 4*) for each harvest date. All parameters of this model (Eq. (5)) were highly significant ($P < 0.0001$) and there was no interaction between distance*date or distance*needle-generation. Three further models were applied to investigate the temporal changes in $\delta^{13}C$ separately for each of the three needle generations (*figure 4*). All factors were highly significant for the models for 1996 and 1997 needle generations ($P < 0.0001$). For the 1998 needle generation only date was a significant factor ($P < 0.005$). The estimate for the parameter distance in the model indicates a positive distance parameter estimate (less negative $\delta^{13}C$ values with increasing distance from the main stem, similar to *figure 3*) of 2.00‰ m^{-1} for the model including the parameter needle generation, and 1.49‰ m^{-1} and 3.67‰ m^{-1} for the 1996 and 1997 needle generations, respectively.

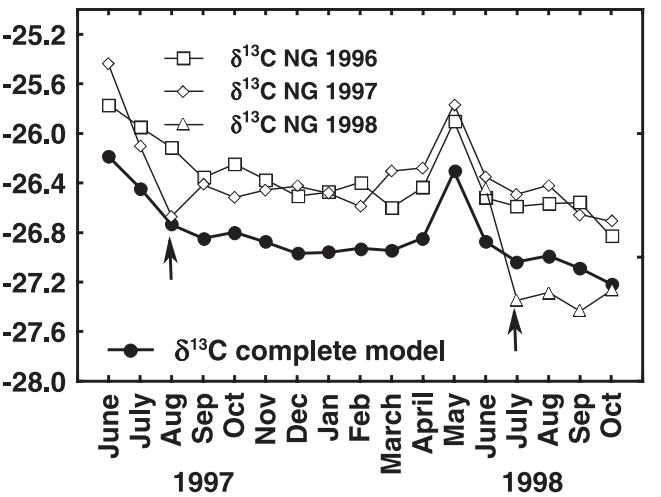

Figure 4. Lsmeans for sampling dates (May 1997 to September 1998) from model 1, including the factors branch, distance and age (complete model, Eq. (5); filled circles, unbroken line); to calculate lsmeans for the needle generations (NG) separately (broken lines, unfilled symbols), the factor age was excluded from the model. Dates were developing needles reached full length are marked by an arrow.

3.5. Temporal behaviour of $\delta^{13}C$ of different needle generations

The needle $\delta^{13}C$ least square means (lsmean) for each sampling date (*figure 4*) as calculated from equation (5) show a steep decrease to more negative $\delta^{13}C$ of 0.6‰ in spring

(for 1997 from June to August and for 1998 from May to July; similar decrease for both years). During summer and autumn the $\delta^{13}C$ values continued slowly to decrease to more negative values and reached a stable minimum in winter (1997). During late winter / early spring (March to May) 1998, the $\delta^{13}C$ increased sharply by about 0.6‰ to less negative values. The spring decrease in 1997 was much steeper for the developing 1997-needle generation compared with the 1996-needle generation; in spring and summer 1998 both needle generations showed a similar drop to more negative $\delta^{13}C$ values. Similarly in 1998, the new 1998-needle generation decreased more than the mature 1996- or 1997-needle generations. The 1997- and 1998-needle generations had reached full length at the August 1997 and July 1998 harvests, respectively.

Figure 4 suggest a general drift to more negative needle $\delta^{13}C$ values over time. For the complete model (Eq. (5); unbroken line in *figure 4*), linear regressions, including or excluding the spring peaks, are significant ($P < 0.05$; coefficients of determination between –0.61 and –0.81; slopes between –0.02 and –0.03‰/month). However, when the needle generations were analysed separately, only the regressions of the 1996 needle generation were significant (spring peak excluded or not; $P < 0.05$; coefficients of determination between –0.55 and –0.85; slopes between 0.03 and 0.04‰/month). For the 1997-needle generation the regression changed between not significant ($P > 0.05$) and significant with the number of data points excluded with the spring peaks, the regressions were not significant for the 1998-needle generation ($P > 0.05$).

3.6. Analysis of influence of climatic variables on needle $\delta^{13}C$

The annual pattern of $\delta^{13}C$ (*figures 4, 5*) consists mainly of a significant peak to less negative $\delta^{13}C$ values in late spring, while there are only minor variations in needle $\delta^{13}C$ during the remainder of the annual cycle. Therefore the spring peaks might bias linear regression models between $\delta^{13}C$ and climatic variables. Linear regression models between $\delta^{13}C$ and radiation, VPD, PET, temperature, humidity and precipitation were tested including data for summer growing season, winter season and from the whole annual cycle excluding the spring-peak periods without any significant results ($P > 0.05$). However, when climatic data for the whole sampling period are plotted along with monthly means of $\delta^{13}C$ (*figure 5*), then the $\delta^{13}C$ spring-peak coincides with the first high radiation in spring (five day integration of radiation before each sampling date) and therefore also with the first spring increase of potential evapotranspiration (PET). For five-day means, significant linear regressions of radiation ($P < 0.005$; $r^2 = 0.425$; inset in *figure 5*) and PET ($P < 0.05$; $r^2 = 0.239$; data not shown) to $\delta^{13}C$ indicate less negative $\delta^{13}C$ values with higher radiation and higher PET.

4. DISCUSSION

4.1. Spatial variation

The investigated spatial variables branch, needle generation and adaxial/abaxial needle position had a significant effect on Scots pine needle $\delta^{13}C$. We further detected a correlation

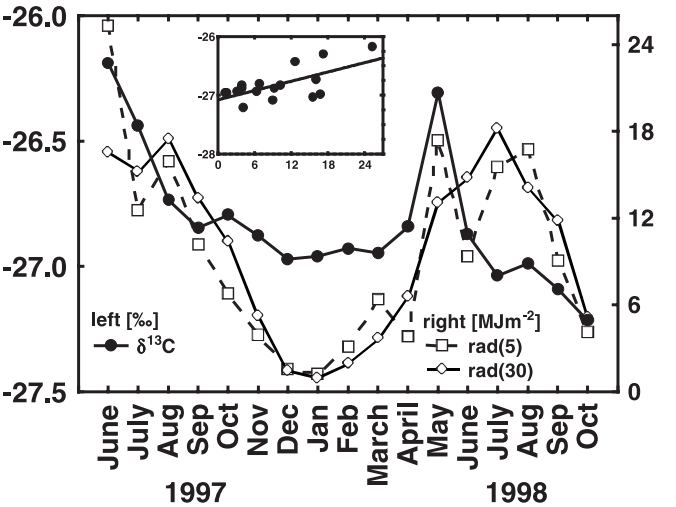

Figure 5. Radiation as 5 (open square) and 30 day means (open diamond) before each sampling date, including the lsmeans for $\delta^{13}C$ for date from model 1 (filled circles, complete model, Eq. (5), see *figure 4*); Inset shows linear regressions of five-day integrations of radiation against $\delta^{13}C$ (significant at $P < 0.005$ with $r^2 = 0.425$; $\delta^{13}C = -27.08+0.03 \times rad$).

between the trunk-needle distance and the needle $\delta^{13}C$ (1.5‰ m^{-1} to 3.7‰ m^{-1}) for distances as short as 30 cm. The trunk-needle distance effect did not change seasonally, as evidenced by the lack of interaction between trunk-needle distance effect and sampling data. Previously published values for among branch comparisons were 0.3‰ m^{-1} to 1‰ m^{-1} branch-length [23, 24, 32–34].

Variation of needle $\delta^{13}C$ with branch length has been attributed to changes in stomatal conductance caused by differences in xylem tension and hydraulic conductivity [23]. After the catenary series model [31], to maintain a constant water potential along the increasing hydraulic resistance of a branch, evapotranspiration needs to be lowered by reducing stomatal conductance. A lower stomatal conductance of more distal needles would lead to less negative $\delta^{13}C$ values. Cernusak and Marshall [6] found for *Pinus monticola* needles less negative $\delta^{13}C$ values with decreasing leaf specific conductivity.

Compression wood contains a higher proportion of lignin than normal wood [30]. As lignin $\delta^{13}C$ is more negative than that of cellulose [35], bulk wood is found to be isotopically lighter than isolated cellulose [20, 21]. The difference of bulk wood $\delta^{13}C$ and cellulose $\delta^{13}C$ can be used as a rough estimator for the lignin content in the bulk wood: the larger the difference the higher the lignin content. We found the difference to be more pronounced for the abaxial side compared to the adaxial side. This result is consistent with the presence of compression wood on the abaxial side of branches. Also, the significantly larger ring width in the abaxial samples is characteristic for compression wood ([27] in [29]).

The $\delta^{13}C$ of abaxial needles was consistently less negative than adaxial ones, a result that we suppose to be caused by reductions in stomatal conductance of abaxial needles. It is plausible that such a reduction could be caused by formation

of compression wood on the abaxial side of the branches [1, 19]. Compression wood is denser than normal wood [30] and in conifers an increase in wood density is often due to a decrease in tracheid lumen [36]. A decrease in tracheid lumen would also decrease specific conductivity. This suggests that hydraulic conductivity in compression wood might be lower than in normal wood, which could result in lower stomatal conductance. Differences of irradiance could contribute to the observed ad- and abaxial differences of $\delta^{13}C$ albeit having the reverse effect on $\delta^{13}C$ to that observed. We doubt that shading of the abaxial side could account for much of the constant difference observed between the $\delta^{13}C$ of adaxial and abaxial needles. The slightly upwards-sloping habit of pine branches, combined in Scotland with a low angle of incident radiation, making a consistent difference in irradiation unlikely. Also the lack of a temporal pattern, for example consistent with the seasonally changing sunlight angle of incidence suggests that radiation is not the major determinant for the adaxial-abaxial difference in needle $\delta^{13}C$. Furthermore a similar significant difference of adaxial versus abaxial needle $\delta^{13}C$ (0.42‰) was found in an independent experiment [2] on a Scots Pine tree, using recent and one-year-old needles from three different twigs (six adaxial / abaxial pairs) with twig orientations from south to west.

The $\delta^{13}C$ of abaxial branch cellulose was significantly less negative than the $\delta^{13}C$ of adaxial branch cellulose, a consistent but smaller difference as found for needle $\delta^{13}C$. However, the physical separation of adaxial and abaxial samples was stricter for needles (±60° of the vertical) than for wood (halves). The smaller difference could also indicate some mixing of carbon exported from adaxial and abaxial needles within the branch wood. That no significant difference was detected between adaxial and abaxial bulk wood $\delta^{13}C$ might be due to the compensatory effect of different lignin concentrations in adaxial and abaxial bulk wood.

The highly significant correlations between needle $\delta^{13}C$ and wood or cellulose $\delta^{13}C$ of the same section could be an indication for a source-sink relationship between needles and adjoining branch sections. Extensive mixing of carbohydrates within the branches would prevent a relationship between needle and wood or cellulose $\delta^{13}C$. Hence, our results suggest, at least for fully exposed, needle bearing branch sections, a high percentage of carbon in branch material that was assimilated by needles in proximity.

4.2. Seasonal variation

The most marked seasonal change in needle $\delta^{13}C$ was a peak of $\delta^{13}C$ enrichment at the time before spring bud break, when also extreme accumulations of starch were observed [12, 17]. As calculated from gas exchange [3], discrimination against ^{13}C during primary assimilation in Scots pine needles can vary by up to 9‰, leading to a large range of $\delta^{13}C$ for primary assimilatory products. It has been shown for several species, that the $\delta^{13}C$ of soluble sugars and starch are closely related to gas exchange parameters and that the starch $\delta^{13}C$ is less negative than the $\delta^{13}C$ of soluble sugars [5]. An isotopic mass balance illustrates the plausible influence of leaf starch $\delta^{13}C$ and concentration on bulk $\delta^{13}C$. Assuming reasonable increases in leaf starch in spring of 15% of needle dry weight (starch in Scots pine needles can rise to values over 25% [12]) and a medium change in starch $\delta^{13}C$ of 5‰ (compared to 9‰ variation observed for instantaneous discrimination [3]), bulk needle $\delta^{13}C$ would change by 5‰ × 0.15 = 0.75‰ to less negative values. This suggests, that the spring ^{13}C-enrichment (0.5‰ over that of mature needles) can be reasonably ascribed to the accumulation of starch translocated from the previous year's needles.

The rapid depletion of ^{13}C in developing needles in spring is consistent with an increasing concentration of lipids (depleted in ^{13}C) with a simultaneous decrease of sucrose concentration during maturation. It is known that following bud break, starch concentrations in developing needles are low, and concentrations of sucrose, glucose and fructose are high; lipid content increases as maturation proceeds [12].

A general trend to less negative $\delta^{13}C$ values with increasing radiation was observed when including the spring period with large changes in needle $\delta^{13}C$. This trend could be caused by the accumulation of starch in the needles in spring, supposing that the rate of accumulation of starch is correlated positively to the increasing radiation in spring.

For the 1996 needle generation a significant decrease to more negative needle $\delta^{13}C$ over time was observed. It is known, than older Scots pine needles have a lower chlorophyll content, maximum Rubisco activity and a lower activity of photosystem II compared to current year needles [14, 18]. This could lead to the here observed depletion in ^{13}C.

4.3. Environmental effects on needle $\delta^{13}C$

The mature 1998- needle generation had more negative $\delta^{13}C$ values than the 1996- or the 1997-needle generations. However, the year 1998 had also significantly higher precipitation than the years 1996 or 1997 (1106 mm in 1998 versus 794 mm in 1997 and 629 mm in 1996). Increased precipitation could on one hand reduce hydraulic restraints and on the other hand correlate with increased cloud cover and hence decreased irradiance. The net result would be more negative $\delta^{13}C$ of primary assimilatory products in needles.

Bulk needle $\delta^{13}C$ was only slightly correlated with most environmental variables, excepting five-day averaged radiation which was positively correlated with less negative $\delta^{13}C$ of needles. This relationship is consistent with other results [3] showing that in Scotland, radiation is a major limiting factor for carbon assimilation in Scots pine.

5. CONCLUSIONS

Whereas temporal variation in needle $\delta^{13}C$ depends on the environmental changes during the annual cycle and the physiology of the investigated species, the quality of spatial variation in needle or leaf $\delta^{13}C$ due to hydraulic effects could be independent of species differences. Compression wood in the abaxial half of conifer branches is widespread and also the presence of tension wood in deciduous tree branches might affect the hydraulic conductivity and thus the $\delta^{13}C$ of leaf material. For the future, not only do sampling strategies need to be standardised to account for these systematic shifts in isotope composition, but we have shown that morphological

constraints as well as environmental conditions both make a major contribution to the carbon isotope signal of pine needles and subtending cellulose in the adjacent branch.

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