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The effect of drought on C and N stable isotopes in different fractions of leaves, stems and roots of sensitive and tolerant beech ecotypes

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ABSTRACT

Beech seedlings from 11 German climatic provenances were exposed to a realistically timed drought treatment in a greenhouse experiment. The stable isotope composition of carbon (C) and nitrogen (N) was analysed in pooled bulk material of roots, stems and leaves, as well as in the aqueous extracts and starch fractions. The δ^{13}C values increased in bulk samples (BS) of roots, stems and leaves by drought, although no leaf growth occurred during the experimental period. A clear drought effect on δ^{13}C in aqueous extracts was detected in leaves. In aqueous extracts of stems and roots as well as in starch fractions of all organs, abundance of δ^{13}C also tended to be increased by drought, but this effect was not statistically significant. For both δ^{13}C and δ^{15}N, enrichment was observed from the site of uptake/source to the site of use/sink. A gradient for δ^{13}C in all fractions from leaves (–29.49, –28.89 and –27.85‰) to stems (–28.81, –27.48 and –26.98‰) and to roots (–27.60, –26.37 and –26.48‰) was detected in BS, aqueous extracts and starch, respectively. An opposite gradient for δ^{15}N was found in BS: 1.59‰, 1.84‰ and 3.05‰ in roots, stems and leaves, respectively. δ^{15}N was neither affected by drought in the BS nor in aqueous extracts, but an effect of provenance was observed. Particularly in roots and stems, drought-sensitive provenances showed the strongest shifts in δ^{13}C induced by drought and the lowest δ^{15}N values. In the present experiment, δ^{13}C values were more affected by the environmental factor drought, while δ^{15}N values were more affected by the genetic factor provenance.

Key-words: drought stress, *Fagus sylvatica*, isotope discrimination, provenances.

INTRODUCTION

Climate change models predict that increasing atmospheric CO_2 partial pressure will cause average surface temperatures to increase by 1–3.5 °C in mid-latitude regions during the next 100 years (Saxe, Ellsworth & Heath 1998; UNEP 1999). As a consequence, precipitation and evaporation patterns will change in Europe, and ecosystems will be exposed to more intense drought and heavy rain events in summer (IPCC 1997; UNEP 1999). Changing climatic conditions will become of particular importance for trees because of their long lifespan. Forests will face altered environmental conditions during their lifetime, with likely consequences for species composition and forest management (IPCC 1997; Saxe *et al*. 2001). Natural regeneration of the drought-sensitive European beech (*Fagus sylvatica* L.) – one of the most important deciduous tree species in Central Europe – may be significantly affected by such climate alterations, specially because the area of distribution includes sites with shallow limestone-derived soils with low water storage capacity (e.g. Schwäbische Alb, Fränkische Alb, Schweizer Jura and French Jura). Therefore, projected short summer drought periods caused by global warming may inhibit natural regeneration in critical habitats.

Owing to its sensitivity towards environmental constraints, δ^{13}C and δ^{15}N are now widely used to assess the effects of changing climatic condition on plant ecophysiology. However, a question that requires further research is which parts, tissues, chemical fractions or compounds of plants reflect the best particular biotic or abiotic conditions. Bulk materials have been isotopically analysed to provide physiological or environmental insights over the lifespan of the plant part sampled (see in review Adams & Grierson 2001; Evans 2001; Dawson *et al*. 2002). Specific individual compounds or groups of compounds such as water extractable C, cellulose, lipids, sugars and starch have been isotopically analysed to provide information about metabolism integrated over particular time integrals (Picon, Ferhi & Guehl 1997; Gleixner *et al*. 1998; Ghashghaie *et al*. 2001; Ponton *et al*. 2001; Terwilliger *et al*. 2001; Arndt & Wanek 2002; Barbour, Walcroft & Farquhar 2002; Damesin & Lelarge 2003). Leaf material is most commonly studied, but axial tissue has also drawn attention (Picon *et al*. 1997; Ponton *et al*. 2001; Arndt & Wanek 2002; Barbour *et al*. 2002; Damesin & Lelarge 2003; Fotelli *et al*. 2003). Not much is known about environmental effects on stable isotope abundance in the roots (Robinson *et al*. 2000; Emmerton *et al*. 2001; Arndt & Wanek 2002; Fotelli *et al*. 2003). Variations

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in water availability may, however, not only change C isotope discrimination by Rubisco but also the downstream processes of C metabolism in autotrophic and heterotrophic tissues and during leaf-to-stem (Damesin & Lelarge 2003) or leaf-to-root allocation (Keitel *et al*. 2003). In addition, changes in N source and distribution of N assimilation between roots and shoots are known to influence intraplant variation in δ^{15}N (Yoneyama *et al*. 1997; Robinson, Handley & Scrimgeour 1998). Thus, the determination of C and N isotopes in different plant parts can give insights into drought effects on the partitioning of C and N metabolism within the plant.

This paper investigates the impacts of drought on δ^{13}C and δ^{15}N from bulk to compound specific levels in an entire plant. It also examines whether there might be a genotypic component to these impacts by subjecting seedlings from different autochthonous provenances to the same drought treatment. Recently, Pritchard & Guy 2005) described that genetic differences in N assimilation and uptake capacity among provenances of white spruce can result in different N isotope fractionation during uptake. Guy & Holowachuk (2001) also observed δ^{13}C values for *Pinus contorta* saplings to increase with their origin from wetter to drier site.

Analysis of different organs was aimed at filling gaps in actual knowledge on the variations in isotopic composition along the plant axis, the potential influences of drought on such gradients and the transport and metabolism of C and N compounds.

The impact of drought treatment and provenance were studied under controlled conditions. To exclude the effects on growth, a summer drought period was simulated after finishing the first phase of shoot tip growth. In addition, summer droughts are an important growth constraint in beech ecosystems in Central Europe; hence, summer droughts are of greater ecological importance compared to water restrictions during spring growth (Fotelli *et al*. 2003).

Our interpretations of the isotopic analyses are enhanced by recent studies on physiological responses to drought sensitivity and nutritional status. Recently, beech ecotypes of different drought sensitivity were identified by physiological parameters among German provenances, spanning latitudinal (53°–47°N) and altitudinal (50–1660 m) gradients. The sensitivity to drought of some provenances ('sees' and 'sont') could be attributed to the climatic conditions in their original habitats, particularly to the amount of rainfall (Peuke *et al*. 2002), and are clearly separated from tolerant provenances ('bov' and 'kloe'). However, exceptions were found where sensitivity do not fit to the climate of the habitat (e.g. 'small'). The drought treatment reduced the substrate's water capacity to 18.5 ± 0.3% relative to the control treatment over the three weeks it was imposed (Peuke *et al*. 2002). The water content was reduced to 97% of controls in leaves and stems and to 92% in the roots. A strong reduction of pre-dawn water potential in roots (5.6-fold) and shoots (2.2-fold of the control) was found (see also Table 1). After drought, provenances showed significant differences in the water potential of the shoots (Peuke *et al*. 2002). The most important effect on macronutrients was that the drought treatment decreased phosphorus and phosphate concentrations in all tissues (Peuke & Rennenberg 2004). The partitioning of all macronutrients was not affected by drought.

Based on the available information, our main working hypotheses were:

1. δ^{13}C in pools with different turnover times should show different sensitivity towards the drought stress applied.
2. Drought-sensitive ecotypes should react more intensively as compared to tolerant.
3. δ^{15}N can be used as an integrator of stress for different beech ecotypes.
4. δ^{13}C and δ^{15}N gradients between above- and below-ground parts of beech and their potential interference with drought can give useful information on the transport and metabolism of C and N compounds.

MATERIALS AND METHODS

Plant material and experimental design

Seeds of beech (*F. sylvatica*) from different autochthonous provenances in Germany that represent differences in mean annual rainfall of the site of origin were used for the present investigation. These provenances were: (1) 'red': Rothemühl, 574 mm rainfall per year; (2) 'kloe': Klötze, 586 mm rainfall per year; (3) 'small': Lüttenhagen, 599 mm rainfall per year; (4) 'goer': Göhrde 630 mm rainfall per year; (5) 'tbb': Tauberbischofsheim, 650 mm rainfall per year; (6) 'bov': Bovenden, 680 mm rainfall per year; (7) 'black': Schwarzach, 800 mm rainfall per year; (8) 'bad': Bad Urach, 890 mm rainfall per year; (9) 'sees': Seesen, 1150 mm rainfall per year; (10) 'harz': Harz, 1400 mm rainfall per year; and (11) 'sont': Sonthofen, 1700 mm rainfall per year (for further details, see Peuke *et al*. 2002).

After germination, seedlings were transferred to 2 L pots (one seedling per pot) filled with a commercial potting soil (Floradur®, Floragard GmbH, Germany), Perlite (Perligran® G, Deutsche Perlite GmbH, Germany) and soil from a natural beech stand near Freiburg (5:5:1, V : V). The seedlings were placed in a completely randomized design in a greenhouse with an artificial light period of 16 h (Osram® HQL 400 Osram GmbH, Munchen, Germany), with 200–250 μmol photons m^{-2} s^{-1} light intensity at plant level during daytime at 20 ± 5 °C and 40–60% RH. These conditions were chosen to simulate the growth of young seedlings and understorey vegetation in a beech stand in summer. Kreuzwieser *et al*. (1997) showed that beech seedlings were not light-limited at the light intensity levels applied here. Every second day the pots were well watered with tap water. Every second week after the first month, the plants were supplied with a commercial fertilizer [0.3% Hakaphos® Blau, Compo GmbH, Germany; 15% N (4% NO_3^--N + 11% NH_4^+-N), 10% P_2O_5, 15% K_2O, 2% MgO].

After 12 weeks, the first period of leaf and shoot tip growth was finished. The population of each provenance was divided into two by selecting homogenous matched pairs and assigning each half to either control or drought

Table 1. Ranking of provenances in order of strength of response to drought treatment in significantly affected parameters in roots, stems and leaves of 4.5 month old beech seedlings (*Fagus sylvatica* L.)

Stomatal conductance (%)	C in H_2O extracts (%)						$\delta^{13}C$ in BS (‰)				$\delta^{13}C$ in H_2O extracts of leaves (‰)	Rain per year	Water potential in shoots after drought [Mpa]	% of control
	Leaves	Stems	Roots	Starch in stems			Leaves	Stems	Roots					
bov	86.3	**bov**	93.7	goer	106.4	**kloe**	102.7	*sont*	97.5	red	101.2	**bov**	100.8	black	100.8	**bov**	104.4	890	bad	−0.27 ± 0.04	119
bad	75.3	goer	94.5	black	109.4	**bov**	103.9	*sees*	104.6	**bov**	100.9	red	99.8	tbb	100.7	small	102.8	586	**kloe**	−0.43 ± 0.04	179
kloe	74.4	**kloe**	100.3	**bov**	112.3	bad	105.7	bad	108.9	*sont*	100.2	tbb	99.5	**kloe**	100.3	black	99.9	680	**bov**	−0.47 ± 0.05	188
red	73.5	red	106.2	*sees*	115.9	small	107.3	goer	109.2	harz	99.3	*sont*	98.6	harz	100.1	red	98.4	630	goer	−0.56 ± 0.11	193
tbb	69.4	tbb	107.2	**kloe**	116.3	tbb	110.8	tbb	121.1	**kloe**	99.0	**kloe**	98.6	**bov**	98.9	tbb	97.5	650	tbb	−0.62 ± 0.09	214
goer	67.8	harz	111.9	bad	117.1	*sees*	111.7	small	128.6	small	98.9	small	98.5	small	98.3	**kloe**	95.4	800	black	−0.46 ± 0.06	219
harz	63.0	black	122.1	red	118.2	*sont*	125.5	**kloe**	132.9	black	98.5	harz	98.3	red	98.2	*sont*	93.0	574	red	−0.49 ± 0.07	223
sees	53.3	*sont*	122.8	harz	123.1	harz	125.9	harz	134.9	tbb	98.3	black	98.1	bad	97.8	harz	92.9	1400	harz	−0.56 ± 0.09	233
black	51.7	*sees*	123.3	*sont*	127.0	red	133.0	red	141.0	goer	97.9	bad	97.3	*sont*	97.8	bad	92.0	1700	*sont*	−0.74 ± 0.14	255
sont	46.1	bad	130.6	tbb	140.7	goer	145.5	black	147.5	*sees*	97.8	goer	96.8	goer	97.0	goer	89.5	1150	*sees*	−0.72 ± 0.22	267
small	41.6	small	136.2	small	146.9	black	147.5	**bov**	160.7	bad	94.8	*sees*	96.1	*sees*	96.6	*sees*	89.4	599	small	−0.81 ± 0.27	326

Shown are the ratios of drought treatment to controls in percentage and the water potentials in shoots of drought-treated plants (data taken from Peuke *et al*. 2002) and the average rainfall per year (mm). The provenances 'sees' and 'sont' originating from wet habitats were identified to be sensitive to drought compared to 'kloe' and 'bov' from dry habitats that were more drought-tolerant ecotypes (Peuke *et al*. 2002). For more details, please see legend Fig. 1.
BS, bulk samples.

treatments. Control plants were watered as before (to field capacity; 2.68 ± 0.02 g water g^{-1} substrate), while in the drought treatment water supply was reduced to a water content of 20% ± 5% (w/w) of the substrate. Water content of pots was adjusted gravimetrically every 1–2 d. Two days before harvest, water supply was stopped. During drought treatment, no leaf growth was possible, and only secondary thickening and root growth may have occurred. For further details regarding the plant material, cultivation and drought treatment, see Peuke *et al*. (2002).

Harvesting

One day before harvest, stomatal conductance was measured using LI-1600 steady state porometer (Li-Cor Inc., Lincoln, NB, USA). The stomatal conductance of leaves 3–5 were used as averages for the entire plant. The measurements were carried out in the greenhouse between 1200 and 1500 h. Before dawn ('pre-dawn'), the seedlings were divided into leaves, stems and roots. Parts of the shoot were carefully washed with deionized water. Roots were first rinsed briefly with tap water, cleaned from substrate particles in 0.1 M sorbitol, briefly rinsed with water again and dried with filter paper. After weighing, the plant parts were immediately frozen in liquid N_2 and stored at −80 °C until analysis.

Chemical analysis

After lyophilization, the dried plant material was ground. Extraction and determination of elements and solutes represent averages of BS of the whole organ of each seedling. Water soluble C compounds were determined in aqueous extracts from lyophilized ground plant material (hereinafter referred to as 'aqueous extracts'). For this purpose, 40 mg of plant powder were mixed with 80 mg PVPP (Sigma Chemie, Deisenhofen, Germany) to remove phenolic compounds during extraction. The dry material was extracted with 2.5 mL deionized water for 1 h in an ice bath under continuous shaking. After boiling for 5 min the extracts were centrifuged for 10 min at 16 000 *g* to precipitate proteins, and the supernatant was frozen immediately in liquid nitrogen.

For starch extraction, the pellets from the sugar extraction procedures were washed once again with deionized water. To digest the starch completely, 2 units of amyloglucosidase from *Aspergillus niger* and 1 mL deionized water were added to the pellets. The mixtures were shaken continuously in a water bath at 37 °C for 1 h. Finally the extracts were boiled and centrifuged again as previously described.

Aliquots of the extracts from aqueous extraction and starch digestion were injected into a Dionex DX 500 HPLC system (Dionex, Idstein, Germany). Separation of sugars was achieved on a 4 × 250 mm CarboPac PA1 column (Dionex, Idstein, Germany) with 36 mM NaOH as mobile phase. Detection and quantification was performed with a pulsed amperometric detector (Dionex ED 40 electrochemical detector, Idstein, Germany). Starch concentration

was expressed on the basis of glucose equivalents [μequiv. glu mg^{-1} BS].

Stable isotope composition

Stable isotope signatures and C and N content were determined in BS, aqueous extracts and starch of leaves, stems and roots of the beech seedlings. The bulk material was oven-dried for 3 d at 65 °C, and 1–2 mg of homogenized powder was transferred into tin capsules (Type A; ThermoQuest, Milan, Italy). For the extracts, 40–120 mL aliquots plus 0.5 mg chromosorb were transferred in tin capsules. The liquid samples were dried overnight at 65 °C before analysis. The samples were injected into an elemental analyser (NA 2500; CE Instruments, Milan, Italy) coupled to an isotope ratio mass spectrometer (Delta Plus; Finnigan MAT GmbH, Bremen, Germany) by a Conflo II interface (Finnigan MAT GmbH, Bremen, Germany). The $\delta^{13}C$ and $\delta^{15}N$ values were defined as:

$$\delta^{XX}E\ (‰) = [(R_{sample}/R_{standard}) - 1] \times 1000,$$

where in the case of C (E = C; XX = 13), R_{sample} and $R_{V\text{-}PDB}$ (1.1237×10^{-2}) are the $^{13}C : {}^{12}C$ ratios of sample and Vienna-Pee Dee belemnite. In the case of N (E = N; XX = 15), R_{sample} and $R_{N2\text{-}atm}$ (3.6764×10^{-3}) are the $^{15}N : {}^{14}N$ ratios of sample and atmospheric N_2, respectively.

Statistics

Twenty seedlings from each provenance were assigned to either drought or control treatments. All statistical calculations were performed with SAS (SAS, Institute Inc., Cary, NG, USA) release 8.2. Two-way analysis of variance (ANOVA, model: 'drought (treatment)', 'provenance' and interactions 'drought*provenance') were performed using the GLM procedure. The adjustment of multiple comparisons according to the Tukey test was chosen for the *P*-values and confidence limits for the differences of least squares of the means (LS-means).

In figures and tables, the means (LS means of entire data set, control or drought treatment, provenance, control or drought treatment of provenance) ± SE are presented. Analysis of correlation was performed on the whole data set using the CORR procedure (K_p: and *P*-value for $H_0 : K_p = 0$).

To test the differences in sensitivities of provenances for $\delta^{13}C$ to the drought treatment, analysis of regression was performed. In the first step of regression analysis by the REG procedure of SAS, a complete model was composed with a number of potential independent variables, which might possibly affect $\delta^{13}C$ in BS of leaves ($_L$), stems ($_A$) or roots ($_R$) (under the assumption that C is taken up by leaves and $\delta^{13}C$ in leaves will therefore affect $\delta^{13}C$ in stems and so on):

$\delta^{13}C$ in $BS_{L/A/R}$ = intercept + stomatal conductance + $\delta^{13}C$ in aqueous extracts (+$\delta^{13}C$ in $BS_{L/A}$ in the case of BS_A or BS_R).

For $\delta^{15}N$ in bulk samples of stems ($_A$) or leaves ($_L$):

$\delta^{15}N$ in $BS_{L/A}$ = intercept + $\delta^{15}N$ in aqueous extracts + d15N in $BS_{A/R}$ in the case of BS_L or (BS_A).

By backwards selection, non-significant independent variables were eliminated (H_0 : estimates = 0). To test for differing sensitivities of provenances to drought, in a selected linear model (GLM procedure of SAS), provenance was introduced as class variable (Model: Y = X $provenance_{class}$ X*provenance) and the homogeneity of slopes was tested.

RESULTS

Stomatal conductance

Stomatal conductance of beech seedlings was decreased significantly by drought and affected by provenance (Fig. 1). Three provenances not significantly affected by drought ('kloe', 'tbb' and 'bov') as well as the provenance 'black' that was significantly affected by drought had stomatal conductance below 100 mmol m^{-2} s^{-1} under control conditions. Mostly, the provenances from a less dry climate responded stronger to drought, with the exception of 'small' which was also strongly inhibited to 42% of the control.

Starch and soluble sugars in leaves, stems and roots

The concentration of C in soluble sugars in beech leaves was affected by both provenance and drought (Figs 2a & 3b). In controls, the difference between provenances was nearly 100% while in drought-treated beeches, the differences between provenances were less pronounced. On

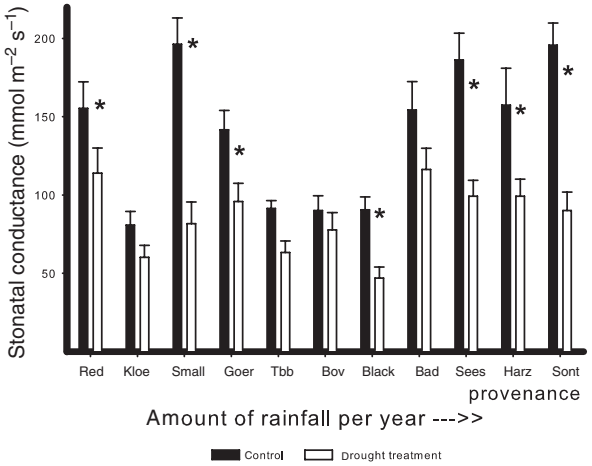

Figure 1. Stomatal conductance in leaves of 4.5 month old beech seedlings (*Fagus sylvatica* L.). The controls were watered every second day in excess throughout while the drought treatment was applied by stopping the watering of the pots three weeks before harvest and keeping the pots at a minimum of about 20 ± 5% water content. Shown are the mean + standard errors. The provenances are sorted in order of annual rainfall in the region. Significance of drought within a provenance is indicated by an asterisk at the columns. Interactions were never significant.

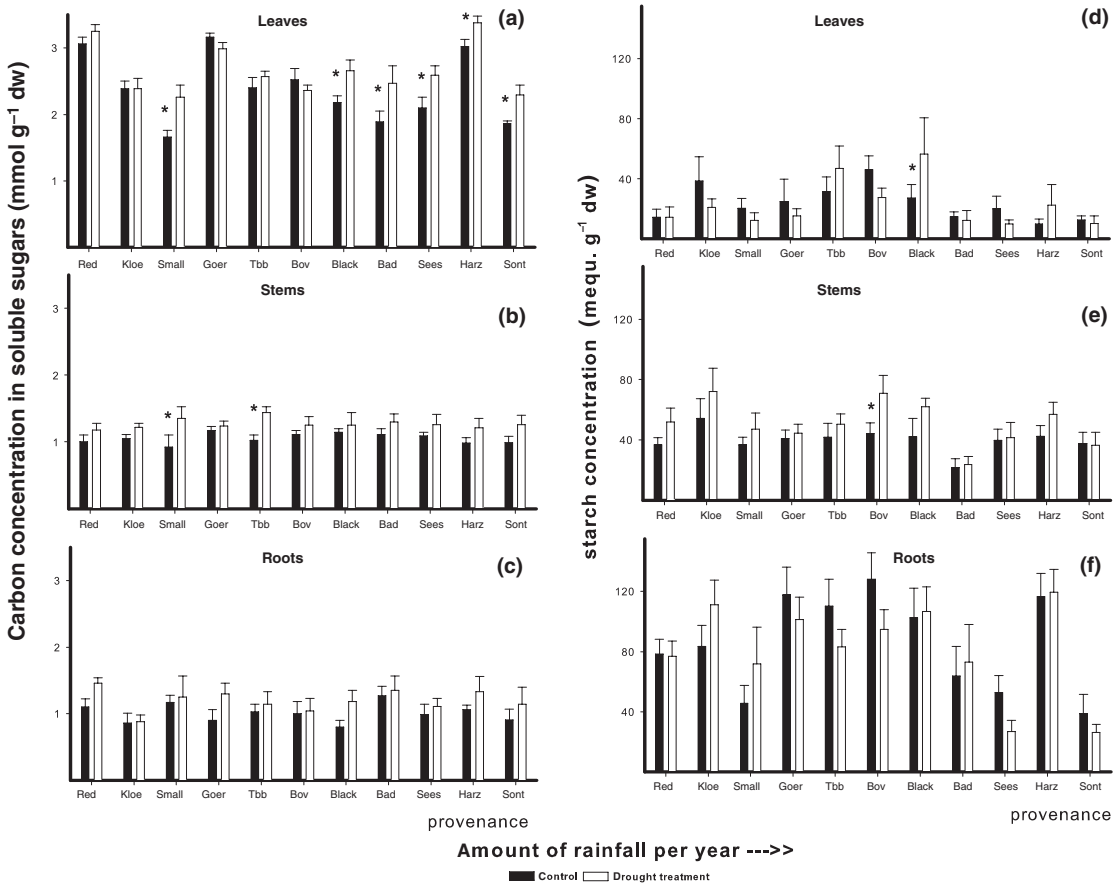

Figure 2. Concentration of carbon (C) in soluble sugars in (a) leaves, (b) stems and (c) roots, and starch concentration in (d) leaves, (e) stems and (f) roots in controls and drought-treated 4.5 month old beech seedlings (*Fagus sylvatica* L.). Data for C in sugars of leaves were recalculated from Peuke & Rennenberg (2004). For further details, see legend Fig. 1.

average, C content in soluble sugars was increased to 111% of controls by drought. With the exception of 'small', the most affected provenances were the ones originating from habitats with the highest rainfall. C content in soluble sugars in stems also increased significantly by drought to 119% of the controls (Figs 2a & 3b). The effect of provenance on stem C content in soluble sugars was not significant. Although differences of approximately 50% in C content in soluble sugars were observed between the roots of provenances, this effect was not significant (Fig. 2c). However, C content in soluble sugars in roots significantly increased to about 116% of the controls by drought (Fig. 3b).

The concentrations of starch in leaves varied strongly between provenances by a factor of nearly six (Fig. 2d). Drought had no significant effect on starch in beech leaves (Fig. 3c). Starch in the stems was affected by both provenance and drought. It was lowest in 'bad' and highest in 'kloe' (Figs 2e & 3c). The average content of starch in stems was increased significantly by drought to 127% of the control (Figs 2f & 3c). Starch in the roots varied significantly by a factor of 5 between provenances. Drought did not affect starch in the roots.

δ^{13}C in BS, aqueous extracts and starch of leaves, stems and roots

The δ^{13}C values in leaves was significantly but only slightly affected by drought (Figs 3d & 4a). Differences between provenances were not observed, but in 'bad' the effect of drought was significant (Fig. 4a). In stems, δ^{13}C was affected by both drought and provenance (Figs 3d & 4b). On average, δ^{13}C was increased in stems slightly by drought. Furthermore, δ^{13}C in the roots of beech seedlings was increased by drought and affected by provenance (Figs 3d & 4c). In general, the effect of drought was not intense and caused only a low increase of δ^{13}C of bulk organic matter.

In the aqueous extract of leaf BS, δ^{13}C was significantly increased by drought (Figs 3e & 4d). When looking at particular provenances, drought resulted in significant ^{13}C enrichment in water soluble organic matter of 'goer' and 'sees'. δ^{13}C in aqueous extract from stems or roots were not affected by drought or provenance (Figs 3e & 4e,f). For the provenance 'tbb', significant ^{13}C enrichment in stem soluble C as consequence of drought was observed.

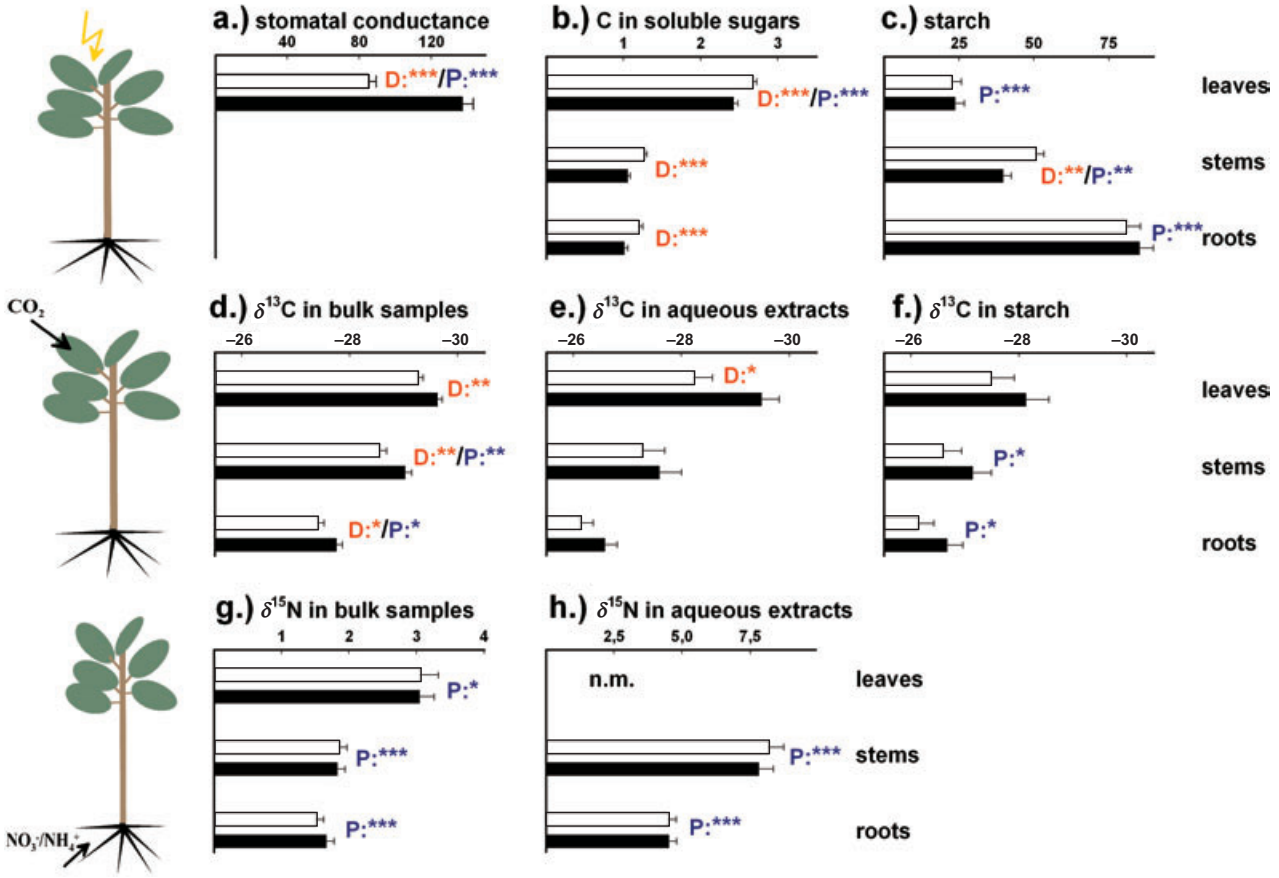

Figure 3. Summary of the main factors drought-treatment (D: in red) and provenance (P: in blue) (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$) and (LS-) means of controls and drought-treated 4.5 month old beech seedlings (*Fagus sylvatica* L.) of (a) stomatal conductance (mmol m^{-2} s^{-1}), (b) carbon (C) concentration in soluble sugars (mmol g^{-1} dw), (c) starch concentration (mequ g^{-1} dw), (d) $\delta^{13}C$ in bulk sample (BS, ‰), (e) $\delta^{13}C$ in water extractable fraction of BS (‰), (f) $\delta^{13}C$ in starch fraction of BS (‰), (g) $\delta^{15}N$ in BS (‰) and (h) $\delta^{15}N$ in water extractable fraction of BS (‰).

The C isotopes composition of starch in leaves of beech seedlings was not affected by drought or provenances (Figs 3f & 4g). In starch from stems and roots, $\delta^{13}C$ was significantly affected by provenance but not by drought (Fig. 3f).

Intense correlation and regression analysis were performed to detect the connection between the measured parameters and also to evaluate provenance-specific sensitivities. Data from earlier studies were also considered. There was a relatively good correlation of $\delta^{13}C$ in the bulk material between stems and leaves ($K_P = 0.73$) as well as roots ($K_P = 0.76$), and between leaves and roots ($K_P = 0.50$). However, correlation for $\delta^{13}C$ between organs was not found in aqueous extracts or starch, as well as between BS, aqueous extracts or starch. There was also no correlation between starch or sugar C concentration and $\delta^{13}C$ in these fractions.

$\delta^{13}C$ values of leaf aqueous extracts were only related to $\delta^{13}C$ values of BS in 'goer' and 'bov' with a poor strength ($r^2 < 0.42$, Table 2) testing by regression analysis sliced for provenances. With regards to modelling $\delta^{13}C$ in BS of leaves versus stomatal conductance, the correlation was significant only in 'black' and 'sees' and the model fitted relatively well for 'sees' ($r^2 = 0.51$). A tight correlation between $\delta^{13}C$ in BS between stems and leaves as well as between roots and stems was found ($r^2 > 0.998$, Table 2). For both regression models, the intercept was not significant. In all presented regression models for $\delta^{13}C$, no significant differences between estimates were found if sliced for the provenances. Additionally, no significant correlations were found with earlier published parameters, namely biomass, water potential, water content, proline or abscisic acid (ABA).

$\delta^{15}N$ in BS and aqueous extracts of leaves, stems and roots

$\delta^{15}N$ in BS of leaves as well as stems and roots was affected only by provenance, but not by drought (Figs 3g & 5a–c). In 'small'and 'sees', the lowest $\delta^{15}N$ values were observed in leaf BS. Nearly twofold higher $\delta^{15}N$ were detected in leaf BS of 'red' and 'harz' than in a group of provenances originating from wet habitats, 'bad', 'sees', and 'sont', and also

Table 2. Results of the regression analysis for $\delta^{13}C$ and $\delta^{15}N$ in BS of leaves, stems or roots sliced for the provenances [Model: Y = (a) + bX1 + (cX2)]

	Red	Kloe	Small	Goer	tbb	bov	Black	Bad	Sees	Harz	Sont
$\delta^{13}C$ -BS_L											
Intercept	–27.90	–27.42	–25.50	–26.53	–27.42	–24.43	–27.59	–25.26	–27.51	–27.07	–27.03
	±3.17	±2.03	±2.33	±1.40	±1.91	±1.57	±1.66	±4.81	±1.09	±1.62	±1.74
	***	***	***	***	***	***	***	**	***	***	***
$\delta^{13}C$ in $extr._L$	0.062	0.067	0.119	0.101	0.070	0.184	0.071	0.132	0.056	0.085	0.094
	±0.107	±0.070	±0.079	±0.046	±0.063	±0.054	±0.056	±0.161	±0.040	±0.054	±0.061
	n.s.	n.s.	n.s.	*	n.s.	**	n.s.	n.s.	n.s.	n.s.	n.s.
r^2	0.022	0.053	0.184	0.222	0.068	0.416	0.095	0.102	0.106	0.129	0.117
	n.s.	n.s.	n.s.	*	n.s.	**	n.s.	n.s.	n.s.	n.s.	n.s.
GLM:	$\delta^{13}Cextr._L$: ***		$r^2 = 0.204$: **								
$\delta^{13}C$ -BS_L											
Intercept	–28.70	–28.20	–28.86	–29.73	–29.06	–29.66	–28.71	–28.39	–28.02	–29.70	–29.15
	±0.62	±0.57	±0.62	±0.60	±0.74	±0.62	±0.42	±0.98	±0.27	±0.46	±0.45
	***	***	***	***	***	***	***	***	***	***	***
stom.conduct.	–0.008	–0.016	–0.001	0.001	–0.006	0.000	–0.014	–0.006	–0.007	0.001	–0.004
	±0.004	±0.008	±0.004	±0.005	±0.009	±0.007	±0.005	±0.007	±0.002	±0.003	±0.003
	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	*	n.s.	***	n.s.	n.s.
r^2	0.177	0.219	0.006	0.004	0.024	0.000	0.334	0.120	0.507	0.002	0.093
	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	*	n.s.	***	n.s.	n.s.
GLM:	stom.cond: **		$r^2 = 0.188$: *								
$\delta^{13}C$ -BS_A											
$\delta^{13}C$ -BS_L	0.991	0.973	0.963	0.970	0.980	0.982	0.981	1.001	0.966	0.998	0.966
	±0.006	±0.004	±0.005	±0.009	±0.005	±0.005	±0.007	±0.011	±0.006	±0.005	±0.004
	***	***	***	***	***	***	***	***	***	***	***
r^2	0.9994	0.9997	0.9997	0.9985	0.9994	0.9995	0.9992	0.9996	0.9992	0.9995	0.9997
	***	***	***	***	***	***	***	***	***	***	***
GLM:	$\delta^{13}C$ BS_L: ***		$r^2 = 0.633$: ***								
$\delta^{13}C$ -BS_R											
$\delta^{13}C$ -BS_A	0.967	0.959	0.965	0.956	0.958	0.952	0.965	0.962	0.961	0.941	0.960
	±0.008	±0.004	±0.008	±0.004	±0.005	±0.004	±0.011	±0.007	±0.003	±0.006	±0.005
	***	***	***	***	***	***	***	***	***	***	***
r^2	0.9989	0.9996	0.9992	0.9996	0.9995	0.9997	0.9977	0.9998	0.9999	0.9992	0.9996
	***	***	***	***	***	***	***	***	***	***	***
GLM:	$\delta^{13}C$ BS_A: ***		proven. ***		$r^2 = 0.620$: ***						
$\delta^{15}N$ -BS_L											
$\delta^{15}N$ -BS_A	1.80	1.37	0.84	1.27	1.22	1.09	1.36	2.01	1.82	1.66	0.26
	±0.37	±0.11	±0.27	±0.14	±0.08	±0.10	±0.13	±0.43	±0.40	±0.20	±1.17
	***	***	***	***	***	***	***	***	***	***	n.s.
r^2	0.56	0.88	0.44	0.82	0.93	0.86	0.85	0.65	0.53	0.79	0.003
	***	***	*	***	***	***	***	***	***	***	n.s.
GLM:	$\delta^{15}N$-BS_A: ***		proven. *	X: **	$r^2 = 0.313$: ***						
$\delta^{15}N$ -BS_A											
$\delta^{15}N$ -BS_R	0.72	0.35	0.48	0.73	0.74	0.55	0.64	1.30	0.66	0.75	0.31
	±0.12	±0.11	±0.38	±0.12	±0.18	±0.12	±0.07	±0.32	±0.10	±0.11	±0.17
	***	**	n.s.	***	***	***	***	**	***	***	n.s.
$\delta^{15}N$ in $extr._A$	0.091	0.172	0.126	0.067	0.081	0.080	0.087	–0.033	0.055	0.096	0.087
	±0.023	±0.033	±0.062	±0.020	±0.032	±0.020	±0.014	±0.040	±0.017	±0.025	±0.044
	***	***	n.s.	**	*	***	***	n.s.	**	**	n.s.
r^2	0.93	0.86	0.53	0.88	0.85	0.90	0.95	0.67	0.81	0.93	0.41
	***	***	*	***	***	***	***	**	***	***	*
GLM:	$\delta^{15}N$-BS_R: ***		$\delta^{15}N$ in $extr._A$ ***		proven. ***		$r^2 = 0.66$: ***				

The estimates of the intercepts and slopes are given ± SE (H_0: estimate = 0; n.s., not significant; $*P < 0.05$; $**P < 0.01$; $***P < 0.001$). The homogeneity of the slopes for the different provenances was tested with the help of a general linear model (GLM) with provenances as class variable (Y = intercept + X + provenance (class) + X*provenance).
Extr., aqueous extracts; BS, dry weight/bulk material; X_L, leaves; X_A, stems; X_R, roots; proven, provenance.

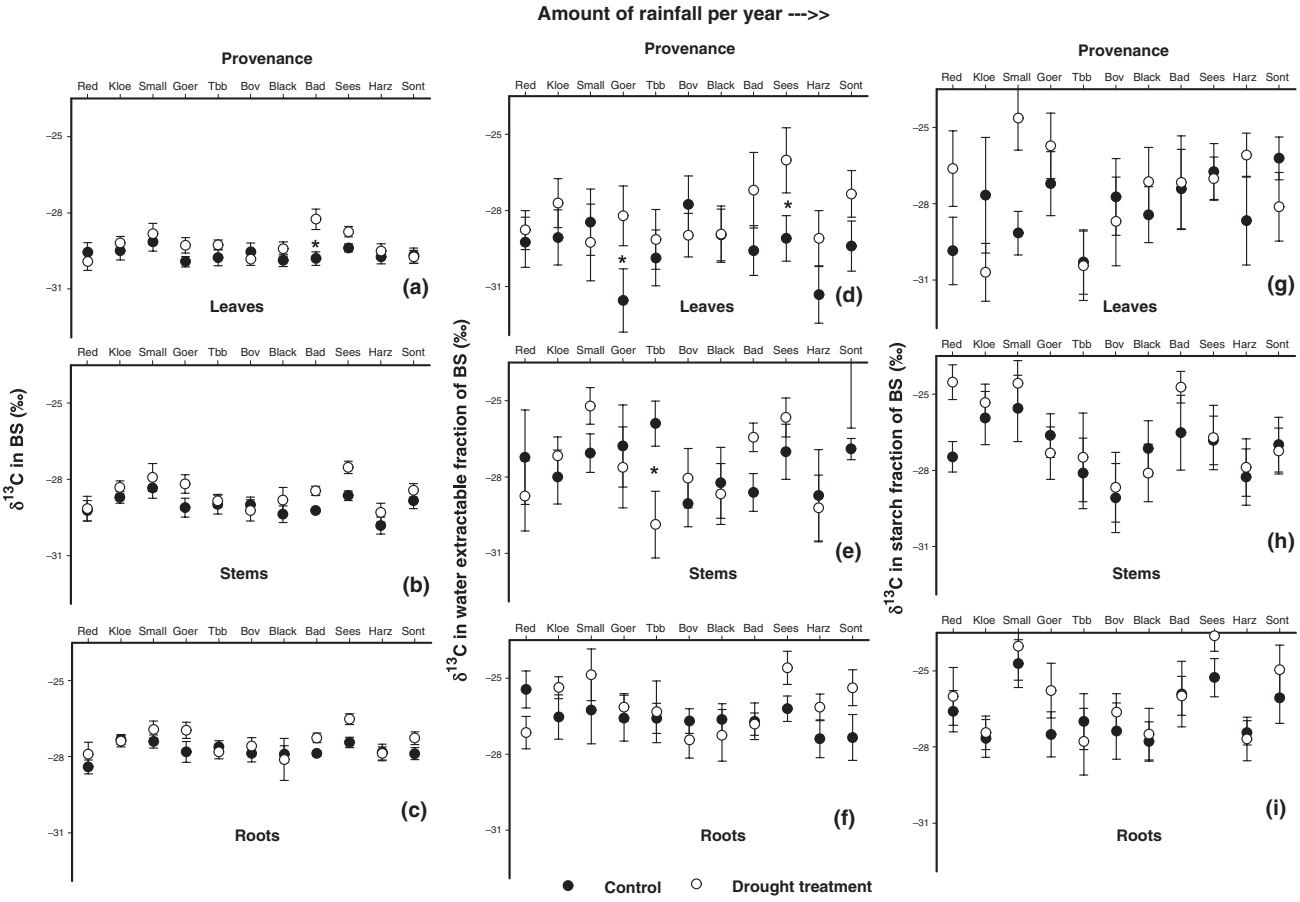

Figure 4. Carbon (C) isotope composition in the bulk samples (BS) of (a) leaves, (b) stems, (c) roots in aqueous extracts of (d) leaves, (e) stems, (f) roots in starch of (g) leaves, (h) stems and (i) roots in controls and drought-treated 4.5 month old beech seedlings (*Fagus sylvatica* L.). For further details, see legend Fig. 1.

'small' from a dry habitat. This group also had lower δ^{15}N in stems and roots compared to the average (Fig. 5b & c).

In aqueous extracts from leaves N, was too low to determine δ^{15}N properly. δ^{15}N in aqueous extracts of stems and roots was affected only by provenance and not by drought (Fig. 3h). Similar to the BS, ^{15}N in aqueous extracts from a group of provenances originating from wet habitats, 'bad', 'sees' and 'sont', but also in 'small' from a dry habitat, showed lower values in extracts from stems and from roots compared to the average (Fig. 5e & f). In the provenance 'black', δ^{15}N in root extract was significantly increased by drought.

A negative correlation was observed between total N (data taken from Peuke & Rennenberg 2004) in stems and δ^{15}N in aqueous extracts of stems ($K_P = -0.57$) and roots ($K_P = -0.70$) as well as a positive correlation ($K_P = 0.63$) between δ^{15}N in BS of stems and roots. Analysis of regression sliced by provenance for δ^{15}N in leaves and stems affected by δ^{15}N in stems and roots, respectively, showed a tight connection (Table 2). Only in 'sont' and 'small' this effect was not significant and of low strength ($r^2 = 0.003$/0.41, respectively). In stems δ^{15}N in BS was additionally affected by δ^{15}N in the water soluble fraction.

DISCUSSION

Drought treatment affects stomatal conductance, sugar and starch contents

The present paper shows that stomatal conductance was reduced to 61% of the control by drought treatment in beech seedlings, similar to the strength and pattern of provenances such as the water potential of shoots (Peuke *et al*. 2002 and Table 1). This indication for a diminished gas exchange was not reflected in the carbohydrate contents of the leaves. Starch was not decreased in leaves and roots; on the other hand, it was even increased in the stems because of drought. In all parts of the plants, C content in soluble sugars increased on a dry weight basis, as previously observed for leaves of oak (Picon *et al*. 1997). The increased sugar concentration in beech leaves following drought treatment (Peuke *et al*. 2002) can be related to the role of sugars as osmoprotectants. Production of osmoprotectants raises osmotic pressure, maintains membrane integrity and stabilizes proteins (Hare, Cress & van Staden 1998). Thus it is considered as a compensating reaction of plants in response to drought.

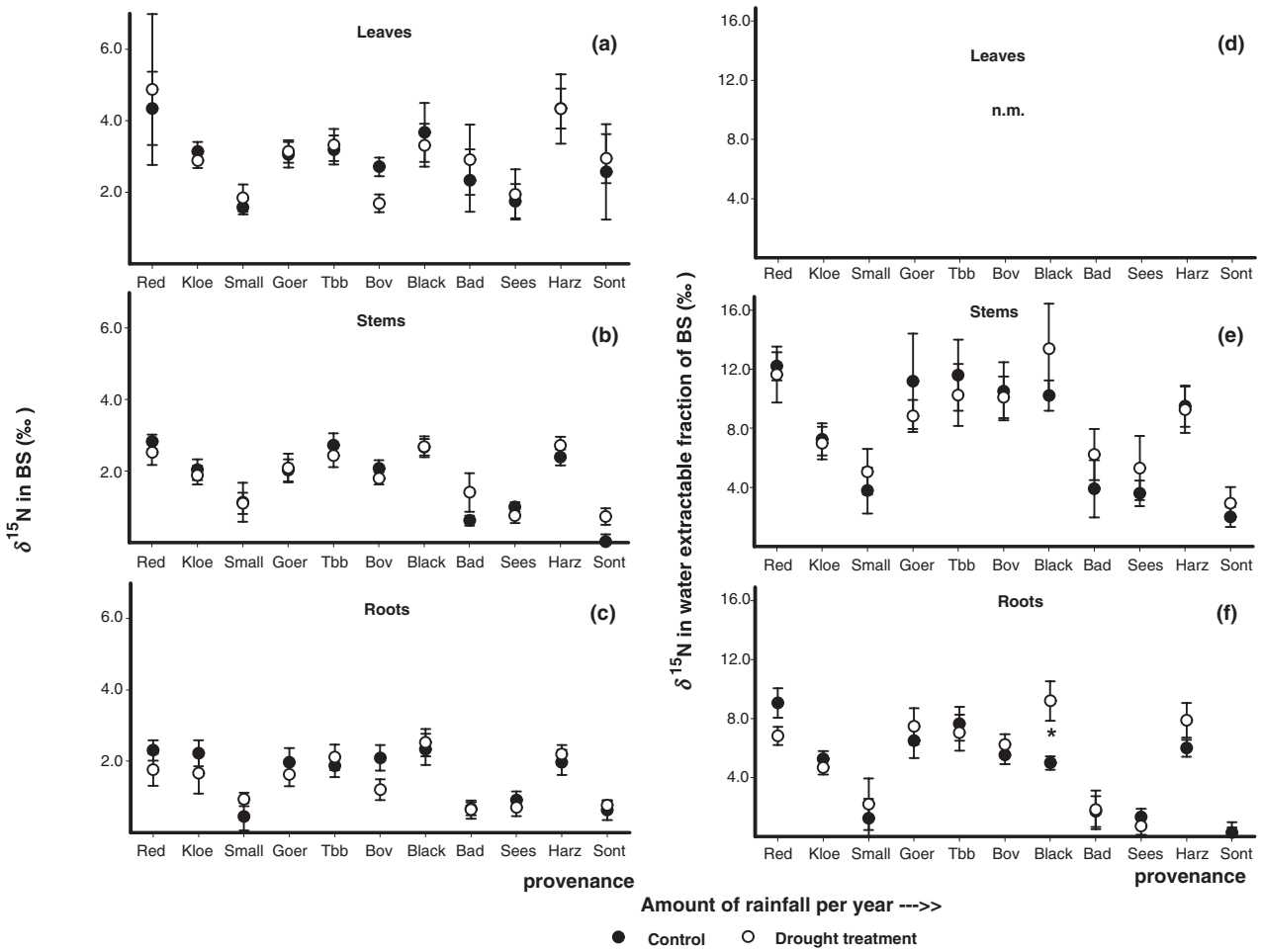

Figure 5. Nitrogen isotope composition in the bulk samples (BS) of (a) leaves, (b) stems, (c) roots in aqueous extracts of (e) stems and (f) roots in controls and drought-treated 4.5 month old beech seedlings (*Fagus sylvatica* L.). In aqueous extracts from leaves (d) N was too low to determine $\delta^{15}N$ properly and was therefore not shown. For further details, see legend Fig. 1.

Effects of drought on isotopic composition

Decreased stomatal conductance (G_s) is generally known to decrease CO_2 concentration in the gaseous spaces within the plant (C_i) and in the chloroplast (C_c), thus reducing C isotope discrimination of Rubisco (Barbour & Farquhar 2000). For beech, strong correlations between G_S and $\delta^{13}C$ of organic matter in short-term turnover pools (phloem sugars, Gessler *et al*. 2004; Keitel *et al*. 2003) and bulk leaf material (Fotelli *et al*. 2003) was observed recently. However, such a general correlation between stomatal conductance and $\delta^{13}C$ was not found in the present study, with the exception of the drought-sensitive provenances 'sees'. No correlation to biomass, water potential, water content, proline or ABA was detected as well (data not shown). Additionally, the drought effect in the present study was probably too mild to cause large isotopic effects.

Generally, it should be assumed that in cases where $\delta^{13}C$ of organic matter remains constant or shows little variation in spite of stronger changes in stomatal conductance, reduced *C* fixation should compensate for changes in C_i or C_c. (Scheidegger *et al*. 2000). This explanation is yet contradicted by the finding of increased sugar *C* and unaltered starch concentrations in leaves as a result of drought.

The well-known effect that $\delta^{13}C$ is increased by water limitation (reviewed by Adams & Grierson 2001; Ehleringer *et al*. 2002; Dawson *et al*. 2002) was observed in the BS of all plant parts, although the overall effect in the present study was very weak: leaves +0.355‰, stems +0.465‰ and roots +0.339‰ compared to the control. Because the drought period applied after leaf growth was finished, the bulk material is a mixture of C assimilated before and during drought. Keitel *et al*. (2003) showed that $\delta^{13}C$ in bulk leaf material is consequently a poor indicator for environmental impacts on adult beech during a 'normal' growing season without extreme weather conditions.

In aqueous extracts of leaves, $\delta^{13}C$ was increased significantly by +1.240‰ compared to the control. This observation is in agreement with the results from Arndt & Wanek (2002) and Brugnoli *et al*. (1988), which demonstrated that

$\delta^{13}C$ of aqueous extracts or leaf tissue sap represented a good short-term integral for water deficit and ratio of internal to atmospheric CO_2, respectively. Gessler *et al*. (2004) also observed a comparable response of $\delta^{13}C$ in sugars exported from leaves of beech to changes in G_s in range as observed here. In the present study, $\delta^{13}C$ was also increased in aqueous extracts of stems (+0.313‰) and roots (+0.443‰) or starch of leaves (+0.644‰), stems (+0.544‰) and roots (+0.535‰ compared to control), but surprisingly without statistical significance. This may be a consequence of higher variation coefficients (ratio of SE to mean values) which were only 0.2–0.3% for bulk material, but 0.6–1.1% for aqueous extracts and starch. Bulk material can be directly introduced after weighing into analysis, but aqueous extracts or starch are manipulated several times during extraction/preparation, which is a source of inaccuracy. For leaf starch, Brugnoli *et al*. (1988) observed a less intensive change in $\delta^{13}C$ with varying C_i/C_a as compared to sugars and a lower regression coefficient. Because starch synthesis includes *C* isotope fractionation steps and may also effect isotopic fractionations in the Calvin cycle (Tcherkez *et al*. 2004), this compound is likely to be a weak direct indicator of environmental stress.

To summarize and refer to hypothesis (1), our results show that leaf sugars are a suitable indicator for moderate drought stress during the growing season. The bulk material of leaves, stems and roots is still slightly influenced by the drought treatment. Contrary to our hypothesis, starch in all tissues and stem and root sugars were not influenced by reduced water supply.

$\delta^{15}N$ was not affected by drought in BS or aqueous extracts of different tissues of beech seedling in the present study. In comparison, whole plant $\delta^{15}N$ of 14 of 30 wild barley genotypes did not respond significantly to drought (Robinson *et al*. 2000). At large spatial scales, foliar $\delta^{15}N$ was observed to mainly reflect ecosystem water availability, which must influence local N cycle processes (Schulze *et al*. 1998). For example, in a global survey of terrestrial vegetation, site-averaged foliar $\delta^{15}N$ decreased significantly as mean annual rainfall increased (Handley *et al*. 1999). However, in these experimental approaches, plant organic matter mainly reflects soil ^{15}N, which is known to be influenced by water availability (Handley *et al*. 1999). There is no direct evidence from the transect studies that water availability influences N isotope discrimination during N uptake and assimilation and, thus, does not contradict the results obtained here.

In contrast to our initial working hypothesis (3), $\delta^{15}N$ has proved to be a poor drought stress integrator for beech.

Effects of provenances on isotopic composition

Genetic variation may influence C isotope discrimination. Ponton *et al*. (2001) demonstrated this by comparing two oak species' (*Quercus robur* versus *Q. petraea*) genetic variations in C isotope discrimination under the same environmental conditions. Among 10 *Pinus contorta* var. latifolia sapling populations, $\delta^{13}C$ decreased with an index of summer dryness and, less so, with increased elevation (Guy & Holowachuk 2001). In the present study, beech provenance affected $\delta^{13}C$ slightly in BS (by a maximum factor of only 1.05 in stems 'harz' versus 'sees') and in starch fraction of stems and roots. Therefore, the genetic effects are lower compared to the environmental impacts. However, when the strength of drought effect in the individual provenances is taken into account (Table 1), the sensitive provenances 'sees' and 'sont' are clearly separated from the tolerant provenances 'bov' and 'kloe'. $\delta^{13}C$ in BS and leaf aqueous extracts of 'sees' was affected very intensively while the tolerant ecotypes were more or less unaffected. In 'sees', $\delta^{13}C$ in leaf BS was also significantly influenced by stomatal conductance (Table 2), as well as 'bad' with poor r^2.

Thus, at least for particular ecotypes, the working hypothesis (2), i.e. drought-sensitive ecotypes react more intensively on a drought treatment, could be verified. Among the five provenances affected most intensively were the four wettest habitats, particularly when looking at the leaf aqueous extracts.

Large differences between the investigated provenances were observed for $\delta^{15}N$ in the present study. In general, it is known that not only variations in N source and its concentration but also the cultivar can influence gradients in $\delta^{15}N$ between external N and plant N (Yoneyama *et al*. 2001). In barley, genotypic effects on $\delta^{15}N$ have been found: the most stress-tolerant genotypes had the most negative whole plant $\delta^{15}N$ (Robinson *et al*. 2000). The present results indicate the opposite effect: the most drought-sensitive provenances ('sees' and 'sont', Peuke *et al*. 2002) had the lowest $\delta^{15}N$ values particularly in stems and roots. There may be also a connection between growth and $\delta^{15}N$. Comparing Fig. 1 in Peuke *et al*. (2002) with Fig. 5a–c of this paper, a similar pattern is observed. However, this could not be verified in a correlation analysis.

Inorganic N taken up is either directly assimilated in the roots or allocated to the shoot whereas a portion of inorganic N is subject to flow out of the roots into the soil. At this metabolic branching point, N isotope discrimination is likely to occur (Robinson *et al*. 1998). Thus, genotypic differences may result from different ratios between inorganic N uptake, assimilation and efflux (Pritchard & Guy 2005).

Isotopic gradients between plant organs and chemical fractions

For $\delta^{13}C$, we observed a gradient from leaves via stems to roots (Fig. 3d–f) with enrichment of ^{13}C for bulk material, aqueous extract and starch. The analysis of regression (Table 1) for $\delta^{13}C$ in BS revealed a strong ($r^2 > 0.9991$) and tight (slopes close to 1) relationship between leaves and stems as well as between stems and roots. Differences between ^{13}C enrichment in bulk material, water soluble *C* (mainly sugars) and starch have also been observed in the leaf material of oak (Picon *et al*. 1997), potato (Gleixner *et al*. 1998), sunflower and tobacco (Ghashghaie *et al*. 2001),

or Neotropical pioneer trees (Terwilliger *et al*. 2001) (for a review see Hobbie & Werner 2004). Differences in $\delta^{13}C$ between leaves and stems/wood and between leaves and roots as shown here were previously observed for beech (Damesin & Lelarge 2003; Fotelli *et al*. 2003) and different other species (reviewed by Hobbie & Werner 2004).

For the observed isotopic gradients, several explanations are possible: (1) in the different plant organs, different compounds with different $\delta^{13}C$ are dominant, thus defining an organ-specific isotopic composition; (2) during transport of organic C from leaves to roots, metabolic branching may result in C isotope fractionation; and (3) transporters may discriminate during loading or unloading of compounds in phloem and xylem.

Ad (1): It can be supposed that the more negative $\delta^{13}C$ values in leaves as compared to stem and roots may be a result of higher lipid content in the membrane-rich chloroplasts. Lipids are depleted in ^{13}C compared to organic matter and acids, sugars and respired CO_2 (Ghashghaie *et al*. 2001; Hobbie & Werner 2004). In contrast to this interpretation, $\delta^{13}C$ was changed in the same way in aqueous extract and starch, in which lipids are not present.

Ad (2): Damesin & Lelarge (2003) claimed that two fractionation steps are responsible for the differences in $\delta^{13}C$ between leaves and sink stems, namely, a first step during export of sugars from leaves to stems and a second step as a consequence of respiration and/or metabolic conversions in sink tissues. Hobbie & Werner (2004) concluded that differing ^{13}C contents of mobile and immobile compounds (sugars versus lignin) result in a ^{13}C enrichment of sugars exported from leaves as compared to bulk leaf material.

A possible explanation of ^{13}C enrichment during phloem transport is given by the dynamic modification of the Münch mass flow model reviewed recently by Van Bel (2003). As part of the sucrose from the sieve tubes is constantly released during phloem transport but only partially reloaded back into the sieve tubes, isotopic composition of the retrieved C may be constantly altered with increasing distance from the source tissue. The conversion of sugars unloaded from the phloem to ^{13}C depleted compounds like lignin (Hobbie & Werner 2004) would cause the remaining sugars – part of them reloaded into the sieve tubes – to carry higher $\delta^{13}C$ signatures.

Ad (3): If isotopic discrimination against the heavier *C* isotope occurs during enzymatic steps like CO_2 assimilation, sucrose metabolization (Gleixner *et al*. 1998) or respiration (in review: Hobbie & Werner 2004), it is not unlikely that transporters in biomembranes also discriminate. However, up to now no information is available concerning isotopic fractionation by channels, carriers or transporters (Adams & Grierson 2001). On the other hand, Gleixner *et al*. (1998) also claimed isotope discrimination during triose-phosphate metabolism. It may, however, be assumed that in contrast to CO_2 assimilation or respiration where a small molecule is processed, in membrane transport bigger molecules are exchanged and the mass difference is hardly important.

For $\delta^{15}N$ in bulk material, we observed a gradient from roots via stems to leaves with an enrichment of the heavier isotope ^{15}N (Fig. 3g). This observation was supported by regression analysis between $\delta^{15}N$ in leaves and stems (Table 1). In aqueous extracts, $\delta^{15}N$ was also higher in stems than in roots (Fig. 3h). An increase in $\delta^{15}N$ from root to shoot has been observed previously for herbaceous plants (Robinson *et al*. 1998, 2000). In subartic plants (Emmerton *et al*. 2001), a tendency of such a $\delta^{15}N$ difference between root and shoot was reported to depend on species, N source and mycorrhiza. In general, bulk $\delta^{15}N$ of plant biomass is determined by $\delta^{15}N$ of the (inorganic) N source and by isotopic discrimination during uptake, assimilation and transport (Evans 2001). Enzymes of nitrate assimilation are known to favour assimilation of the lighter N isotopes (for a review see Werner & Schmidt 2002). If ammonium and/or nitrate taken up from the soil are/is assimilated exclusively in the roots, the potential efflux of inorganic N into the soil may result in ^{15}N depletion of organic matter. This efflux depends mainly on the relation between N supply and N demand (Kolb & Evans 2003), but is not supposed to result in gradients within the plant (Yoneyama *et al*. 1997). Earlier studies in beech suggest that nitrate seems to be almost exclusively assimilated in the roots, as nitrate was virtually absent in the xylem of beech (Geßler *et al*. 1998) and nitrate concentrations in beech leaves were very low (Peuke & Rennenberg 2004). Consequently, the main transport forms of N in the xylem of beech are Gln, Asp and Asn (Geßler *et al*. 1998). These amino compounds have generally higher ^{15}N abundances as all the other amino acids (Werner & Schmidt 2002). Another potential cause for ^{15}N enrichment in the shoot compared to the roots could be the loss of ^{15}N depleted assimilated N from above-ground tissues (e.g. photorespiration). NH_3 lost from organic matter is known to have $\delta^{15}N$ values of down to –40‰ (Handley *et al*. 1999). As a consequence of both, supply with ^{15}N enriched compounds (Gln, Asp and Asn) in combination with a potential loss of ^{15}N depleted NH_3 the bulk N pool in the shoot could be ^{15}N enriched as compared to roots.

CONCLUSIONS

The present experiments show that $\delta^{13}C$ was more affected by environmental factors while $\delta^{15}N$ is more affected by genetic factors. For both isotope signatures, the bulk material was an indicator for these factors in the young beech seedlings, although the first phase of tip growth was finished before drought treatment. Effects were observed in all organs indicating impact of transport. For both elements, a gradient from the site of uptake (source) to the sink/end of the transport pathway was observed with enrichment of the heavier isotope. A clear-cut explanation for this observation requires further investigation on isotope fractionation during transport and related metabolic processes.

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