Eur J Forest Res (2006) 125: 1–14
DOI 10.1007/s10342-005-0103-5

ORIGINAL PAPER

Michael Nahm · Thomas Holst · Andreas Matzarakis
Helmut Mayer · Heinz Rennenberg · Arthur Geßler

Soluble N compound profiles and concentrations in European beech (*Fagus sylvatica* L.) are influenced by local climate and thinning

Received: 11 October 2004 / Accepted: 11 July 2005 / Published online: 14 December 2005

Abstract We assessed seasonal changes of total soluble nonprotein nitrogen compounds (TSNN) in adult European beech trees (*Fagus sylvatica*, L.) growing under different local climate during the growing season immediately following a thinning treatment and 3 years later. In both years, samples of leaves, xylem sap and phloem exudates from beech trees growing in thinned and unthinned (control) stands on a dry, warm SW exposed and a cooler, moist NE exposed site were collected in May, July and September. In May of both years, asparagine (Asn) and glutamine (Gln) were most abundant in leaves and xylem, respectively, whereas arginine (Arg) dominated in the phloem. In July, TSNN concentrations decreased in all tissues and sites, but differences in water availability between aspects were reflected in TSNN concentrations. In September, differences in the increase of Arg concentration in the phloem were related to differences in the onset of senescence between treatments. Thinning treatment increased amino compound concentrations of beech tissues in July on both aspects, particularly at the NE thinned site. It is supposed that, the N balance of adult beech is favoured by both, the thinning treatments as well as the cool-moist climate prevailing at the NE aspect.

Communicated by Franz Makeschin

M. Nahm · H. Rennenberg · A. Geßler
Chair of Tree Physiology, Institute of Forest Botany and
Tree Physiology, Albert Ludwigs University of Freiburg,
Georges-Köhler-Allee, Gebäude 053/054,
79110 Freiburg, Germany

T. Holst · A. Matzarakis · H. Mayer
Meteorological Institute, Albert Ludwigs University of Freiburg,
Werderring 10, 79085 Freiburg, Germany

Present address: A. Geßler (✉)
School of Forest and Ecosystem Science,
University of Melbourne, Water Street,
3363 Creswick, VIC, Australia
E-mail: arthur.gessler@sonne.uni-freiburg.de
Tel.: +61-3-53214151

Keywords Amino compounds · Sap flow density · N flow in the xylem · Adult European beech · Thinning · Climate · N remobilisation

Introduction

In Central Europe, European beech (*Fagus sylvstica* L.) is the dominant deciduous tree species of the potential natural vegetation (Ellenberg 1992). Whereas beech forests have been replaced by conifer plantations during the last two centuries, beech is recently favoured by forest policy to substitute parts of monocultural spruce forests (Dertz 1996), because these monocultures are ecologically of low value and have shown sensitivity to calamities caused by storms and insects. For instance, the state of Baden-Württemberg (Germany) aims at amplifying the relative portion of beech forests from ca. 20% to more than 30% of the forested area within this century (Moosmayer 2002).

Beech is a drought-sensitive species, and its southern area of distribution is mainly limited by water availability (Ellenberg 1992). Since climate projections for Central Europe predict elevated temperatures and prolonged drought periods during summer within the next decades (IPCC 2001; Schär et al. 2004), beech trees are supposed to experience environmental conditions that impair their water balance and reduce nutrient uptake in future (Fotelli et al. 2002a; Spiecker et al. 2001). Thinning or selective felling as a frequently applied forest management practice to promote adult target trees or natural regeneration in deciduous forests (Tarp et al. 2000) also changes the microclimate in forest stands by increasing incoming radiation (Mayer et al. 2002). Especially beech stands growing on shallow soils of low water storage capacity derived from limestone (Rendzina soils) might be negatively affected by both, dry future climate and silvicultural treatments (Geßler et al. 2001), since water balance of beech can be impaired by thinning under unfavourable climatic conditions such as drought periods during the growing season (Čermak et al. 1993; Breda et al. 1995; Geßler et al. 2001). Since nutrient uptake in beech is largely dependent on water availability (Fotelli et al. 2001, 2002b), additional effects of drought on mineral nutrition have to be assumed.

Among mineral nutrients, N is a major growth-limiting factor in natural forest ecosystems with low anthropogenic N input (Rennenberg et al. 1998), and changes in N availability or uptake influence growth patterns of beech (Marschner 1995). Fotelli et al. (2004) found that thinning affected nitrate uptake of natural beech regeneration differently under different climatic conditions. It increased pedospheric N uptake in cool and moist beech stands, whereas uptake was decreased in dry and warm stands. For adult trees, positive effects of thinning on the remaining trees, e.g. improved growth parameters (Yu et al. 2003), nutrient availability (Thibodeau et al. 2000), or drought resistance (Laurent et al. 2003) have frequently been observed. However, the combined effects of climate and thinning on the N balance of adult beech trees growing on lime stone derived soils have not yet been assessed and an integrative physiological measure to predict nutritional responses of adult beech trees to these factors is lacking.

In previous studies, the patterns of total soluble nonprotein nitrogen compounds (TSNN) cycling within the plant have been used to characterise the N balance of trees (Fotelli et al. 2002a; Geßler et al. 1998b; Nordin et al. 2001). The pool of amino compounds cycling between roots and shoot is involved in N translocation, storage and remobilisation and in the regulation of pedospheric N uptake (Millard 1996; Caputo and Barneix 1997; Geßler et al. 2003; Collier et al. 2003). Studies with different plant species (wheat, rye, Scots pine, Norway spruce, European beech) support the hypothesis that allocation of specific amino compounds (especially Gln) between shoot and root and vice versa is influenced by the ratio between N supply and N demand (Cooper and Clarkson 1989; Gezelius and Näsholm 1993; Muller et al. 1996; Schneider et al. 1996; Kreuzwieser et al. 1997; Geßler et al. 1998b). In addition, the assessment of seasonal changes in the amount of Arg can be used to characterise N remobilisation and storage processes in spring and autumn, respectively (Geßler et al. 1998a; Fotelli et al. 2002a). Due to translocation and signalling functions, the pool of soluble N compounds reacts rapidly to changes in N availability and demand and, hence, is supposed to be a sensitive indicator of the plant's internal N status and growth potential (Rennenberg and Geßler 1999; Fotelli et al. 2002a; Novitskaya et al. 2002; Foyer et al. 2003), in contrast to total N concentration which does not vary intensively over a wide range of different N availability (Geßler and Rennenberg 2000).

Therefore, seasonal changes in TSNN composition and concentration in various tissues of adult beech trees grown in the field under different local climatic conditions and in different thinning treatments were examined in the present study. With this approach we wanted to assess the applicability of TSNN analysis for characterisation of the N nutrition status of adult beech trees as was assessed previously for beech seedlings (Fotelli et al. 2002a). The main hypothesis based on the water balance of adult beech trees (Geßler et al. 2001; Keitel et al. 2003) and the N nutrition of beech seedlings (Fotelli et al. 2002b, 2004) at the same field sites was that the N balance of adult trees was affected by both local climate and silvicultural treatment. We expected better N supply and, hence, increased TSNN concentrations in various tissues of beech grown on the NE slope compared to the dry-warm SW slope—which is supposed to be a local climatic model for the general climatic conditions in Central Europe within the next 50–100 years (Geßler et al. 2004)—and higher TSNN concentrations on the thinned stands compared to the controls. To clarify if potential effects of thinning on the N status of adult beech are transient or persisted over a longer time period, TSNN composition and concentrations were determined in the growing season immediately following the thinning treatment and 3 years later.

Material and methods

Experimental sites

The experimental sites of this study are located in southern Germany, about 100 km south–southwest from Stuttgart (longitude: 8°45′E; latitude: 48°00′N) in a low mountain range (Schwäbische Alb, 740–760 m above sea level). Long-term mean annual regional air temperature measured at a climate station of the German Weather Service (DWD) about 6 km from the experimental sites, is about 6.6°C, and mean temperature during the growing season (May to October) is about 11.5°C. Mean annual precipitation is 856 mm with monthly maxima in June and July, the total precipitation during the growing season (May to end of September) amounts to mean values of 410 mm. In the growing season of 1999, mean air temperature was 14.8°C and precipitation reached 478 mm, whereas the temperature in 2002 was slightly lower (14.3°C) and the total precipitation amounted to 551 mm (Table 1).

The experimental sites are located on two opposing slopes of a narrow valley, less than 1,000 m apart from each other. One of the sites faces to the north-east (NE) and the other to the south-west (SW). Forests on both sides of the valley are dominated by European beech (*F. sylvatica* L.) aged 70–80 years, contributing to >90% of the basal area of adult trees. Soil profiles are characterised as Rendzic Leptosols (WRB-classification, ISSS 1998) derived from limestone (Weissjura beta and gamma series) and are shallow on both sites, averaging less than 0.20 m depth of topsoil before becoming dominated by parent rock interspersed with pockets of organic matter and mineral soil. The soil profile at the SW site is especially rocky, containing more than 40% (volumetric basis) rocks and stones (>60 mm diameter) in the top 0.20 m of the soil and rising to 80% below

Table 1 Climate characteristics of the experimental sites during growing season (May to end of September) 1999 and 2002

	Tuttlingen climate station	NE aspect		SW aspect	
Mean air temperature 1999 (°C)	14.8^{a}				
Total precipitation 1999 (mm)	478				
Mean air temperature 2002 (°C)	14.3^{a}	13.9^{b}		13.9^{b}	
Total precipitation 2002 (mm)	551	602^{c}		784^{c}	
Climate characteristics of the four sites in 2002		Control	Treatment	Control	Treatment
Mean T_{air} in 1.5 m height (°C)		13.8	13.6	13.8	13.9
Mean midday T_{air} in 1.5 m height (°C)^{d}		15.9	16.2	16.9	17.7
Mean T_{soil} in 5 cm depth (°C)		12.3	13.0	13.3	13.9
Mean T_{soil} in 10 cm depth (°C)		12.2	12.8	13.2	13.7
Mean RH (%)		78	81	80	80
Mean PAR in 1.5 m height (μmol m^{-2} s^{-1})		8.9	126.3	18.7	80.7
Total throughfall (mm)^{e}		366	484	232	376
Basal area of adult trees (m^{2} ha^{-1})		27	10	20	10

The experiments were carried out on two opposing slopes in a small valley, facing north-east (NE) and south-west (SW) and were either subjected to thinning (T) or remained unthinned as controls. In 1999, climate data could only be obtained from the climate station Tuttlingen of the German Weather Service, 6 km east of the sites. Mean values shown are averages of daily (24 h) means

[a] Measurement at 2 m a.g.l

[b] Measurement above the canopy

[c] Measurement above the canopy (42 m a.g.l.)

[d] Data are means of temperatures measured between 13:00 and 15:00 CET

[e] Total throughfall refers to the amount of precipitation passing through the canopy of the trees and reaching the forest floor

0.50 m. The soil at the NE site contains 15% rocks and stones in the uppermost 0.20 m of the soil and ca. 30% below 0.50 m. Soil pH (H_2O) is 5.7 in the organic surface layer and 7.5 at 60 cm depth.

The difference in aspects (NE, SW) produces a difference in radiation interception per m^{2} of inclined surface area with higher energy available on the SW site (Geßler et al. 2001; Mayer et al. 2002). According to the retrospective analyses of meteorological data, as well as the growth and water status of adult beech trees (Geßler et al. 2001) and beech seedlings (Fotelli et al. 2002a), the SW-exposed site has permanently lower water availability (Keitel et al. 2003) as well as increased soil temperatures near ground (Holst et al. 2004a; Table 1). Thus, the understorey vegetation differs between the two sites (Paul 2003) and the classification of the stand on the NE site is a *Hordelymo-Fagetum* and on the SW site, a *Carici-Fagetum* (Oberdorfer 1992).

To assess the effects of thinning, selective felling of trees was performed in March 1999 on both sides of the valley. The total basal area (BA) of trees on the untreated control plots varied between sites; on the NE site the mean BA in control plots was 27 m^{2} ha^{-1}, while on the SW site the control plot BA was about 20 m^{2} ha^{-1}. Thinning reduced BA of adult trees to ca. 10 m^{2} ha^{-1} on both aspects (for further details see Fotelli et al. 2002a). It resulted in increased PAR on the forest floor (Mayer et al. 2002; Table 1) and, hence, in higher soil temperatures on both aspects (Holst et al. 2004a; Table 1). Precipitation throughfall in 2002 was notably higher on the SW than on the NE aspect; both slopes received more precipitation than the adjacent DWD climate station Tuttlingen (Table 1). Throughfall at the forest floor was higher in the thinned stands as compared to the controls on both aspects (Table 1). Elemental analysis of leaves performed in July 1999 (Table 2) provided no indications of nutrient deficiencies and/or imbalances (cp. Bauer et al. 2000; Rothe 1998) on both aspects and in both silvicultural treatments. Thinning decreased plant area index [PAI (m^{2} m^{-2})] from 5.2 (control) to 1.7 in the thinning treatment on the NE aspect and from 5.1 to 2.1 on the SW aspect in the first year after the treatment (Holst et al. 2004b). Until 2002 PAI increased to approximately 2.7 in the thinning treatment of both aspects and remained constant in the controls.

Plant material

Twigs and leaves from sun crowns of adult beech trees growing in thinned stands (T) and an unthinned control stand (C) were harvested on both aspects of the valley. Sampling campaigns were conducted during the growing seasons of the years 1999 and 2002 in May (immediately after bud break: 6 to 7 May 1999 and 5 to 7 May 2002), July (in the middle of the growing season: 15 to 16 July 1999 and 1 to 2 July 2002) and in September (at the end of the growing season: 6 to 17 September 1999 and 18 to 19 September 2002).

Figure 1a and b show the cumulative diameter distribution (diameter at breast height, DBH) of trees in the control treatment of the NE and SW aspect whereas Fig. 1c and d show the same parameter on the thinned plots. In the thinning treatment mainly trees with a low DBH were removed and, thus thinning increased the mean DBH of the trees at these plots. Due to the different long-term growing conditions the median of the DBH values was lower on the SW as compared to the

Table 2 Elemental concentrations of leaves of adult beech trees grown on two opposing slopes of a narrow valley, being exposed to NE and SW, respectively

Site	N	S	P	Mg	Ca	Na	K
NEC	1.6 ± 0.4	44.0 ± 8.2	38.2 ± 5.2	64.3 ± 9.7	278.3 ± 59.4	0.6 ± 0.1	176.0 ± 68.1
NET	1.7 ± 0.3	45.7 ± 1.2	40.8 ± 4.7	66.8 ± 6.6	380.8 ± 72.3	0.8 ± 0.1	164.9 ± 47.7
SWC	1.8 ± 0.2	48.2 ± 3.5	34.1 ± 4.0	65.0 ± 9.9	352.7 ± 87.2	0.7 ± 0.2	171.1 ± 36.5
SWT	1.7 ± 0.1	45.2 ± 4.5	32.5 ± 5.0	58.4 ± 10.4	338.6 ± 101.1	1.3 ± 0.5	182.7 ± 46.0

On both slopes, the forest stand was subjected to thinning (T), whereas another stand remained untreated and is referred to as control stand (C). Concentrations were determined in samples collected in July 1999 and are given in μmol g^{-1} dry wt. with standard deviation, except concentrations of Nitrogen, which are given in mmol g^{-1} dry wt.

NE aspect. In order to compare comparable trees between treatments and to account in addition for the different long-term growing conditions between aspects our selection criterion was the following: we have chosen for both, control and treatment, trees with DBH values above the median of the respective control treatment (NE or SW) but excluded the 10% of trees with highest DBH values.

Thus, we have only selected dominant trees within the diameter range of 27–33 cm in both treatments at the NE aspect and within the diameter range 21–26 cm in both treatments on the SW aspect. The DBH values of the particular trees examined are given in Table 3. During all campaigns in 1999 and 2002 the same trees were sampled.

The twigs from the adult trees were obtained by professional tree climbers cutting two twigs from each of four trees per site and treatment during each sampling campaign. Leaves, phloem exudates and xylem sap were collected from each twig. During each measurement campaign the samples were taken between 09:00 and 11:00 to minimise influences of diurnal changes in

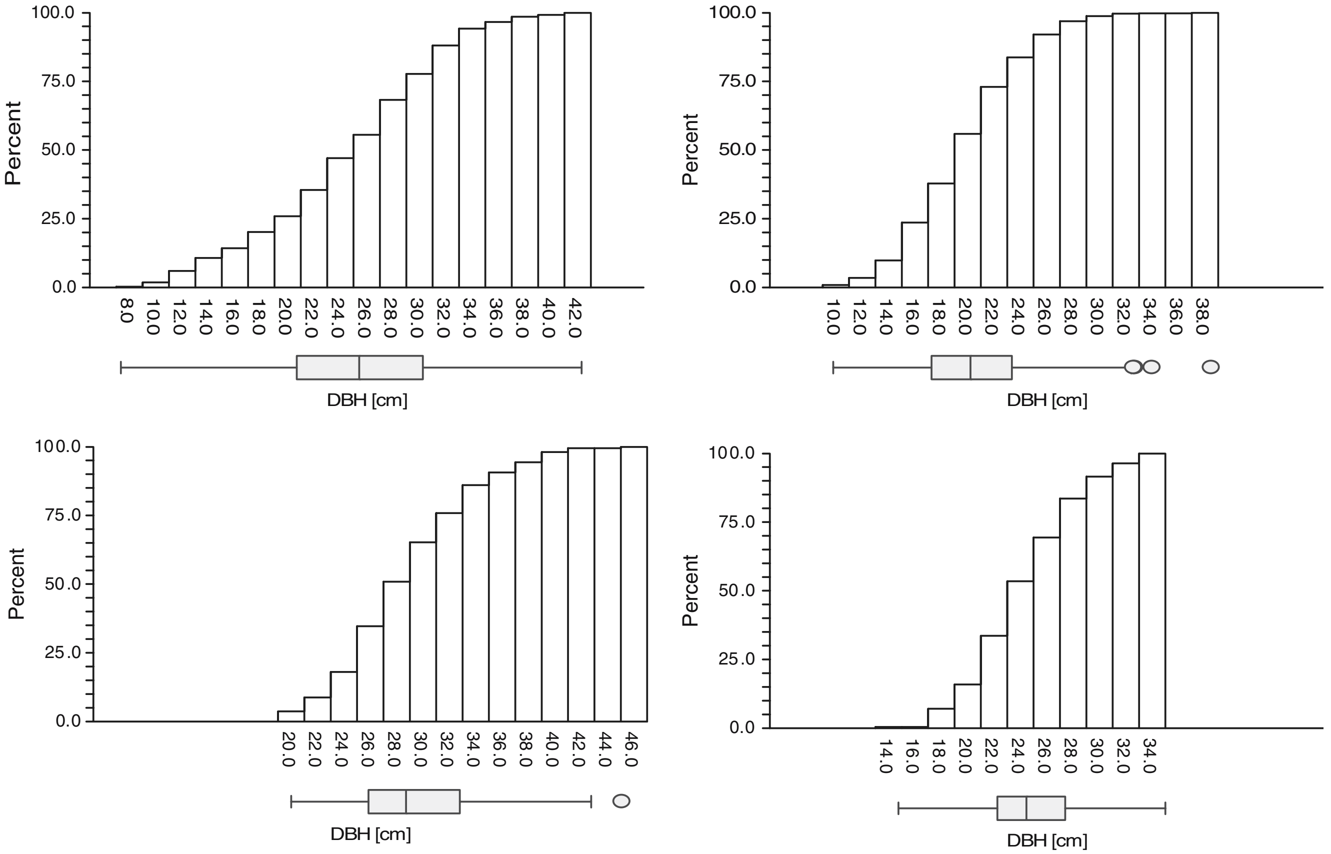

Fig. 1 Cumulative frequency distribution of diameter in breast height (DBH) of the unthinned control plots of the NE (**a**) and SW (**b**) aspect and the thinned plots of the NE (**c**) and SW aspect (**d**) of the experimental site. Measurement of DBH was performed in February 1999. Total number of trees (*N*) was 625 (SW control), 468 (NE control), 226 (SW treatment) and 216 (NE treatment). For each bar the mean value (*x* cm) of a diameter group is given. The group ranges from $x - 1.5$ to $x + 1.4$ cm. In addition a box plot displays median, twenty-fifth and seventy-fifth percentiles as well as upper and lower adjacent values and outliers. (Source: Institute of Forest Growth and Institute of Silviculture, Albert-Ludwigs Universität Freiburg)

TSNN. Leaves and xylem sap were immediately frozen in liquid N_2 and stored at −80°C.

Collection of phloem exudate

Phloem exudates of twigs from adult beech trees were obtained applying the EDTA-technique described by Schneider et al. (1996). Small pieces of bark (c. 300 mg FW) were cut from the twig and incubated for 5 h in 2 ml exudation solution containing 10 mM EDTA and 0.015 mM chloramphenicol at pH 7. After 5 h, the bark pieces were removed from the solution and the remaining exudate was stored in liquid N_2. Previous studies (Schneider et al. 1996) showed that contamination of phloem exudates of beech with cellular constituents can be neglected under the experimental conditions applied.

Collection of xylem sap

Xylem sap of roots was collected by the modification of the procedure of Scholander et al. (1965) described by Schneider et al. (1996). After removing the bark from the cut end at a length of ca. 30 mm, twigs of 30–50 cm length were placed in a pressure chamber with the cut end protruding ca. 10 mm. Subsequently, increasing pressure was applied at a rate of 0.1–0.2 MPa min^{-1}. The pressure at which xylem sap first was visible at the cut end was recorded as current water potential of the twig. The outflow of xylem sap was collected with pipettes and transferred into Eppendorf vials, which were frozen in liquid N_2. Contamination with cellular components was checked under the experimental conditions applied by measuring luminometrically the ATP concentrations of the xylem sap (Schupp 1991; Rennenberg et al. 1998) and was found to be below 0.5% (Schneider et al. 1996; Geßler et al. 1998a).

Extraction of amino compounds, ammonium and nitrate

Samples of frozen leaves were ground in liquid N_2 with mortar and pestle. For extraction of amino compounds and ammonium, aliquots of 0.1 g of the frozen powder were homogenised in 0.2 ml buffer (pH 7.0) containing 20 mM Hepes, 5 mM EGTA and 10 mM NaF, in 1 ml chloroform: methanol (1.5:3.5, v:v) according to Winter et al. (1992). The homogenate was incubated for 30 min at 4°C and subsequently, water-soluble metabolites were extracted twice with 0.6 ml double-demineralised water after centrifugation at 4°C for 5 min at 16,000*g*. The aqueous phases were combined, centrifuged for 10 min again and then freeze-dried (Alpha 2–4, Christ, Osterode, Germany).

For the extraction of nitrate in leaves aliqouts of 0.025 g of the frozen powder were incubated for 1 h with 1.5 ml double-demineralised water and 70 mg PVPP at 5°C to remove phenolic compounds. Subsequently, the sample was heated at 95°C for 5 min, shortly stored on ice for recovery to room temperature and centrifuged for 5 min at 16,000*g*. The supernatant was extracted and stored at −80°C.

Phloem exudates were treated similarly for nitrate analyses: 100 ml of the exudate were incubated in 900 ml double-demineralised water with 70 mg PVPP for 1 h, heated at 95°C for 5 min and centrifuged afterwards.

Table 3 DBH values (cm) of the trees examined on the different aspects and treatments as determined in 1999

NE-C	NE-T	SW-C	SW-T
27.85	27.90	21.4	21.3
28.95	28.05	23.35	23.25
31.05	29.05	24.4	24.2
32.95	32.35	25.85	25.7

Determination of TSNN

Freeze-dried leaf samples were dissolved in 1 ml lithium citrate buffer (0.2 M, pH 2.2). The pH values of this solution as well as aliqouts of the phloem exudate were adjusted to pH 2.2 before centrifugation at 4°C for 5 min at 16,000*g*. An aliquot of 20–75 μl of each sample was injected into an automated amino acid analyser (Biochrom, Pharmacia LKB, Freiburg, Germany). Xylem sap was directly injected into the amino acid analysator without pre-treatment.

The amino acids were separated on a PEEK column (Ultrapac 8 Resin, Lithium 250×4.6 mm, Biochrom, Pharmacia, Freiburg, Germany) working with a system of five lithium citrate buffers generating a pH gradient from pH 2.8 to 3.55 to separate the amino compounds as described by Geßler et al. (1998b). After separation, the different amino acids and ammonium were derivatised with ninhydrin and detected spectrophotometrically at 440 and 570 nm. The peaks were identified and quantified using a standard solution containing 39 amino acids and ammonium (Sigma Chemie, Deisenhofen, Germany).

For nitrate analysis, aliquots of 0.5 ml of the supernatants were injected into an ion exchange chromatography system (DX 100, Dionex, Idstein, Germany). Anions were separated on an IonPac® column (AS9-Sc 250×4 mm, Dionex, Idstein, Germany) using a solution containing 1.8 mM Na_2CO_3 plus 1.7 mM $NaHCO_3$ at a flow rate of 1.0 ml min^{-1}. Nitrate was detected with a conductivity detector module (CDM, Dionex, Idstein, Germany). The detection limit of this method is <0.3 nmol ml^{-1}.

In the phloem exudates determination of nitrate concentrations was performed with a UV-VIS detector (SPD-6AV, Shimadzu, Duisburg, Germany) at 210 nm as described by Hayashi and Chino (1985). The detection limit of this method is <0.3 nmol ml^{-1}.

Stand transpiration, sap wood area and mass flow densities

Stand transpiration was calculated using the water balance model WBS3 (Schmidt 1990), a forest-hydrological model that requires daily mean values of air temperature and daily precipitation as meteorological inputs. Time-independent input variables of the WBS3-simulations are: basal area of the stand, mixing ratio of deciduous trees, mixing ratio of coniferous trees, type of soil, slope angle, slope direction and geographical latitude. For evapotranspiration, transpiration and interception of forests, validations of WBS3 showed a good agreement between results from model calculations and measurements for different areas and slopes (Fritsch 1998; Matzarakis et al. 2000). Model calculations for both years were conducted with data from the nearby DWD climate station Tuttlingen, starting at May 15 since model applicability depends on full leaf expansion.

For a validation of the model we plotted the sums of daily transpiration of the two control plots calculated with the WBS 3 model for the growing season 2000 against values obtained from up-scaled xylem flow measurements (Fig. 2) with Granier-style sensors (see Keitel et al. 2003). There was a reasonable good agreement between the two approaches for both aspects, even though the control stands on the two slopes differed in stem density and timber volume (Geßler et al. 2004).

On both aspects, sap wood areas (SA in m^2 sapwood m^{-2} forest floor) of 25 trees were determined using flourescent berberine-chlorid solution following the method described in Glavac et al. (1989).

On the basis of SA and stand transpiration (ST in $1\ m^{-2}\ day^{-1}$), sap flow densities (SD in ml H_2O $cm^{-2}\ day^{-1}$) were calculated according to the following equation:

$$SD = \frac{ST}{SA}.$$

Mass flow of N (mmol $cm^{-2}\ day^{-1}$) was obtained by multiplying sap flow density with the TSNN concentration of the xylem.

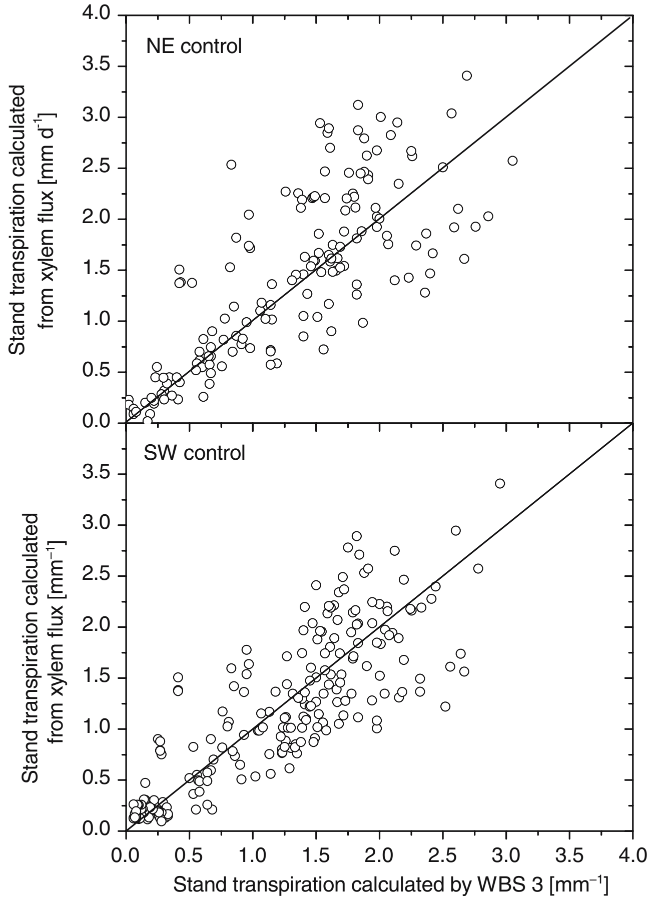

Fig. 2 The daily sums of stand transpiration obtained from the WBS 3 model for the control plots of the SW and NE aspect during the growing season 2,000 plotted against stand transpiration calculated from sap flux measurements. The data for xylem flux densities up-scaled from singles tree to the stand transpiration level were taken from Keitel et al. (2003)

Recovery rates

For validation of the extraction methods applied the recovery rates of ammonium, aspartic acid (Asp), asparagine (Asn), glutamic acid (Glu), glutamine (Gln) and arginine (Arg) applied as internal standards have previously been determined in the phloem exudates and in leaves of beech. Recovery rates amounted to between ca. 82 ± 16% and 108 ± 12% for the different TSNN compounds in the different tissues (Geßler 1999).

Data analysis

Statistical analysis was performed using SPSS 11.0 (SPSS, Inc., USA). The effect of aspect and thinning treatment on TSNN and on the concentrations of the most abundant amino compounds were assessed using a one-way ANOVA.

Results

Sap flow densities

During both the growing seasons in 1999 and 2002, the sap flow raised from mid May to reach maximum values of ca. 200 ml H_2O $cm^{-2}\ day^{-1}$ at the thinned stands and maximum values of ca. 160 ml H_2O $cm^{-2}\ day^{-1}$ at the control stands in mid summer, and subsequently decreased again until the end of September (Fig. 3). Throughout the two growing seasons, trees grown on the thinned stands constantly displayed higher sap flow densities compared to the control stands on either aspect. During both years, lowest sap flow densities were

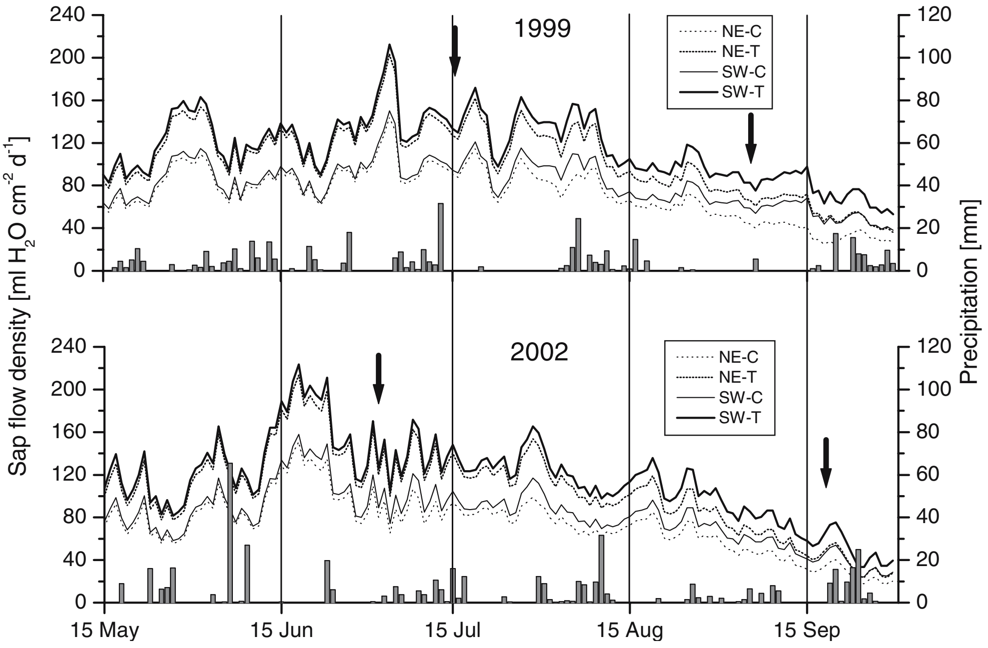

Fig. 3 Precipitation and sap flow densities of adult European beech trees grown at two differently exposed sites (NE vs. SW) in control stands (C) and in stands subjected to thinning (T) during the vegetation periods 1999 and 2002 when leaves were fully expanded. *Arrows* indicate the times of sampling campaigns in July and September. The May campaigns are not indicated since they took place before full leaf expansion

observed with trees of the NE control stand, whereas highest sap flow densities were displayed by trees grown on the dry-warm SW thinned stand—particularly after the middle of July. In mid summer months sap fluxes were enhanced during periods of low precipitation and long sunshine duration, especially during the dry period in June 2002.

TSNN compound profile and concentrations in the xylem

During the growing seasons 1999 and 2002 there was a clear trend to a decrease in TSNN concentration from the beginning to the end of the growing season (Fig. 4). In May 1999, TSNN concentrations varied between 19.7

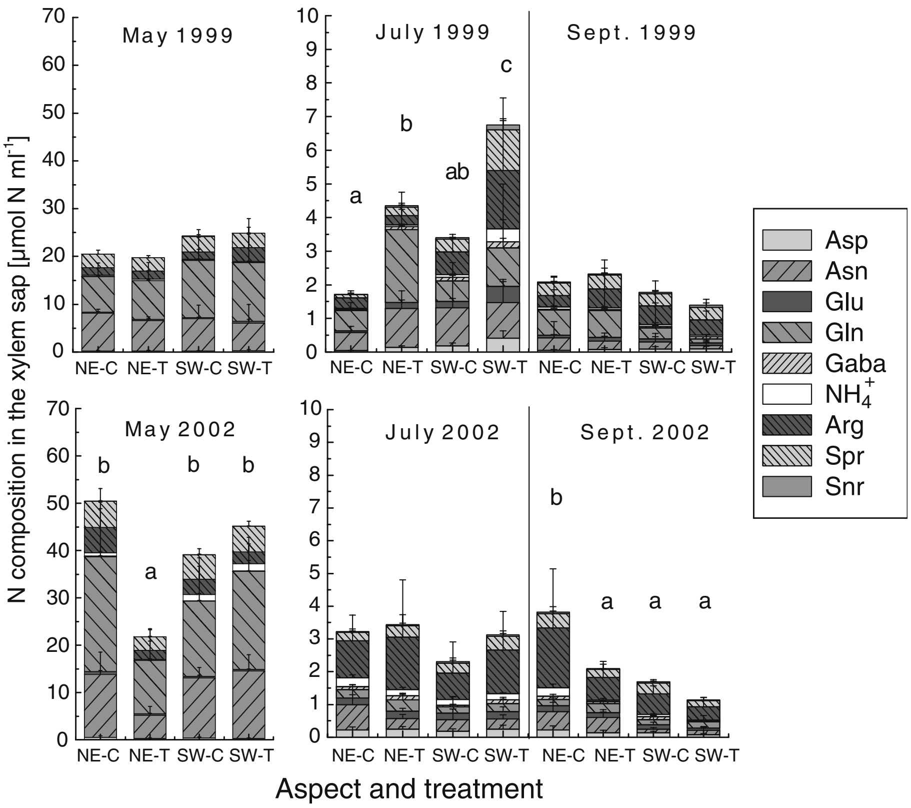

Fig. 4 TSNN composition and concentrations in twig xylem sap of adult European beech trees grown in control stands (C) and in stands subjected to thinning (T) at two differently exposed sites (NE vs. SW). The concentrations of the six most abundant amino compounds and ammonium are shown, as well as the sum of all other proteinogenic (Spr) and nonproteinogenic (Snr) amino compounds including nitrate Gaba: γ-aminobutyric acid. Xylem sap was collected at six sampling campaigns in May, July and September 1999 and 2002. Statistical analysis was performed by one-way Anova followed by a Tukey post hoc test. *Different letters* indicate differences in TSNN concentration between aspects and treatments ($P < 0.05$). The values shown are means (+SD) of four trees

and 24.9 μmol N ml^{-1}, but no significant effect of treatment or aspect on TSNN or a particular compound was observed. Independent of site or treatment, Gln and Asn were the most abundant amino compounds comprising ca. 44% and 30% of TSNN, respectively. In July 1999, TSNN decreased notably on both aspects and in both treatments. The lowest concentration of 1.7 μmol N ml^{-1} was observed at the NE control site whereas the July maximum (6.8 μmol N ml^{-1}) was detected at the SW thinned site. The decrease between May and July was due to a reduction of all major components. A significant difference in TSNN concentrations was observed in both thinned stands as compared to the respective control sites. Comparing the same treatments on the two aspects, TSNN concentrations were higher on the SW aspect, though differences were significant only for the thinned stands. In September 1999, TSNN of xylem sap decreased slightly compared to July amounting to 1.4–2.3 μmol N ml^{-1}. Neither TSNN nor single compounds showed significant differences between aspect and treatment.

In May 2002, TSNN concentrations were higher in both treatments on the SW aspect and on the control treatment of the NE aspect as compared to 1999. TSNN amounted to between 22.0 μmol N ml^{-1} (NET) and 50.5 μmol N ml^{-1} (NEC). The relative composition of TSNN was comparable to 1999 with Gln (46%) and Asn (28%) as most abundant compounds. The significantly lower TSNN concentration in the NE thinned stand as compared to the other stands was mainly a consequence of reduced Asn and Gln concentrations. In July 2002, TSNN decreased drastically compared to May, but a significant influence of site or treatment was not detected. In September 2002, TSNN concentrations of the NE thinned stand and both SW stands decreased slightly compared to July, amounting to between 1.1 and 3.8 μmol N ml^{-1}. Highest TSNN concentrations were observed in the xylem of trees from the NE control site, mainly as a consequence of high Arg concentrations. Throughout the two growing season, nitrate in xylem amounted to < 1% of TSNN.

Nitrogen mass flow in the xylem

In July 1999, N mass flow reached values between 0.15 and 0.9 mmol N cm^{-2} day^{-1}, and was significantly higher on both thinned stands compared to the control stands (Fig. 5). Moreover, xylem N flow was significantly enhanced on the SW thinned site compared to the NE thinned site. In September 1999, N flow decreased to similar values of ca. 0.1 mmol N cm^{-2} day^{-1} on all four sites. In July 2002, N flow amounted to 0.21 and 0.56 mmol N cm^{-2} day^{-1}. As in July 1999, the thinned stands displayed higher N flow than the control stands, but the only significant difference was observed between the NE thinned stand and the SW control stand. In September 2002, N flow was again reduced to similar values of ca. 0.1 mmol N cm^{-2} day^{-1} on all four sites examined.

TSNN compound profile and concentrations in the leaves

As in the xylem, there was a clear trend to a decrease in leave TSNN concentration in leaves from the beginning to the end of the growing season in both years (Fig. 6).

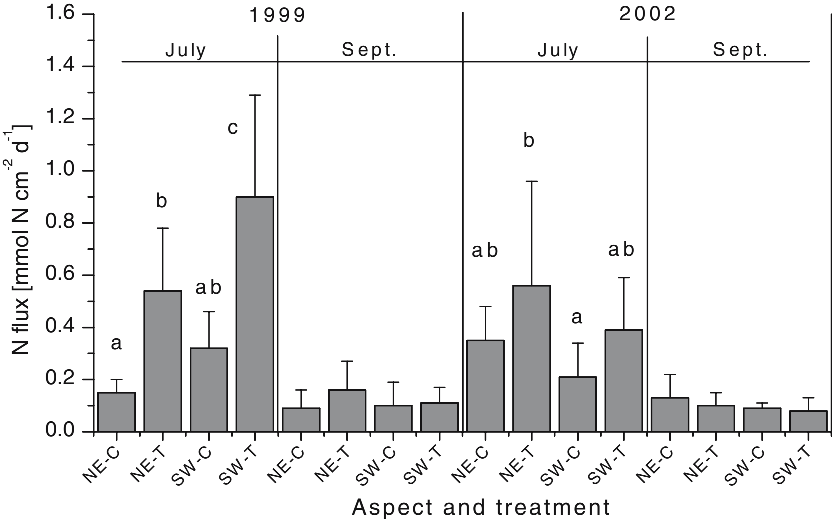

Fig. 5 N flux in the xylem of adult European beech trees grown in control stands (C) and in stands subjected to thinning (T) at two differently exposed sites (NE vs. SW). N flux was calculated on the basis of total soluble N (TSNN) in the xylem, sap wood area of the trees and stand transpiration after full leaf expansion; thus the May campaigns are excluded. Xylem sap was collected from beech twigs at four sampling campaigns in July and September 1999 and 2002. Statistical analysis was performed by one-way Anova followed by a Tukey post hoc test. *Different letters* indicate differences in TSNN concentration between aspects and treatments ($P < 0.05$). The values shown are means (+SD) of four trees

In May 1999, TSNN varied between 26.8 and 86.9 μmol N g^{-1} f.wt. Asn was the prevailing amino compound with ca. 26% of TSNN. Concentrations of TSNN, Asn, ammonium displayed a significant exposition effect, i.e. an increase on both SW sites compared to the NE sites. Moreover, the concentration of Arg was significantly increased on the SW thinned site compared to the NE thinned site. In July 1999, TSNN was drastically reduced compared to May, amounting to between 3.6 and 5.7 μmol N g^{-1} f.wt. Similar to May, TSNN of the SW thinned stand significantly exceeded TSNN of the NE thinned stand. TSNN in the leaves amounted to between 0.5 and 0.7% of total N (data not shown). In September 1999, TSNN decreased slightly to values from 2.8 to 3.4 μmol N g^{-1} f.wt. Effects of aspect or treatment were not detected. Throughout the growing season, Nitrate was almost absent in the leaves (< 1% of TSNN).

In May 2002, TSNN varied from 26.6 to 35.9 μmol N g^{-1} f.wt. Main amino compounds were Asn with 29% of TSNN and Glu with ca. 16%. In contrast to May 1999, no significant differences in TSNN were observed between aspect and treatment. In July 2002, TSNN was markedly reduced to between 3.2 and 7.7 μmol N g^{-1} f.wt, a range comparable to July 1999. This effect could be attributed to a reduction of Asn and Glu. The predominant amino compound was Glu (24%), followed by Asp (13%). In the thinned stands, TSNN, Asp, Glu, Gln and ammonium were significantly higher on the NE aspect, a pattern inverse to that observed 3 years before. TSNN amounted to between 0.5 and 1.2% of total N in the leaves (data not shown). In September 2002, TSNN decreased slightly compared to July. It amounted to between 2.8 μmol N g^{-1} f.wt in the SW thinned stand and 5.1 μmol N g^{-1} f.wt in the NE thinned stand. In the SW thinned stand, TSNN was significantly lower as compared to the SW control stand and the NE thinned stand, whereas TSNN was significantly increased on the NE thinned stand. Throughout the two growing season, nitrate in leaves amounted to < 1–2% of TSNN.

TSNN compound profile and concentrations in the phloem

Comparable to the trend of TSNN concentrations in xylem and leaves, there was a decrease in phloem TSNN concentration from the beginning to the end of the growing season 1999 (Fig. 7). By contrast, phloem TSNN concentrations in 2002 reached a maximum in September.

In May 1999, TSNN varied between 4.0 and 15.4 μmol N g^{-1} f.wt. The dominant amino compound was Arg, comprising ca. 51% of TSNN. A significant treatment and exposition effect with increased concentrations of TSNN, Asn, Gln and Arg was observed on the NE thinned stand with significantly higher values compared to the NE control stand and the SW thinned stand. In July 1999, TSNN declined to between 1.4 μmol N g^{-1} f.wt (SW-T) and 2.6 μmol N g^{-1} f.wt (NE-C), which was primarily due to a decrease of Arg.

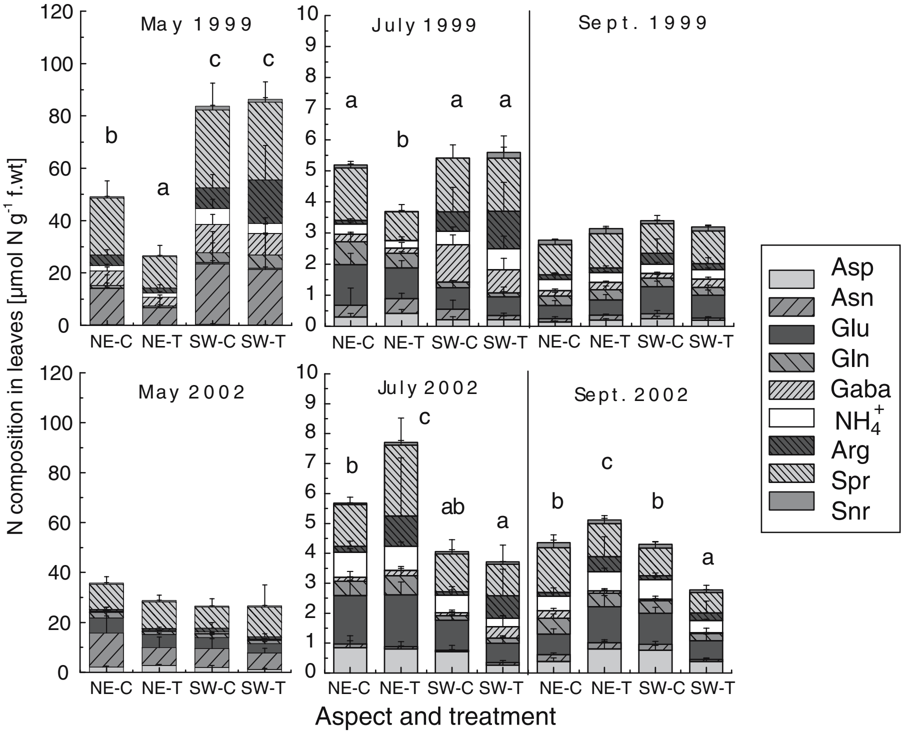

Fig. 6 TSNN composition and concentrations in leaves of adult European beech trees grown in control stands (C) and in stands subjected to thinning (T) at two differently exposed sites (NE vs. SW). The concentrations of the six most abundant amino compounds and ammonium are shown, as well as the sum of all other proteinogenic (Spr) and nonproteinogenic (Snr) amino compounds including nitrate. The leaves were collected at six sampling campaigns in May, July and September 1999 and 2002. Statistical analysis was performed by one-way Anova followed by a Tukey post hoc test. *Different letters* indicate differences in TSNN concentration between aspects and treatments ($P < 0.05$). The values shown are means (+ SD) of four trees

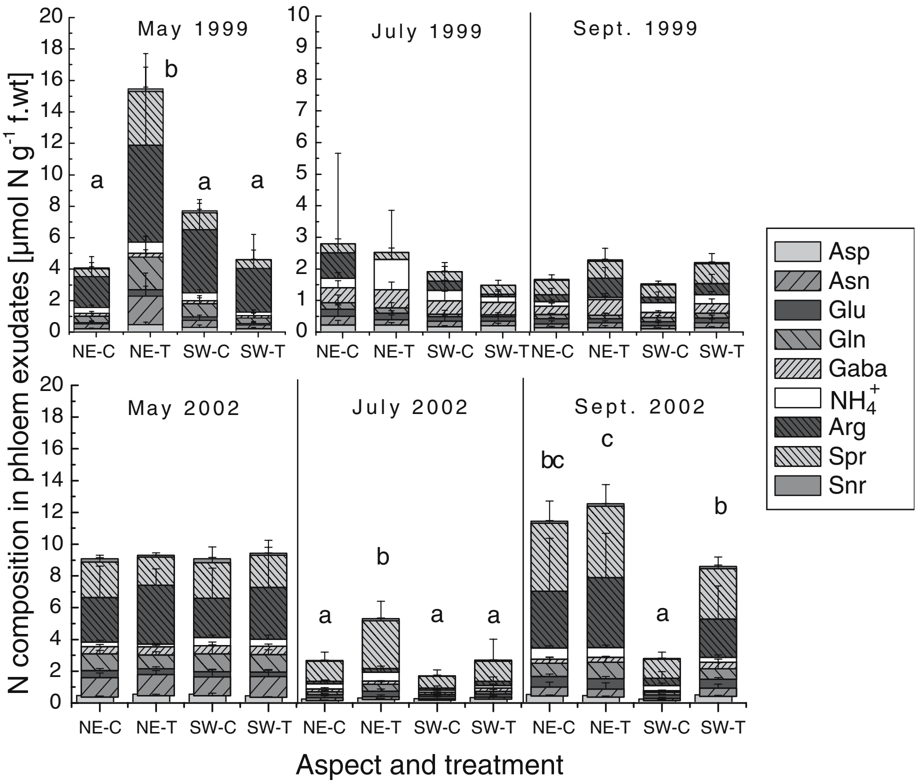

Fig. 7 TSNN composition and concentrations in twig phloem sap of adult European beech trees grown in control stands (C) and in stands subjected to thinning (T) at two differently exposed sites (NE vs. SW). The concentrations of the six most abundant amino compounds and ammonium are shown, as well as the sum of all other proteinogenic (Spr) and nonproteinogenic (Snr) amino compounds including nitrate. Phloem sap was collected at six sampling campaigns in May, July and September 1999 and 2002. Statistical analysis was performed by one-way Anova followed by a Tukey post hoc test. *Different letters* indicate differences in TSNN concentration between aspects and treatments ($P < 0.05$). The values shown are means (+SD) of four trees

Differences between aspects or treatments were not observed. In September 1999, TSNN varied between 1.5 and 2.2 µmol N g^{-1} f.wt and did not show any significant differences between aspects and treatments.

In May 2002, TSNN amounted to ca. 9 µmol N g^{-1} f.wt at all aspect and treatment. The most abundant amino compounds were Arg (33% of TSNN) and Asn (14%). In July 2002, TSNN decreased to 1.7 µmol N g^{-1} f.wt at the SW control stand and to 5.3 µmol N g^{-1} f.wt at the NE thinned stand. At this time of the year, the most abundant amino compound was Ala, comprising 24% of TSNN and 49% of the proteinogenic rest. A significant treatment effect was observed on the NE aspect with increased concentrations of TSNN, Glu, and Gln in the thinned stand. In addition, TSNN, Gln, and ammonium were significantly higher on the NE thinned stand as compared to the SW thinned stand. In September 2002, TSNN raised again on three stands, amounting to highest concentrations measured during the growing season 2002 in phloem at the NE thinned site with 12.5 µmol N g^{-1} f.wt. The minimum remained at 2.8 µmol N g^{-1} f.wt at the SW control site. The dominant amino compound was Arg (28% of TSNN), followed by Ala (18% of TSNN and 48% of proteinogenic rest). Effects of exposition and treatment were detected on the SW control site. TSNN and all amino compounds with the exception of ammonium were decreased in the phloem of trees from the SW control site as compared to the NE control site and the SW thinned site, respectively. Moreover, TSNN concentrations of NET exceeded that of SWT. Throughout the two growing seasons in 1999 and 2002, nitrate in the phloem was below the limit of detection.

Discussion

In the present study we assessed the effects of thinning under different meso-climatic conditions (temperature, water availability, radiation) on TSNN in xylem, leaves and phloem of adult beech trees during the growing seasons in 1999 and 2002. The two different years were chosen in order to compare the nitrogen balance shortly after a thinning treatment was applied and 3 years later. The experimental sites were located on opposing slopes with different exposition (NE vs. SW) of a narrow valley differing in local climatic conditions. Previous studies revealed that only trees growing on the SW aspect were subjected to water shortage during periods of low precipitation in summer, due to higher soil and air temperatures combined with lower water storage capacity of the soil compared to the NE aspect (Geßler et al. 2001). Findings for beech regeneration suggested that N status and uptake was improved on the cool moist NE aspect but impaired under the warm dry conditions on the SW aspect (Fotelli et al. 2002a, 2004). Since not only air temperatures but also prolonged drought periods during summer are expected to increase in frequency in Central Europe (IPCC 2001; Schär et al. 2004), this experimental design is regarded appropriate to assess the influence of climate change on the nitrogen and, thus, nutrient status of adult beech trees grown on limestone (Geßler et al. 2004a).

Differences between aspects and treatments in TSNN concentrations in phloem, xylem and leaves at the beginning of the growing season indicate differences in the timing of N remobilisation

During both the years of measurement, TSNN concentrations of xylem and leaves reached their maximum in May, and also phloem TSNN concentrations were generally increased compared to summer. Similar patterns were observed by Fotelli et al. (2002a) and Schneider et al. (1996) for beech. The high TSNN concentrations at the onset of the growing season can be attributed to xylem and phloem loading of N compounds from storage tissue shortly before and during bud break in order to supply newly developing leaves.

In May 1999, no significant differences in TSNN concentrations were observed in the xylem (Fig. 4), but considerable differences in TSNN concentrations were found in leaves (Fig. 6) and phloem (Fig. 7). TSNN concentrations in leaves or phloem on the NEC, SWC and SWT sites were similar and only trees from the NET site showed divergent patterns. In the phloem, TSNN was markedly increased mainly due to high Arg, Gln and Asn concentrations, whereas concentration was decreased in the newly developing leaves. In May 2002 such differences were not observed. Then in contrast, TSNN concentration in the xylem sap was lowest in trees from the NET site.

The observed patterns of TSNN concentrations of xylem sap and phloem exudates in May 1999 and 2002 may be attributed to differences in timing of N remobilisation between the plots. Early in the growing season immediately before and during bud break, phloem transport is supposed to contribute to acropetal translocation of remobilised N compounds (Da Silva and Shelp 1990). This view is supported here by the finding that Arg, a major N storage compound in beech (Geßler et al. 1998b), is the most abundant N compound of the phloem exudates in spring (Fig. 7). After unfolding of leaves and onset of transpiration, transport of soluble N compounds via the xylem is supposed to be the main N source for the developing leaves (e.g. Peuke and Kaiser 1996).

Geßler (1999) observed a strong increase in TSNN concentrations in the phloem exudates of beech about 2 weeks before bud break, which decreased again sharply immediately before leaves started to unfold. Comparable peaking of TSNN concentrations was also found in leaves and xylem sap, but showed a time lag with maximum values during bud break. Since average air temperatures measured during the 4 weeks before sampling in May 2002 were lowest on the NE aspect, particularly at the thinned site (NET: 6.4°C; NEC: 6.6°C, SWT: 6.9°C; SWC: 6.9°C) it is assumed that the start of N remobilisation was retarded at the NE thinned site. As a consequence the peak in TSNN concentration in the xylem sap was, in contrast to the other sites, not yet reached when the samples were taken. As the temperature patterns determined in spring 2002 were also observed in other years (Keitel 2004), it is concluded that a delay in spring remobilisation of stored N was also the reason for the low TSNN concentration in leaves and the maximum values in the phloem detected in the thinning treatment of the NE aspect in 1999. In view of the fact that Geßler (1999) observed a time-lag between TSNN spring maximum in phloem exudates and leaves; it is concluded that the trees from the NEC, SWC and SWT sites had already been passed the spring peak in the phloem and reached the maximum of TSNN concentrations in breaking buds whereas the individuals from the NET site were harvested when they showed maximum TSNN concentrations in the phloem. These observations are supported by the assessment of leaf phenology (budding stage, leaf development stage) during the growing season in 2000 (Kirchgäßner 2001). It was observed that bud break and unfolding of leaves occurred earlier in the control treatments of the NE aspect as compared to the respective thinning treatment.

TSNN concentrations in mid summer reflect environmental differences between years, treatments and aspects

In July 1999 and 2002, TSNN in xylem, leaves and phloem decreased heavily compared to May of both years, a finding also observed in other studies with beech regeneration (Fotelli et al. 2002a) or adult beech and spruce (Schneider et al. 1996). Lowered TSNN concentrations in all tissues during mid-growing season can be explained by lower N demand of the already developed leaves.

The thinned stands displayed significantly higher N mass flow and TSNN concentrations on both aspects in the xylem sap in July 1999 and showed the same tendency, although not significant, in July 2002. Higher N transport rates in xylem in both thinned stands point to higher N availability and uptake or to intensive phloem-to-xylem exchange, both leading to increased xylem loading with amino compounds. Consistent with the first assumption, increasing soil nutrient abundance within the first years after thinning have been reported (Thibodeau et al. 2000) and better water supply for the remaining trees has been suggested (Laurent et al. 2003). Moreover, N uptake in beech roots is positively correlated with soil temperature (Geßler et al. 1998a), which was elevated in the thinned stands of each aspect (Table 1). Hence, pedospheric N uptake might have been facilitated particularly in the first year after thinning, owing to large canopy gaps permitting increased precipitation throughfall and radiation perception (Table 1). Highest TSNN concentrations 3 years after the thinning treatment in phloem, leaves and xylem were found in the thinned stand of the NE aspect, indicating most favourable growth conditions.

Geßler et al. (2005) showed that nitrate uptake potential as well as pedospheric availability of inorganic N was generally higher at the NE as compared to the SW aspect. Due to higher soil temperatures (Table 1; Fotelli et al. 2003; Holst et al. 2004a) and reduced intraspecific competition, effective nitrate uptake is supposed to be highest in the NE thinning treatment, thus leading to the increased soluble N concentrations observed in 2002. This result is consistent with findings from Fotelli et al. (2004) who observed thinning to cause increased N concentrations in beech seedling on the NE aspect.

In July 1999, however, TSNN concentrations in leaves were lower in the thinning treatment on the NE site compared to the respective control and even on both SW sites, despite higher N mass flow in the xylem on both thinned stands and, thus, putative higher N supply of the crown. Since thinning induces a strong increase in crown biomass in the first year after treatment (Mitscherlich and Ganssen 1951), the reduced foliar TSNN concentration may be attributed to a 'dilution' of nitrogen within the expanding crown, an effect not observed 3 years after thinning treatment, when PAI was increased particularly at the thinned sites (Holst et al. 2004b) and canopy structure was largely closed again.

Phloem TSNN concentrations in late summer are sensitive to treatment-specific onset of senescence

In September 2002, TSNN and particularly Arg concentrations in the phloem on the SW site were significantly higher in the thinned stand compared to the control stand. Comparable albeit not significant patterns were observed in the same year at the NE aspect and in September 1999 at both aspects. In 2002, it is obvious that leaf TSNN concentrations of trees from the NEC, NET and SWT stands decreased between July and September, but showed on the contrary a strong increase in TSNN and Arg in the phloem within the same period of time. Raised Arg concentrations in phloem were frequently observed at the end of the growing season, and are supposed to indicate remobilisation of N from decomposed proteins in leaves (Geßler et al. 1998b; Fotelli et al. 2002a; Schneider et al. 1996). Phenological studies of Kirchgäßner (2001) showed that leaf senescence (i.e. leaf yellowing) started slightly earlier in the thinned stands. Thus, the significantly higher phloem TSNN concentration of the SW thinned stand as compared to the control treatment in September 2002 may be a result of the earlier onset of phloem transport of remobilised N from the senescing leaves to the storage tissues in bark and wood.

Similarly, Garcia-Plazaola and Becerril (2001) observed antecedent onset of leaf senescence in sun exposed beech leaves as compared to shade leaves, owing to earlier chlorophyll degradation. Moreover, the beginning of leaf senescence is triggered by a variety of different biotic and abiotic factors such as CO_2 concentrations in the atmosphere (Sigurdsson 2001), nutrient supply (Sigurdsson 2001), metabolic imbalance between source and sink at a whole plant level (Paul and Foyer 2001) or water availability (Amlin and Rood 2003; Pic et al. 2002). Kirchgäßner (2001) concluded that the earlier initiation of leaf senescence in the thinned stands was due to increased midday air temperatures (Table 1), leading to increased evapotranspiration. As a consequence soil water availability could be reduced after periods of low rainfall in late summer as in fact observed for the thinning treatments on the SW aspect (Geßler et al. 2001), hence, leading to impaired growth conditions. Yet, a variety of parameters might interact in the regulation of senescence and related N storage processes.

Conclusions

Previous studies with beech revealed that concentrations and compound profile of TSNN can be a sensitive indicator of short-term physiological responses to environmental influence on trees (Geßler et al. 1998b; Fotelli et al. 2002a), reflecting changes in their internal nitrogen status in much more detail than total N concentrations, which are constant over a wide range of climatic and nutritional conditions (Rennenberg et al. 1998; Geßler and Rennenberg 2000).

In the present study we showed that TSNN reflects seasonal changes in the N balance of adult beech as influenced by local climate and silvicultural treatment. Taking xylem sap flow densities into account, the assessment of the TSNN concentrations and composition can be used as a tool to characterise N remobilisation and storage patterns. It was shown that the thinning treatment on the cool-moist NE aspect resulted in a retarded onset of N remobilisation, probably due to a reduction of air temperatures in early spring. In mid summer, thinning resulted in enhanced acropetal N flow, indicating improved N availability for the trees. This effect was more pronounced in the year following the silvicultural treatment than 3 years later. Yet, thinning accelerated remobilisation of structural N from leaves via the phloem to storage tissues on the warm-dry SW-aspect in late summer, possibly due to impaired growth conditions. The patterns of remobilisation and storage fitted well with phenological observation made at the experimental sites.

In July 2002, during advanced closure of the stand canopy, trees grown on the thinned stand on the NE aspect displayed maximum TSNN concentrations in all tissues. Thus, it is supposed that the N balance of adult beech is favoured by thinning treatments as well as the cool-moist climate prevailing on the NE aspect. On the other hand, lower N concentrations found in trees grown on the warm-dry SW-aspect may reflect the reduced tree growth as observed by Geßler et al. (2001).

Acknowledgements This reasearch was part of a project funded by the Deutsche Forschungsgemeinschaft under contract numbers

SFB 433 and Re515/13-1. The data for the frequency distribution of BDH were provided by J. Huss (Institute of Silviculture; University of Freiburg) and H. Spiecker (Institute of Forest Growth, University of Freiburg).

References

Amlin NM, Rood SB (2003) Drought stress and recovery of riparian cottonwoods due to water table alteration along Willow Creek, Alberta. Tree Struct Funct 17:351–358

Bauer GA, Persson H, Mund M, Hein M, Kunnetz E, Matteucci G, van Oene H, Scarascia-Mugnozza G, Schulze E-D (2000) Linking plant nutrition and ecosystem processes. In: Schulze E-D (ed) Ecological studies 142: carbon and nitrogen cycling in european forest ecosystems. Springer, Berlin Heidelberg New York, pp 63–98

Breda N, Granier A, Aussenac G (1995) Effects of thinning on soil and tree water relations, transpiration and growth in an oak forest (Quercus-Petraea (Matt) Liebl). Tree Physiol 15:295–306

Caputo C, Barneix AJ (1997) Export of amino acids to the phloem in relation to N supply in wheat. Physiol Plant 101:853–860

Cermak J, Matyssek R, Kucera J (1993) Rapid response of large, drought-stressed beech trees to irrigation. Tree Physiol 12:281–290

Collier MD, Fotelli MN, Nahm M, Kopriva S, Rennenberg H, Hanke DE, Gessler A (2003) Regulation of nitrogen uptake by *Fagus sylvatica* on a whole plant level—interactions between cytokinins and soluble N compounds. Plant Cell Environ 26:1549–1560

Cooper HD, Clarkson DT (1989) Cycling of amino-nitrogen and other nutrients between shoots and roots in cereals—a possible mechanism integrating shoot and root in the regulation of nutrient-uptake. J Exp Bot 40:753–762

Da Silva MC, Shelp BJ (1990) Xylem-to-phloem transfer of organic nitrogen in young soybean plants. Plant Physiol 92:797–801

Dertz W (1996) Buchenwälder im Zielkatalog der Forstwirtschaft. In: Buchenwälder - ihr Schutz und ihre Nutzung. Stiftung Wald in Not, Bonn, Germany, pp 2–8

Ellenberg H (1992) Vegetation Mitteleuropas mit den Alpen. Eugen Ulmer, Stuttgart, Germany

Fotelli MN, Gessler A, Peuke AD, Rennenberg H (2001) Drought affects the competitive interactions between *Fagus sylvatica* seedlings and an early successional species, *Rubus fruticosus*: responses of growth, water status and delta C-13 composition. New Phytol 151:427–435

Fotelli MN, Nahm M, Heidenfelder A, Papen H, Rennenberg H, Gessler A (2002a) Soluble nonprotein nitrogen compounds indicate changes in the nitrogen status of beech seedlings due to climate and thinning. New Phytol 154:85–97

Fotelli MN, Rennenberg H, Gessler A (2002b) Effects of drought on the competitive interference of an early successional species (*Rubus fruticosus*) on *Fagus sylvatica* L. seedlings: N-15 uptake and partitioning, responses of amino acids and other N compounds. Plant Biol 4:311–320

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

Fotelli NM, Rienks M, Rennenberg H, Geßler A (2004) Climate and forest management affect 15N-uptake, N balance and biomass of European beech (*Fagus sylvatica* L.) seedlings. Trees 18:157–166

Foyer CH, Parry M, Noctor G (2003) Markers and signals associated with nitrogen assimilation in higher plants. J Exp Bot 54:585–593

Fritsch J (1998) Energiebilanz und Verdunstung eines bewaldeten Hanges im Hochschwarzwald. Ber. Meteor. Inst. Univ. Freiburg Nr. 1, 186 pp

Garcia-Plazaola JI, Becerril JM (2001) Seasonal changes in photosynthetic pigments and antioxidants in beech (*Fagus sylvatica*) in a Mediterranean climate: implications for tree decline diagnosis. Aust J Plant Physiol 28:225–232

Geßler A, Schneider S, Weber P, Hanemann U, Rennenberg H (1998a) Soluble N compounds in trees exposed to high loads of N: a comparison between the roots of Norway spruce (*Picea abies*) and beech (*Fagus sylvatica*) trees grown under field conditions. New Phytol 138:385–399

Geßler A, Schneider S, Von Sengbusch D, Weber P, Hanemann U, Huber C, Rothe A, Kreutzer K, Rennenberg H (1998b) Field and laboratory experiments on net uptake of nitrate and ammonium by the roots of spruce (*Picea abies*) and beech (*Fagus sylvatica*) trees. New Phytol 138:275–285

Geßler A (1999) Untersuchungen zum Stickstoffhaushalt von Buchen (*Fagus sylvatica*) in einem stickstoffübersättigten Waldökosystem. PhD Thesis, University of Freiburg, Germany

Geßler A, Rennenberg H (2000) The effect of liming on the soluble nitrogen pool in Norway spruce (*Picea abies*) exposed to high loads of nitrogen. Phyton 40:51–64

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

Geßler A, Weber P, Schneider S, Rennenberg H (2003) Bidirectional exchange of amino compounds between phloem and xylem during long-distance transport in Norway spruce trees (*Picea abies* [L.] Karst). J Exp Bot 54:1389–1397

Geßler A, Keitel C, Nahm M, Rennenberg H (2004) Water shortage affects the water and nitrogen balance in Central European beech forests. Plant Biol 6:289–298

Geßler A, Augustin S, Hildebrand E, Gasche R, Heidenfelder A, Papen H, Börner E, Metzler B, Rennenberg H (2005) Climate and forest management influence nitrogen balance of beech forests: microbial N transformations and N uptake by mycorrhizal roots. Eur J For Res 124:95–111

Gezelius K, Nasholm T (1993) Free amino-acids and protein in scots pine—seedlings cultivated at different nutrient availabilities. Tree Physiol 13:71–86

Glavac V, Koenis H, Jochheim H, Ebben U (1989) Mineralstoffe im Xylemsaft der Buche und ihre jahreszeitlichen Konzentrationsveränderungen entlang der Stammhöhe. Angew Botanik 63:471–486

Hayashi H, Chino M (1985) Nitrate and other anions in rice phloem sap. Plant Cell Physiol 26:325–330

Holst T, Mayer H, Schindler D (2004a) Microclimate within beech stands—Part II: thermal conditions. Eur J For Res 123:13–28

Holst T, Hauser S, Kirchgäßner A, Matzarakis A, Mayer H, Schindler D (2004b) Measuring and modelling plant area index in beech stands. Int J Biometeorol 48:192–201

IPCC. Climate change 2001: impacts, adaptation and vulnerability. http://www.IPCC.ch/wg2SPM.pdf

ISSS Working Group (1998) World reference base for soil resources. In: Deckers JA, Nachtergaele FO, Spaargaren OC (eds) Introduction. International Society of Soil Science (ISSS), International Soil Reference and Information Centre (ISRIC) and Food and Agriculture Organization of the United Nations (FAO). ACCO, Leuven

Keitel C, Adams MA, Holst T, Matzarakis A, Mayer H, Rennenberg H, Gessler A (2003) Carbon and oxygen isotope composition of organic compounds in the phloem sap provides a short-term measure for stomatal conductance of European beech (*Fagus sylvatica* L.). Plant Cell Environ 26:1157–1168

Keitel C (2004) Isotope signatures (δ^{13}C, δ^{18}O, δ^{15}N) as a measure of environmental effects on the physiology of trees in the Northern and Southern Hemispheres. PhD Thesis, University of Freiburg, Germany

Kirchgäßner A (2001) Phänoklimatologie von Buchenwäldern im Südwesten der Schwäbischen Alb. Ber. Meteor. Inst Univ Freiburg Nr 7:295

Kreuzwieser J, Herschbach C, Stulen I, Wiersema P, Vaalburg W, Rennenberg H (1997) Interactions of NH^{4+} and L-glutamate with NO^{3-} transport processes of non-mycorrhizal *Fagus sylvatica* roots. J Exp Bot 48:1431–1438

Laurent M, Antoine N, Joel G (2003) Effects of different thinning intensities on drought response in Norway spruce (*Picea abies* (L.) Karst.). For Ecol Manage 183:47–60

Marschner H (1995) Mineral nutrition of higher plants. Academic, London

Matzarakis A, Mayer H, Schindler D, Fritsch J (2000) Simulation des Wasserhaushaltes eines Buchenwaldes mit dem forstlichen Wasserhaushaltsmodell WBS3. Ber Meteor Inst Univ Freiburg Nr 5:137–146

Mayer H, Holst T, Schindler D (2002) Mikroklima in Buchenbeständen - Teil 1: Photosynthetisch aktive Strahlung. Forstwissenschaftliches Centralblatt 121:301–321

Millard P (1996) Ecophysiology of the internal cycling of nitrogen for tree growth. Zeitschrift für Pflanzenernährung und Bodenkunde 159:1–10

Mitscherlich G, Ganssen R (1951) Die Ergebnisse zweier Buchendurchforstungsversuche in höheren Lagen des Schwarzwaldes. Allgemeine Forst- und Jagdzeitung 123(1):1–15

Moosmayer H-U (2002) Langfristige regionale Waldbauplanung in Baden-Württemberg - Grundlagen und Ergebnisse. Landesforstverwaltung Baden-Württemberg, Stuttgart

Muller B, Touraine B, Rennenberg H (1996) Interaction between atmospheric and pedospheric nitrogen nutrition in spruce (*Picea abies* L Karst) seedlings. Plant Cell Environ 19:345–355

Nordin A, Uggla C, Nasholm T (2001) Nitrogen forms in bark, wood and foliage of nitrogen-fertilized *Pinus sylvestris*. Tree Physiol 21:59–64

Novitskaya L, Trevanion SJ, Driscoll S, Foyer CH, Noctor G (2002) How does photorespiration modulate leaf amino acid contents? A dual approach through modelling and metabolite analysis. Plant Cell Environ 25:821–835

Oberdorfer E (1992) Süddeutsche Pflanzengesellschaften Teil IV: Wälder und Gebüsche. Fischer-Verlag, Stuttgart

Paul MJ, Foyer CH (2001) Sink regulation of photosynthesis. J Exp Bot 52:1383–1400

Paul T (2003) Die Vegetation in Kalkbuchenwäldern in Abhängigkeit von Standort und forstlicher Nutzung. PhD-Thesis, University of Freiburg, Germany

Peuke AD, Kaiser WM (1996) Nitrate or ammonium uptake and transport, and rapid regulation of nitrate reduction in higher plants. Prog Bot 57:93–113

Pic E, de la Serve BT, Tardieu F, Turc O (2002) Leaf senescence induced by mild water deficit follows the same sequence of macroscopic, biochemical, and molecular events as monocarpic senescence in pea. Plant Physiol 128:236–246

Rennenberg H, Kreutzer K, Papen H, Weber P (1998) Consequences of high loads of nitrogen for spruce (*Picea abies*) and beech (*Fagus sylvatica*) forests. New Phytol 139:71–86

Rennenberg H, Gessler A (1999) Consequences of N deposition to forest ecosystems—recent results and future research needs. Water Air Soil Pollut 116:47–64

Rothe A (1998) Einfluß des Baumartenanteils auf Zuwachsleistung, Wasserhaushalt, Bodenzustand und Stofflüsse eines Fichten-Buchen Mischbestandes am Standort Höglwald. PhD Thesis, University of Munich, Germany

Schär C, Vidale PL, Lüthi D, Frei C, Häberli C, Liniger MA, Appenzeller C (2004) The role of increasing temperature variability in European summer heatwaves. Nature 427:332–336

Schmidt J (1990) Überlegungen zur Erfassung und Beschreibung von Wachstumsgängen am Beispiel der Durchmesserzuwachsentwicklung der letzten Jahrzehnte von Fichtenbeständen in Bayern unter besonderer Berücksichtigung witterungsbedingter Zuwachsreaktionen. Forstl. Forschungsber. München Nr 104:162

Schneider S, Gessler A, Weber P, von Sengbusch D, Hanemann U, Rennenberg H (1996) Soluble N compounds in trees exposed to high loads of N: a comparison of spruce (*Picea abies*) and beech (*Fagus sylvatica*) grown under field conditions. New Phytol 134:103–114

Scholander PF, Hammel HT, Bradstre ED, Hemmings EA (1965) Sap pressure in vascular plants—negative hydrostatic pressure can be measured in plants. Science 148:339–345

Schupp R (1991) Untersuchungen zur Schwefelernährung der Fichte (Picea abies L.): Die Bedeutung der Sulfatassimilation und des Transportes von Thiolen. Wissenschaftsverlag Dr. Wigbert Maraun, Frankfurt/Main 86–90

Sigurdsson BD (2001) Elevated [CO_2] and nutrient status modified leaf phenology and growth rhythm of young *Populus trichocarpa* trees in a 3-year field study. Tree Struct Funct 15:403–413

Spiecker H, Kahle H-P, Hauser S (2001) Klima und Witterung als Einflußfaktoren auf das Baumwachstum in Laubwäldern: Retrospektive Analysen und Monitoring. In: Buchendominierte Laubwälder unter dem Einfluss von Klima und Bewirtschaftung: Ökologische, waldbauliche und sozialwissenschaftliche Analysen. Abschlussbericht des SFB 433. University of Freiburg, Germany pp. 307–334

Tarp P, Helles F, Holten-Andersen P, Larsen JB, Strange N (2000) Modelling near-natural silvicultural regimes for beech—an economic sensitivity analysis. For Ecol Manage 130:187–198

Thibodeau L, Raymond P, Camire C, Munson AD (2000) Impact of precommercial thinning in balsam fir stands on soil nitrogen dynamics, microbial biomass, decomposition, and foliar nutrition. Can J For Res-Revue Canadienne de Recherche Forestiere 30:229–238

Winter H, Lohaus G, Heldt W (1992) Phloem transport of amino acids in relation to their cytosolic levels in barley leaves. Plant Physiol 99:996–1004

Yu S, Chambers J, Tang Z, Barnett J (2003) Crown characteristics of juvenile loblolly pine 6 years after application of thinning and fertilization. For Ecol Manage 180:345–352