Research paper

Impacts of drought on mineral macro- and microelements in provenances of beech (*Fagus sylvatica* L.) seedlings

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Beech seedlings originating from 11 German provenances with different climatic conditions were grown in pots and cultivated in a greenhouse. The composition of macro- and microelements in roots, axes and leaves was measured after half of the seedlings were subjected to a simulated summer drought. The recently described sensitivity of these provenances to drought was compared with drought-mediated changes in the elemental and ionic composition in organs of the seedlings; in addition, partitioning between roots and shoots was evaluated. A number of element concentrations were decreased in roots due to drought (K 94% of control, Mg 94%, Mn 75% and Zn 85%). However, chloride concentration increased in all organs (115–125%) and was the only element affected in leaves. Some changes in ionome can be related to sensitivity of provenances, but it is difficult to decide whether these changes are a result of, or a reason for, drought tolerance or sensitivity. Observed increases in chloride concentration in all plant parts of drought-treated beech seedlings can be explained by its function in charge balance, in particular since the level of phosphate was reduced. As a result of chloride accumulation, the sum of added charges of anions (and cations) in water extracts of leaf and root material was similar between drought and control plants. Since only the partitioning of Ca and Al (both only in axis) as well as Mn was affected and other elements (together with previously observed effects on C, N, S and P) remained unaffected by drought in all provenances, it can be concluded that direct effects by means of mass flow inhibition in xylem and phloem are unlikely. Secondary effects, for example on the pH of transport sap and the apoplastic space, cannot be excluded from the present study. These effects may affect partitioning between the apoplast and symplast and therefore may be significant for drought sensitivity.

Keywords: anions, beech, cations, plant nutrition, water stress.

Introduction

European beech (*Fagus sylvatica* L.) is one of the dominant tree species in Central Europe that is known to be drought sensitive (Backes and Leuschner 2000, Gessler et al. 2004). Climate models predict an increase in air temperature and changing precipitation patterns, which in combination can increase the risk of severe drought periods in the current range of distribution of beech (IPCC 2007). During the 2003 summer drought, beech forests in Central Europe were among those forests that expressed the largest reductions in net ecosystem productivity (Ciais et al. 2005). Drought affects tree function in many ways, including gas exchange, cell growth and division, phytohormone levels, metabolism and transport processes (Hsiao 1973). Owing to drought, gradually decreasing stomatal conductance, predawn leaf water potential, assimilation and growth are commonly observed in trees, accompanied by a stimulation of fine root growth (Leuzinger et al. 2005). In addition, both greater temperatures and periods with limited water supply are thought to alter the ability of terrestrial ecosystems to take up elements. Leaves, roots and stems generally differ in their sensitivity to drought. Therefore, a whole-tree approach rather than restricted studies at the leaf level is required to properly address the consequences of drought on tree function (Leuschner et al. 2001).

Previous research investigating drought effects on tree physiology focused on water relations, gas exchange, assimilation and growth (Ciais et al. 2005, Leuzinger et al. 2005). Information on the nutritional status of trees following water shortage is relatively scarce. Potted saplings of spruce were exposed to drought for several vegetation periods, which led to significant effects on mineral elements (Wallin et al. 2002). However, changes in soil water content could become both beneficial (increased Mn and P availability) and harmful (decreased Zn availability) to plant nutrition (Misra and Tyler 1999).

In addition to the metabolized mineral elements N, P and S, plants require Ca, Mg and K in relatively large amounts (>0.1% of dry mass), and each of these so-called macroelements is an essential plant nutrient (Marschner, H. 1995, Maathuis 2009). Mineral macroelements play important roles in a wide field of functions in plants, including photosynthesis, enzyme activation, plant structure and growth; as counter-cation for inorganic and organic anions in the vacuole; as a link for environmental signals and osmotic potential; and as counter-ion to inorganic ions and organic bio-polymers. Essential microelements like B, Cl, Fe, Mn and Zn were found as ligands in >1500 proteins where they fulfil catalytic, (co-)activating and/or structural functions (Clarkson and Hanson 1980, Hänsch and Mendel 2009). Regardless of special functions in physiology and structure, Cl and K are important for osmoregulation and charge balance (Clarkson and Hanson 1980, Maathuis 2009). The overall Cl concentration in the whole plant is insufficient to be an effective osmoregulator. However, Cl can accumulate in certain tissues or single cells to a level sufficient to mediate such a function. For example, stomatal aperture is mediated by the flux of K^+ and anions, such as malate and chloride, in and out of guard cells (Hänsch and Mendel 2009, Maathuis 2009). Potassium is the most abundant cation in the cytosol, xylem and phloem sap (Clarkson and Hanson 1980, Peuke 2010), but it can be functionally replaced in the vacuole by other cations (Marschner 1995).

A large osmoregulatory capacity contributes to the maintenance of positive turgor at low water potentials. The same species of trees originating from different provenances probably developed diverse strategies to tolerate drought, and osmotic adjustment facilitated by changes in mineral concentration can be one of them. Nguyen-Queyrens and Bouchet-Lannat (2003) discussed how osmotic adjustment contributes to differences in drought tolerance among provenances of *Pinus pinaster*. Large quantities of minerals accumulated in tomato plants and the resulting dilution of organic matter explained why the estimated construction cost of biomass was low compared with other crop species (Gary et al. 1998). Osmolyte accumulation may be a key mechanism to increase the yield of crops subjected to drought (Serraj and Sinclair 2002). They found that maintenance of root development in order to reach new water sources in the soil poses a mechanism for beneficial yield

responses. Möttönen et al. (2001) concluded that increased drought resistance in seedlings is achieved by adequate boron supply.

Long-distance transport and partitioning of nutrients within the plant—in addition to nutrient availability and nutrient uptake—have to be considered to understand plant function under drought conditions. It is well known that transpiration is inhibited by drought as previously observed for beech seedlings (Peuke et al. 2002). Whereas nitrogen uptake does not follow transpiration in *Festuca* (Gastal and Saugier 1989), the uptake and partitioning of K^+, Ca^{2+} and Mg^{2+} were not affected by decreased transpiration (Tanner and Beevers 2001). Apparently, efficient transpiration is not essential for long-distance transport of minerals and the remaining 7% of transpiration is sufficient to sustain long-distance transport of these cations (Tanner and Beevers 2001).

Ecotypes of different drought sensitivity were identified among German beech provenances, which were partly attributed to climatic conditions of their habitats, particularly with respect to annual precipitation (Peuke et al. 2002). The drought-stress-sensitive cluster originating from regions with high annual precipitation had low water potential and transpiration rates, as well as high concentrations of fructose, abscisic acid (ABA) and proline after drought. Drought had no effect on the dry weight of roots, axes or leaves in the different clusters. Particularly, total phosphorus and phosphate concentrations had decreased in all tissues after 3 weeks of drought treatment, which was discussed in terms of lower phosphate mobility in the substrate due to lower water availability (Peuke and Rennenberg 2004). The partitioning of C, N, S and P was not affected by drought, although concentrations of total phosphorus and phosphate were significantly lowered.

In the present paper on beech seedlings, the effect of drought treatment, simulating a summer drought period after finishing the first phase of shoot tip growth, on mineral macro- and microelement concentration and partitioning in leaves, axes and roots, is reported. The aims of this study were (i) to describe the effect of reduced water availability on the ionic composition of plant organs in beech provenances differing in drought sensitivity, (ii) to identify how well changes in the ionome of different provenances correlate with drought tolerance and sensitivity to drought, e.g., that a high amount of minerals can be related to the tolerance of beech, and (iii) to analyse whether the partitioning of mineral elements is affected by drought.

Materials and methods

Plant material, cultivation and drought treatment period

Seeds of European beech (*F. sylvatica* L.) from different autochthonous provenances in Germany, representing sites with particular differences in annual precipitation (rainfall

(mm)) and named after the state forest service ('abbreviation') (Peuke et al. 2002), were used for the present investigation: Rothemühl 574 mm 'Red', Klötze 586 mm 'Kloe', Lüttenhagen 599 mm 'Small', Göhrde 630 mm 'Goer', Tauberbischofsheim 650 mm 'Tbb', Bovenden 680 mm 'Bov', Schwarzach 800 mm 'Black', Bad Urach 890 mm 'Bad', Seesen 1150 mm 'Sees', Harz 1400 mm 'Harz' and Sonthofen 1700 'Sont'. Relatively drought-tolerant ecotypes were provenances 'Kloe' and 'Bov', while drought-sensitive ecotypes were 'Sees' and 'Sont' (see Peuke et al. 2002).

Seeds were germinated and seedlings were transferred to 2 l pots (one seedling per pot) filled with commercial potting soil (Floradur®; Floragard GmbH, Germany), Perlite (Perligran® G; Deutsche Perlite GmbH, Germany) and soil of a natural beech stand near Freiburg (5:5:1, v:v:v). The seedlings were placed in a randomized design in a greenhouse with supplemental artificial light for 16 h (Osram® HQL 400, 200–250 µmol photons m^{-2} s^{-1}) at 20 ± 5 °C and 40–60% RH. Pots were well watered every second day with tap water. Following the first month of growth, plants were supplied with a commercial fertilizer (0.3% Hakaphos® Blau; Compo GmbH, Germany) every second week.

After 12 weeks the first period of leaf and shoot growth had finished. Plants from each provenance were selected in 'matched pairs' by visual assessment. Each pair was assigned to either the control or the treatment group. This selection approach guaranteed a homogeneous plant distribution between groups prior to the experimental phase. Control plants were watered as before, while water supply to plants in the drought treatment was reduced to achieve a water content of substrate of ~20 ± 5% (w/w) of maximum water-holding capacity. Two days before harvest, water supply was stopped. For further details of plant material, cultivation and drought treatment, see Peuke et al. (2002).

Harvesting

The seedlings were divided into leaves, axes and roots. Shoot parts were carefully washed with deionized water; roots were first rinsed with tap water before being cleaned of substrate particles in 100 mM sorbitol to avoid leaching of solutes. Surface moisture was carefully removed and sample weight was recorded before being frozen in liquid N$_2$ and stored at −80 °C until analyses.

Chemical analysis

After lyophilization, the entire plant material of each sample was finely ground. Aliquots of the bulked material were used to extract and identify mineral elements and ions. Ca, K, Mg, Mn, B, Fe, Al and Zn in dried plant material were determined after digestion with nitric acid under pressure for 10 h at 170 °C using an inductively coupled plasma atomic emission spectrometer (JY 70 plus; ISA, Instrument S.A. Division Jobin-Yvon, France).

Concentrations of chloride and other anions were measured in aqueous extracts of ground material. Extracts were generated by continuously shaking sample material in 2.5 ml of water for 1 h. Phenolic compounds were removed by adding 80 mg of polyvinylpolypyrrolidone (Sigma Chemie, Deisenhofen, Germany) to 40 mg of ground plant material. Finally, samples were boiled for 5 min, extracts were centrifuged for 10 min at 16,000g to precipitate proteins and clear supernatants were injected into a high-performance liquid chromatography system (DX 120; Dionex, Idstein, Germany). Anions were separated using an ion exchange column (AS12A, 4 mm; Dionex, Idstein, Germany) with 2.7 mM Na$_2$CO$_3$ and 0.3 mM NaHCO$_3$ as mobile phase. Ion concentrations were detected and quantified using a pulsed amperometric detector (Electrochemical detector ED 40; Dionex, Idstein, Germany). Sodium chloride was used as a standard. The water extracts were also used for the determination of other soluble compounds like sugars and cations (data taken from Peuke et al. 2002, Peuke and Rennenberg 2004).

Calculation of partitioning

The total element or ion content in a particular organ (leaves, axes or roots) was calculated by the product of concentration in dry matter and dry weight of the organ (dry weights of organs: see Peuke et al. 2002). Partitioning between leaves, axes and roots was related to whole-plant content of the element of interest.

Statistics

Ten seedlings per treatment and provenance were cultivated in individual pots. Pots were placed randomized in the greenhouse at daily changing position in the chamber. All statistical calculations were performed with SAS release 9.2. Normal distribution of data was tested and confirmed by the Shapiro–Wilk test, which investigates group residuals (procedure general linear model, GLM) by the UNIVARIATE procedure. Two-way analysis of variance (ANOVA, model: 'drought (treatment)', 'provenance' and 'drought × provenance') was calculated using a GLM. Adjustment of multiple comparisons according to Tukey was chosen for the P values and for the confidence limits of differences of least squares means. Significance in all tests was given at $P < 0.05$.

To assess patterns in the ionome of the provenances, cluster analysis was used with the procedure CLUST using the complete linkage of element concentrations in root, axis and leaf material.

Results

Element concentrations in plant tissues

In root tissue of beech seedlings, concentrations of Ca (169 ± 4 µmol g^{-1} dw, mean of the whole data set independent of provenance or treatment) and K (167 ± 3 µmol g^{-1}

dw) were similar, followed by Mg (76.1 ± 1.3 μmol g^{-1} dw), Al (9.8 ± 0.6 μmol g^{-1} dw), Fe (4.67 ± 0.20 μmol g^{-1} dw), Mn (1.64 ± 0.11 μmol g^{-1} dw), B (0.72 ± 0.01 μmol g^{-1} dw) and Zn (1.01 ± 0.00 μmol g^{-1} dw) (Table 1). Chloride concentrations (134 ± 4 μmol g^{-1} dw) were similar to Ca and K concentrations. Element concentrations, except for Ca, varied strongly across provenances; differences were particularly large for Mn ('Bov' vs. 'Sees' in controls). Plant roots subjected to drought contained 94% K and Mg, 85% Zn and 75% Mn compared with control plant root concentrations, but chloride concentrations increased to 115% in treated plant roots. At the single provenance level, the greatest number of statistically significant effects on element concentrations was found in the provenance 'Black' (K, Mg, B, Fe, Zn, Al and Cl).

Calcium was the most abundant cation in axis (293 ± 6 μmol g^{-1} dw) followed by K (166 ± 3 μmol g^{-1} dw) (Table 2). With the exception of B the investigated elements differed significantly across provenances. Similar to roots, Mn concentration showed the greatest decrease in axes of drought-treated plants ('Bov' vs. 'Bad' in controls). Concentrations of Ca (94% of controls), B (93%), Zn (90%) and Al (74%) decreased due to reduced water supply. No effect of drought treatment was observed for K and Mg. The concentration of Fe and Cl in drought-treated axes increased to 132 and 112% of the control plants. As in roots, a number of elements in the provenance 'Black' were particularly affected by drought (Table 2).

In leaf dry matter, Ca was strongly accumulated (408 ± 9 μmol g^{-1} dw) compared with K (187 ± 4 μmol g^{-1} dw) or Mg (99.6 ± 2.1 μmol g^{-1} dw) (Table 3). All investigated elements, except for Fe, varied significantly across provenances. As in roots and stems, the concentration of Mn varied strongly in leaves across provenances with a maximum factor of around sevenfold ('Small' vs. 'Bov' in controls) (Table 3). Only Cl concentrations were significantly elevated (125%) in leaves of drought-treated compared with control plants.

Partitioning of elements within the whole plant

Element content was mostly greatest in the plant shoot, although with big differences between elements (Figure 1). For example, roots contained just 8.9 ± 0.3% B or 12.0 ± 0.5% Mn of total plant content, but accumulated 74 ± 1% Al in that tissue type. Fe and Zn in roots represented 46 ± 2% of total plant Fe and Zn. Significant differences across provenances in partitioning of elements were observed for all elements with the exception of Ca and Fe, where the ratio ranged from 17.8% ('Black') to 21.0% ('Tbb') and from 41% ('Bad' and 'Kloe') to 55% ('Tbb'), respectively. Different distribution of B between root and shoot among provenances resulted in differences of 8.0% ('Tbb') to 12.6% ('Bad') of B accumulated in root tissue. The drought treatment solely affected partitioning of Mn between root and shoot: the ratio decreased significantly from

12.5 ± 0.5 to 10.9 ± 0.5%. On a single provenance level, this effect was significant for 'Black' and 'Sees'.

Distribution of mineral elements was similar between axes and leaves. Only the relative distribution of Al (7.1 ± 0.3%), Cl (9.5 ± 0.3%) and B (14.2 ± 0.4%) was lower in axis compared with other plant parts. The partitioning between axes and leaves of all elements investigated showed statistically significant differences across provenances with the exception of Fe. Accumulation of Al or Mn in axes was reduced to 72 or 93% of control, respectively, but Mn increased to 109% in leaves. Additionally, less Ca was accumulated in axes (94% of control) due to the drought treatment.

Water-soluble compounds, charges and multivariate analysis

The contributions of sugars (data taken from Peuke et al. 2002, 2006), anions (data taken in part from Peuke and Rennenberg 2004) and cations to total solute concentration were compared using water extracts of leaf and root material. The overall proportion of sugars in leaf material amounted to 35% (myo-inositol, glucose, fructose and sucrose), 24% anions (Cl$^-$, NO$_3^-$, Pi, SO$_4^{2-}$ and malate + oxalate) and 41% cations (Na$^+$, K$^+$, Mg^{2+} and Ca^{2+}) (Figure 2a). The drought treatment increased the total solute concentration to ~105% of the control, and statistically significant differences across provenances were observed. The solute concentration in drought-treated seedlings was lower in some provenances, including tolerant ecotypes (provenances 'Goer' and 'Bov'), but the majority of provenances that originated from areas with >800 mm of rainfall ('Bad' <) contained greater solute concentrations.

The proportion of sugars (19%) was relatively low compared with anions (33%) and cations (49%) in water extracts of root material (Figure 2b). Drought treatment had no significant effects on this pattern. Differences across provenances in solute concentrations of root extracts were observed, but were not as pronounced as for leaves.

The sum of added charges of anions and cations in water extracts of leaf and root material was not affected by drought (Figure 3). Although provenances showed statistically significantly different positive charges in roots and leaves in response to drought, this effect was low for negative charges in roots and absent in leaves. However, in root extracts a statistically significant interaction occurred. The sum of positive charges in root extracts from the provenances 'Kloe' and 'Black' decreased in response to drought (tendency also for 'Bov' and 'Tbb'), contrary to 'Sont' where it increased. An increasing trend was generally observed for provenances from wet habitats, but this trend was not significant compared with provenances originating from dryer habitats. Chloride was the dominant anion in extracts and contributed 57% of total anion concentration across the entire plant (Figure 2). In detail, the negative charge of chloride was responsible for 44% of total

Table 1. Concentration of elements and ions in root dry matter in 4.5-month-old beech seedlings (*F. sylvatica* L.) originating from 11 German provenances.

Treatment	Red	Kloe	Small	Goer	Tbb	Bov	Black	Bad	Sees	Harz	Sont	Prov.	Drought	Prov. × drought
Annual rainfall	574	586	599	630	650	680	800	890	1150	1400	1700			
	→ → →													
Calcium (μmol g^{-1} dw)														
Control	153	179	168	166	177	149	155	151	176	165	173	–	–	–
±SE	8	12	13	13	11	9	8	9	10	8	9			
Drought	187	179	174	161	178	172	148	183	176	170	179			
±SE	12	10	10	7	7	14	20	4	9	14	8			
	*	–	–	–	–	–	–	–	–	–	–			
Potassium (μmol g^{-1} dw)														
Control	168	157	176	151	158	168	193	237	186	156	184	***	*	–
±SE	7	10	10	6	7	6	14	35	11	6	15	(94%)		
Drought	157	146	183	151	142	148	150	218	184	144	198			
±SE	9	4	7	5	7	7	6	12	5	4	7			
	–	–	–	–	–	–	*	–	–	–	–			
Magnesium (μmol g^{-1} dw)														
Control	78.7	77.8	78.0	68.2	75.7	74.8	90.6	93.7	80.7	72.3	81.7	**	*	–
±SE	5.1	4.9	3.2	4.0	2.9	3.0	5.6	9.5	4.1	3.0	8.4	(94%)		
Drought	73.3	76.0	81.2	71.4	66.6	67.6	66.8	86.1	80.4	70.4	81.3			
±SE	2.2	3.8	4.5	3.3	2.6	4.4	4.2	5.7	1.8	2.9	2.6			
	–	–	–	–	–	–	*	–	–	–	–			
Manganese (μmol g^{-1} dw)														
Control	1.08	1.22	3.37	2.14	1.11	0.91	1.67	2.64	3.46	1.13	2.62	***	**	–
±SE	0.14	0.15	1.21	0.87	0.26	0.21	0.24	0.47	0.54	0.18	0.26	(75%)		
Drought	1.15	1.76	1.63	1.38	1.22	0.95	0.88	1.57	2.47	1.00	2.06			
±SE	0.10	0.36	0.17	0.14	0.17	0.13	0.13	0.07	0.32	0.16	0.21			
	–	–	*	–	–	–	–	–	*	–	–			
Boron (μmol g^{-1} dw)														
Control	0.69	0.72	0.78	0.65	0.66	0.74	0.80	0.93	0.80	0.64	0.78	**	–	–
±SE	0.04	0.03	0.04	0.03	0.03	0.03	0.03	0.05	0.07	0.02	0.04			
Drought	0.69	0.67	0.77	0.65	0.65	0.69	0.68	0.86	0.78	0.63	0.81			
±SE	0.03	0.02	0.06	0.04	0.04	0.03	0.04	0.09	0.05	0.03	0.05			
	–	–	–	–	–	*	–	–	–	–	–			
Iron (μmol g^{-1} dw)														
Control	4.04	2.76	4.44	3.71	5.77	3.60	6.38	6.07	5.65	3.68	5.15	***	–	–
±SE	0.37	0.22	0.47	0.38	1.04	0.40	1.45	1.06	0.73	0.35	0.37			
Drought	4.85	4.48	5.15	4.07	4.70	4.42	4.55	7.08	5.36	3.55	5.72			
±SE	0.55	0.33	0.55	0.42	0.95	0.49	0.42	0.35	0.45	0.27	0.18			
	–	*	–	–	–	–	*	–	–	–	–			
Zinc (μmol g^{-1} dw)														
Control	0.90	1.08	1.41	0.84	1.15	1.06	1.45	1.37	1.20	0.74	1.12	***	***	–
±SE	0.07	0.10	0.20	0.06	0.13	0.10	0.11	0.28	0.14	0.05	0.06	(85%)		
Drought	0.78	0.93	0.85	0.91	1.12	0.88	0.96	1.43	1.03	0.78	0.86			
±SE	0.07	0.07	0.09	0.09	0.18	0.01	0.13	0.28	0.09	0.06	0.06			
	–	–	*	–	–	–	*	–	–	–	–			
Aluminium (μmol g^{-1} dw)														
Control	8.9	5.5	9.7	8.0	11.6	8.7	16.7	11.9	8.5	7.5	10.0	**	–	–
±SE	0.9	0.6	0.8	1.0	1.9	1.2	5.1	2.7	1.3	1.0	1.1			
Drought	10.2	9.7	9.2	8.3	11.2	10.6	10.5	17.6	9.1	6.5	10.9			
±SE	1.5	0.9	1.3	1.0	3.2	1.7	1.1	3.1	1.3	0.7	0.6			
	–	–	–	–	–	–	*	–	–	–	–			
Chloride (μmol g^{-1} dw)														
Control	125	104	105	115	136	143	108	124	142	117	140	**	***	–
±SE	6	5	11	12	5	13	8	13	18	10	10	(115%)		
Drought	150	129	90	162	156	162	154	145	131	167	123			
±SE	4	10	16	14	19	10	16	13	16	16	14			
	–	–	–	*	–	–	*	–	–	–	–			

Controls were watered every second day in excess throughout the experiment; drought treatment was induced by keeping the pots 3 weeks before harvest at 20 ± 5% water content. Shown are means ± standard errors. Significant effects of the main factors provenance (Prov.), drought (treatment) and the interactions (prov. × drought) and for single provenances (below the column) are given in the table: –, not significant; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. If drought was significant, the ratio of drought to control is expressed in parenthesis.

Table 2. Concentration of elements and ions in axis dry matter of well-watered (control) and drought-treated 4.5-month-old beech seedlings (*F. sylvatica* L.) originating from 11 German provenances (for further details see the footnote of Table 1).

Treatment	Provenances											Significances		
	Red	Kloe	Small	Goer	Tbb	Bov	Black	Bad	Sees	Harz	Sont	Prov.	Drought	Prov. × drought
Annual rainfall	574	586	599	630	650	680	800	890	1150	1400	1700			
	→ → →													
Calcium (µmol g⁻¹ dw)														
Control	272	355	288	303	305	260	293	323	296	307	320	***	*	–
±SE	14	15	13	18	20	11	17	6	25	21	14		(94%)	
Drought	271	339	262	270	285	268	266	283	278	282	324			
±SE	12	20	17	12	13	11	19	27	13	14	20			
	–	–	–	–	–	–	–	–	–	–	–			
Potassium (µmol g⁻¹ dw)														
Control	169	158	170	155	166	160	177	158	211	144	195	***	–	–
±SE	7	7	14	5	9	7	6	14	15	10	16			
Drought	156	158	155	154	142	157	168	186	193	140	196			
±SE	6	3	7	6	4	8	7	17	10	4	13			
	–	–	–	–	*	–	–	–	–	–	–			
Magnesium (µmol g⁻¹ dw)														
Control	71.5	84.1	56.5	70.5	74.4	72.2	86.1	79.7	63.9	74.1	70.6	***	–	–
±SE	2.5	3.4	2.7	3.7	4.3	3.2	4.1	1.7	4.0	3.7	3.2			
Drought	72.3	76.5	65.5	68.3	67.6	69.5	69.0	77.8	62.0	69.2	70.8			
±SE	3.2	2.6	4.0	2.9	3.1	2.5	3.4	7.9	2.6	2.9	3.5			
	–	–	–	–	–	*	–	–	–	–	–			
Manganese (µmol g⁻¹ dw)														
Control	3.58	4.57	9.85	7.52	3.79	2.42	3.71	10.97	8.61	3.98	9.73	***	***	–
±SE	0.35	0.56	2.71	1.96	0.42	0.28	0.47	0.77	0.62	0.57	0.92		(79%)	
Drought	3.56	5.43	5.97	4.47	3.79	3.07	2.60	5.42	8.08	4.45	7.76			
±SE	0.19	0.58	0.75	0.44	0.31	0.29	0.20	0.32	0.57	0.92	0.45			
	–	–	*	*	–	–	–	*	–	–	–			
Boron (µmol g⁻¹ dw)														
Control	1.18	1.15	1.04	1.21	1.19	1.12	1.24	1.35	1.20	1.16	1.23	–	**	–
±SE	0.06	0.05	0.10	0.06	0.07	0.05	0.04	0.02	0.03	0.05	0.06		(93%)	
Drought	1.17	1.13	1.10	1.11	1.07	1.09	1.11	1.08	1.14	1.10	1.13			
±SE	0.08	0.04	0.07	0.06	0.05	0.03	0.06	0.14	0.04	0.05	0.05			
	–	–	–	–	–	–	–	–	–	–	–			
Iron (µmol g⁻¹ dw)														
Control	2.91	3.50	5.59	2.89	2.68	4.05	4.31	4.67	2.99	2.63	3.26	***	*	(*)
±SE	0.59	1.18	2.23	0.65	0.49	1.14	0.82	2.16	0.49	0.58	0.51		(132%)	
Drought	4.81	5.68	3.85	3.03	1.76	3.30	4.04	15.15	4.23	2.31	4.12			
±SE	1.54	1.66	1.42	0.98	0.23	0.70	0.92	4.06	0.91	0.63	0.95			
	–	–	–	–	–	–	–	*	–	–	–			
Zinc (µmol g⁻¹ dw)														
Control	0.51	0.67	0.42	0.51	0.61	0.56	0.61	0.46	0.44	0.58	0.53	***	**	–
±SE	0.03	0.04	0.03	0.05	0.04	0.03	0.05	0.10	0.03	0.04	0.03		(90%)	
Drought	0.51	0.52	0.42	0.45	0.48	0.49	0.50	0.60	0.43	0.49	0.46			
±SE	0.03	0.02	0.03	0.04	0.03	0.02	0.03	0.10	0.03	0.01	0.03			
	–	*	–	–	*	–	*	–	–	*	–			
Aluminium (µmol g⁻¹ dw)														
Control	1.57	1.08	0.47	1.71	0.62	0.81	0.89	0.57	0.65	1.51	0.53	***	**	–
±SE	0.23	0.18	0.08	0.42	0.07	0.11	0.12	0.12	0.17	0.12	0.12		(74%)	
Drought	0.77	0.95	0.57	0.86	0.55	0.76	0.78	0.75	0.60	0.71	0.44			
±SE	0.06	0.14	0.11	0.13	0.05	0.10	0.17	0.12	0.09	0.08	0.09			
	***	–	–	***	–	–	–	–	–	***	–			
Chloride (µmol g⁻¹ dw)														
Control	23.8	17.2	34.7	28.7	26.2	23.4	23.5	40.8	28.1	25.9	35.9	***	*	–
±SE	2.3	1.4	4.4	6.3	3.8	2.1	1.7	4.4	3.1	2.8	3.0		(112%)	
Drought	38.4	22.4	29.0	32.0	32.8	26.5	34.4	34.2	28.0	34.9	33.2			
±SE	3.2	2.4	3.0	3.4	5.3	2.8	2.2	7.9	2.5	3.8	2.8			
	*	–	–	–	–	–	*	–	–	–	–			

Table 3. Concentration of elements and ions in leaf dry matter of well-watered (control) and drought-treated 4.5-month-old beech seedlings (*F. sylvatica* L.) originating from 11 German provenances (for further details, see the footnote of Table 1).

| Treatment	Provenances											Significances		
	Red	Kloe	Small	Goer	Tbb	Bov	Black	Bad	Sees	Harz	Sont	Prov.	Drought	Prov. × drought
Annual rainfall	574	586	599	630	650	680	800	890	1150	1400	1700			
	→ → →													
Calcium (µmol g⁻¹ dw)														
Control	397	360	441	394	358	344	430	434	442	394	445	*	–	–
±SE	15	22	26	29	16	19	26	17	52	15	35			
Drought	437	374	429	414	390	413	404	424	436	406	461			
±SE	24	19	31	29	34	21	38	55	21	23	25			
	–					–	–	–	–	–	–			
Potassium (µmol g⁻¹ dw)														
Control	186.9	149.6	219.0	216.4	175.2	140.2	134.1	230.7	182.6	204.2	207.3	*	–	–
±SE	15.1	9.3	26.8	17.7	17.1	6.8	8.4	13.3	14.6	14.8	14.0			
Drought	181.9	143.9	198.0	198.8	147.9	122.9	153.6	238.0	199.4	209.5	240.4			
±SE	20.0	20.2	29.8	14.7	10.5	16.5	17.9	32.9	10.9	14.0	17.6			
	–	–	–	–	–									
Magnesium (µmol g⁻¹ dw)														
Control	104.3	59.8	110.6	92.2	71.6	70.1	77.1	134.6	103.7	96.1	101.0	***	–	–
±SE	6.5	3.2	14.5	8.5	5.4	4.8	8.3	8.0	12.6	6.8	6.7			
Drought	106.0	59.4	104.7	99.8	66.9	70.2	74.3	117.1	112.0	99.0	116.5			
±SE	9.1	7.0	7.1	7.9	9.2	6.6	7.1	8.0	6.7	12.9	8.0			
	–	–	–	–	–	–	–	–	–	–	–			
Manganese (µmol g⁻¹ dw)														
Control	4.9	5.3	16.7	6.3	4.5	2.4	3.4	11.7	11.8	4.4	16.2	***	–	–
±SE	0.6	1.0	3.1	1.2	0.9	0.2	0.3	1.6	1.5	0.3	2.3			
Drought	5.4	4.8	14.9	5.5	4.4	3.9	4.9	17.6	13.3	6.5	12.9			
±SE	0.7	0.6	3.5	0.4	0.6	0.5	1.6	9.1	0.7	1.5	0.7			
	–	–	–	–	–	–	–	–	–	–	–			
Boron (µmol g⁻¹ dw)														
Control	7.2	8.1	5.8	7.6	6.8	4.9	8.0	4.7	5.4	8.5	7.3	**	–	–
±SE	0.6	1.0	0.7	1.0	0.6	0.4	0.8	0.7	0.6	0.6	1.0			
Drought	7.5	11.6	5.2	5.9	7.2	6.4	6.6	4.2	5.6	7.3	5.3			
±SE	0.7	4.5	0.7	0.5	1.0	0.5	1.2	1.0	0.5	0.6	0.4			
	–	*	–	–	–	–	–	–	–	–	–			
Iron (µmol g⁻¹ dw)														
Control	2.5	1.8	1.8	2.0	1.6	1.8	2.3	3.9	3.2	2.8	2.2	–	–	–
±SE	0.6	0.2	0.1	0.2	0.1	0.2	0.4	1.4	1.4	0.9	0.3			
Drought	2.9	3.0	2.4	1.9	1.9	2.0	2.9	3.1	2.3	2.8	2.7			
±SE	0.5	0.7	0.4	0.1	0.2	0.2	0.3	0.9	0.2	0.5	0.6			
	–	–	–	–	–	–	–	–	–	–	–			
Zinc (µmol g⁻¹ dw)														
Control	0.65	0.80	0.48	0.68	0.75	0.55	0.90	0.47	0.43	0.76	0.51	***	–	*
±SE	0.03	0.07	0.06	0.07	0.05	0.06	0.09	0.04	0.03	0.06	0.02			
Drought	0.68	0.69	0.42	0.55	0.67	0.75	0.71	0.51	0.43	0.76	0.43			
±SE	0.07	0.06	0.03	0.05	0.06	0.07	0.05	0.07	0.02	0.05	0.03			
	–	–	–	–	–	*	*	–	–	–	–			
Aluminium (µmol g⁻¹ dw)														
Control	2.3	4.0	1.1	2.6	2.5	1.5	3.4	0.5	1.1	3.5	0.9	***	–	–
±SE	0.3	0.8	0.3	0.4	0.3	0.2	0.4	0.1	0.2	0.5	0.2			
Drought	2.0	3.9	1.1	1.9	3.2	2.9	3.4	1.2	1.1	2.7	0.7			
±SE	0.4	0.4	0.2	0.3	0.5	0.8	0.7	0.7	0.1	0.4	0.2			
Chloride (µmol g⁻¹ dw)														
Control	152	112	129	129	132	104	113	110	125	163	144	***	***	–
±SE	13	11	12	15	19	14	4	8	15	21	13		(125%)	
Drought	211	145	150	160	175	148	170	94	129	211	175			
±SE	29	16	12	21	24	19	23	12	8	30	19			
	*	–	–	–	–	–	*	–	–	–	–			
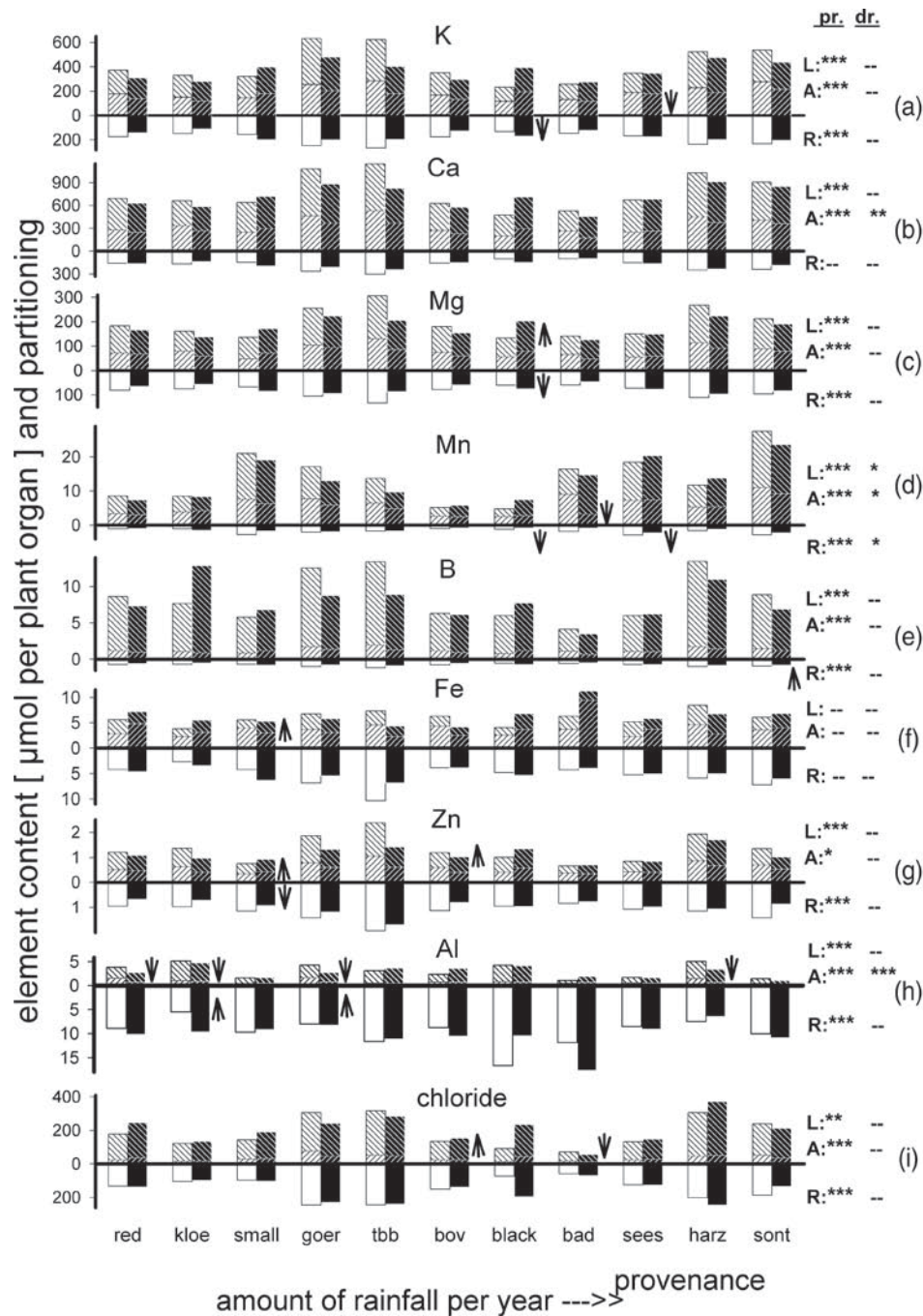
Figure 1. Content and partitioning of (a) potassium, (b) calcium, (c) magnesium, (d) manganese, (e) boron, (f) iron, (g) zinc, (h) aluminium and (i) chloride in and between roots (R, below *x*-axis, white and black), axes (A, left to right up-hatched) and leaves (L, left to right down-hatched) of 4.5-month-old beech seedlings (*F. sylvatica* L.). Control plants (left of bar group) were watered every second day in excess throughout. Drought treatment (right of bar group) was induced by keeping pots 3 weeks before harvest at 20 ± 5% water content. Values shown are means. The provenances are sorted in order of annual rainfall in the region. Significance of main factors (pr., provenances; dr., drought treatment) is indicated by asterisks; effects between treatments within a provenance are represented by arrows (up, increase by drought; down, decrease) at the columns. Interactions of provenances and drought treatment were not significant and therefore not shown.

negative charge in root extracts, 66% in axis and 53% in leaf extracts. K^+ was the major cation in all extracts. The contributions of K^+ to total cation or positive charges were 45 or 36% in roots, and 48 or 35% in leaves. The mean positive to negative charge ratio was 1.49 in root extracts, but with 2.05 much

greater in shoots. This ratio was not affected by the drought treatment, despite significant differences between leaf extracts from different provenances. The positive to negative charge ratio found in leaves was remarkably lower (1.4–1.6) in more drought-tolerant ('Kloe', 'Bov', 'Goer' and 'Black') compared
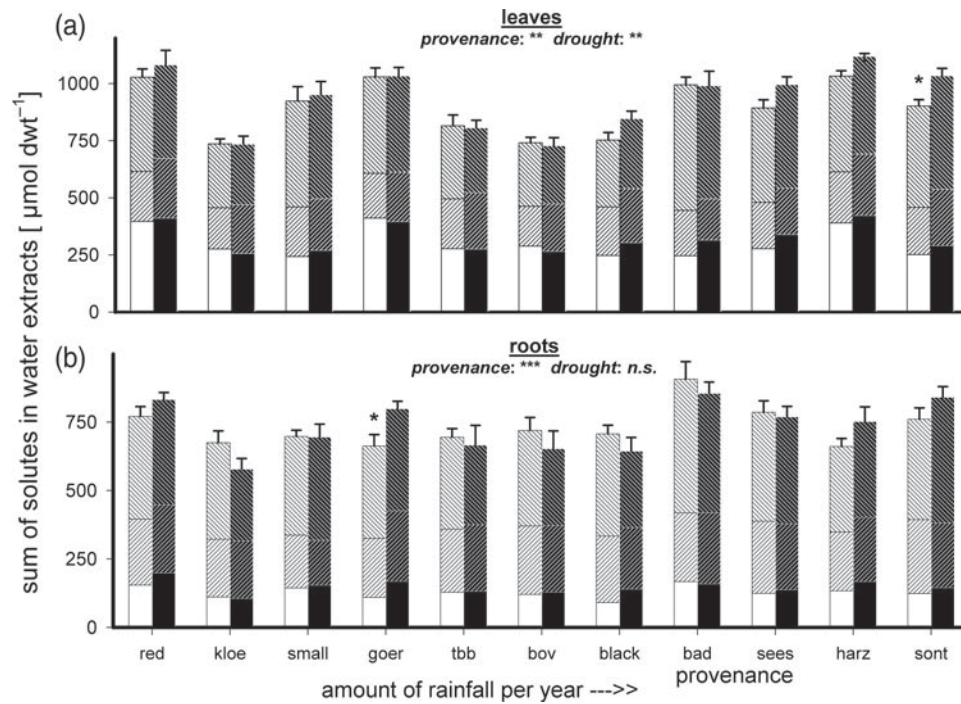
Figure 2. Concentration of solutes in water extracts from (a) leaves and (b) roots of 4.5-month-old well-watered (control) and drought-treated beech seedlings (*F. sylvatica* L.). Sugars (white and black), anions (left to right up-hatched) and cations (left to right down-hatched). Significance of the main factors 'provenance' and 'drought' (treatment) is indicated by asterisks and effects between treatments within a provenance are shown as an asterisk at the columns. For further details see the legend for Figure 1.
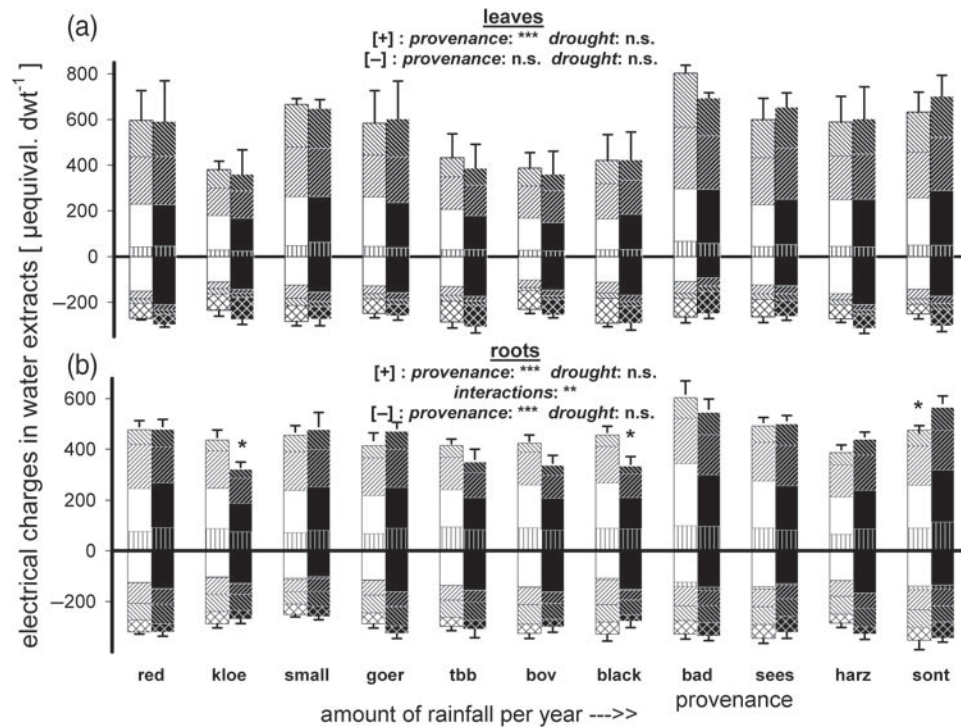

Figure 3. Concentration of positive (Na^+, K^+, Mg^2 and Ca^{2+}) and negative (Cl^-, NO_3^-, Pi, SO_4^{2-} and malate + oxalate) charges in water extracts from (a) leaves and (b) roots of 4.5-month-old well-watered (control, white/light) and drought-treated (black/dark) beech seedlings (*F. sylvatica* L.). Coding of elements: white or black = Cl^-/K^+; left to right up-hatched = Pi/Mg^{2+}; left to right down-hatched = SO_4^{2-}/Ca^{2+}; vertical-hatched = NO_3^-/Na^+; cross-hatched = organic acids. Significance of the main factors 'provenance' and 'drought' (treatment) is indicated by asterisks and effects between treatments within a provenance are shown with an asterisk at the columns. For further details, see the legend of Figure 1.
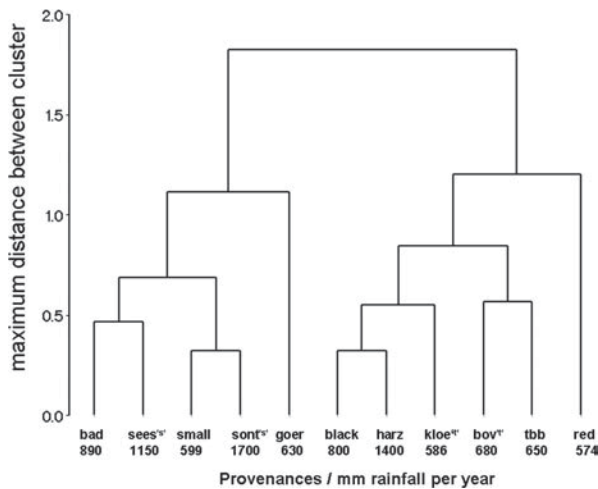
Figure 4. Tree presentation of cluster analysis. The codes of provenances are given in Materials and methods, rainfall per year (mm) and drought-tolerant ecotypes are indicated by 't' and the sensitive ecotypes by 's'. The cluster analysis was calculated using the CLUST procedure of SAS (V9.2) using the complete linkage of mineral concentration in dry matter of leaves, axes and roots of 4.5-month-old well-watered and drought-treated beech seedlings (*F. sylvatica* L.).

with drought-sensitive ecotypes (provenances 'Sees' and 'Sont' 2.5).

A cluster analysis of all measured mineral elements in bulk dry material did not reveal a clear-cut result regarding drought sensitivity since differences between clusters were not pronounced (Figure 4). The drought-tolerant ecotypes (provenances 'Kloe' and 'Bov'), but also 'Goer' and 'Black' assembled in one half, and sensitive ecotypes like the provenances 'Sees' and 'Sont' were found on the other twig. On the other hand, the relatively sensitive provenance 'Harz' from a very wet habitat was clustered together with the tolerant provenance 'Black'. Therefore, ionomic data alone are not sufficient to discriminate between drought-sensitive and drought-tolerant ecotypes.

Discussion

Effects of provenances

Statistically significant differences in concentrations of mineral elements were observed across provenances, reaching approximately twofold above average of all plants. The greatest variability of a single element was found for Mn with a maximum factor of ~5 in leaves ('Small' compared with 'Bov'). Similarly, Duquesnay et al. (2000) found in beech that C was relatively constant, while N and P showed low, K and Ca medium, and Mn and Mg high variability between trees, sampled from >100 stands and over ~30 years. Also Bauer et al. (1997) found that element concentrations (N, S, P, Ca, K and Mg per dry weight) in *Picea abies* and *F. sylvatica* showed little variation between central Italy and southern Sweden. In general, the element concentrations in potted beech seedlings presented are in

accordance with data from the literature (Clarkson and Hanson 1980, Bauer et al. 1997).

The present data do not support the hypothesis that a high amount of minerals can be related to drought tolerance of a beech provenance. Nguyen-Queyrens and Bouchet-Lannat (2003) observed in *P. pinaster* that solute accumulation was ~ 2.3 times greater in provenances from dry compared with wet sites. Osmotic adjustment was achieved in leaves of tomato mainly by Cl^- and to some extent by K^+ under drought (Plaut et al. 2004). However, drought-tolerant provenances of beech did not express a greater element concentration as a consequence of drought. Sugars contributed significantly to total amounts of water-extractable solutes in beech leaves. Additionally, the concentration of specific elements such as B did not reflect the performance of provenances in response to drought. Contrary to this finding, stands reduced height growth in spruce seedlings under drought and B deficiency (Möttönen et al. 2001).

On the other hand, the ionome of various beech provenances is not entirely uncoupled from stress performance. In contrast to former hypotheses, elevated solute concentrations in water extracts of stress-sensitive ecotypes were observed (see Figure 2) and sensitive ecotypes showed a significantly higher surplus of positive charges in leaf extracts. Similarly, the results of the cluster analysis presented here did not match with identification of drought-sensitive and drought-tolerant provenances based on annual precipitation. While a previous cluster analysis used physiological parameters and organic compounds (relative water content in leaves, axes and roots, transpiration rate, predawn water potential in root and shoot, sucrose, proline and ABA concentration in leaf dry matter; Peuke et al. 2002), the present cluster analysis was based on elemental/inorganic composition of tissues. Nevertheless, the relationships were not mixed up totally and matched in part the drought tolerance. In this study, only German provenances were studied. Marginal beech provenances may exhibit a better drought adaptation (Rose et al. 2009).

The present study focused primarily on the effects of water availability on beech seedlings. The provenances were selected based on the mean precipitation in their natural habitats. No data were available about nutrient supply at these stands; thus, we cannot exclude the fact that nutrient availability may also affect development of ecotypes in parallel with drought sensitivity. The development of 'nutrient' ecotypes may additionally interact with the observed elemental composition of beech seedlings.

Effects of drought

In the present study, the concentration of elements in at least one organ was affected by drought and mostly decreased. This is consistent with previous findings of Wallin et al. (2002) in spruce saplings after drought treatments that lasted for several growing seasons. A significant decrease in P and B was found

in the needles. In contrast, the concentration of Ca, Mg, Mn and S increased. However, the latter observation could be attributed to the lower growth of these trees, resulting in reduced biomass accumulation. In the present study, the period of shoot tip and leaf growth had finished and there was no indication of root growth in the experimental period (see Peuke et al. 2002). Observed changes of element concentrations as a result of drought were not pronounced and reached a minimum of 75% of the control in the present but also in a former study (Wallin et al. 2002).

Low water availability in soils may have different effects on soil nutrient concentration, although nutrient concentration may increase when the volume of soil water declines and leads to improved uptake by plants for a short period. However, in a greenhouse experiment, soil solution P, Mn and pH increased while the concentration of Ca, Mg and Zn decreased when soil moisture increased (Misra and Tyler 1999). Here, concentrations of P, Zn and Mn in plants were related to soil solution concentrations (Misra and Tyler 1999).

In the present study, only the concentration of chloride increased in all plant parts. Chloride is a mobile anion and most of its functions in plants are related to electrical charge balance (Clarkson and Hanson 1980, Hänsch and Mendel 2009). Such changes were observed when the nitrogen source changed from nitrate to ammonium, and also under limited supply of nitrate, phosphate or potassium (Peuke 2010). Since cationic minerals were mostly reduced in the studied beech seedlings, but negative charges were not affected by drought (Figure 3), the increase in chloride can be explained by its electrical charge balance function. This function is required in beech exposed to drought due to reduced levels of phosphate (Peuke and Rennenberg 2004).

The effects of drought on the chemical composition of mineral nutrients declined from roots to leaves. While concentrations of K, Mg, Mn, Zn and Cl were affected in roots, and concentrations of Ca, Mn, B, Fe, Zn and Cl were affected in axes, only chloride concentrations changed in leaves. This shows a clear gradient in effects from site of uptake and stress perception to site of primary production and may indicate a protection of photosynthetic activity. While the concentration of chloride increased in all parts of drought-stressed beech seedlings by up to 25% compared with controls, the concentration of Mn was most reduced in roots (by 25%). On the single provenance level the drought effects were only frequently statistically significant, probably due to the lower number of replicates. A correlation with the former observed sensitivity can hardly be detected. Most statistically significant changes were observed in the provenance 'Black', which was rather tolerant.

Although elemental concentration was least affected by drought in one plant part, the partitioning of elements was only affected in the case of Mn in all organs and Ca as well as Al in the axis. This appears surprising, since transpiration was strongly reduced (60% of controls) in the investigated seedlings (Peuke et al. 2002). Lower transpiration should result in a reduced volume flow in xylem, which might inhibit solute transport. The present observation can be explained by an increased solute concentration in the xylem sap, which can compensate lower volume flow. It thus appears that (i) convective water transport in xylem by root pressure (guttation), (ii) water for growth processes and (iii) Münch's phloem counter-flow were sufficient to maintain long-distance transport of minerals and that full transpiration is not required for this function as previously observed in sunflower (Tanner and Beevers 2001).

Distribution, partitioning and abundance of elements are affected by a coordinated action of xylem and phloem transport (Peuke 2010). Since some ions like K^+ and Cl^- are also highly mobile in the phloem (like C, N, P and S), effects in xylem transport may be compensated by a synergistic action of the phloem. Most of the major nutrients are redistributed readily from mature/senescent to young leaves by phloem when plants are subjected to nutrient deficiency. This is indicated by losses of N, P, K and, in some instances, S from older leaves (Clarkson and Hanson 1980, Blevins and Lukaszewski 1998). In contrast, Ca and B are not considered phloem mobile and are usually not effectively redistributed. In the current study partitioning of Ca and Mn was affected. However, Mn moves primarily as the free ion, can partially be mobilized from leaves, can be leached from leaves and seems to be partially phloem mobile (Clarkson and Hanson 1980).

The partitioning of less soluble minerals (Mn, Al and Ca) was significantly affected by drought in the present study. This may, combined with increasing concentrations of Fe in the shoot, point to secondary effects of the drought treatment. Xylem sap alkalization in response to drought stress has been found repeatedly in plants (Sharp and Davies 2009). This may regulate the partitioning between the apoplast and symplast for compounds that possess greater solubility at lower pH (Marschner 1995).

Conclusion

Concentrations of cationic mineral elements in drought-treated beech seedlings generally decreased. The overall effect was—with minimum concentrations of 75% compared with controls—low and was not always statistically significant, particularly at the provenance level. The effects of drought on mineral concentration in roots, shoots and leaves seem to vary randomly across different provenances of beech. However, a trend of overall decreasing effects from roots to leaves was detected, possibly maintaining more stable ionic/elemental conditions at the site of photosynthesis. A further compensating effect was shown by increased chlo-

ride concentration, apparently to achieve charge balance. The ionome of the provenances does not seem to be totally uncoupled from stress performance. Greater solute concentrations in extracts and a significantly higher surplus of positive charges in leaf extracts were observed in stress-sensitive ecotypes.

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