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ORIGINAL ARTICLE

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Climate and forest management affect ^{15}N-uptake, N balance and biomass of European beech seedlings

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Abstract We investigated the effect of (a) different local climate and (b) thinning of the forest canopy on growth and N status of naturally regenerated European beech seedlings in a beech forest on shallow rendzina soil in southern Germany. For this purpose, a ^{15}N-tracing experiment was conducted during the growing season of the year 2000 with beech seedlings growing on a warm, dry SW-exposed site and a cooler, moist NE-exposed site, and in a thinned and a control stand at each site. Biomass, ^{15}N uptake and partitioning and total N concentrations of beech seedlings were determined. Site and thinning produced clear differences, particularly at the end of the growing season. Biomass and cumulative ^{15}N uptake of beech seedlings then increased due to thinning on the NE site and decreased on the SW site. Total N concentrations in leaves, roots and stems of beech seedlings responded similarly. Therefore, growth and N status of beech seedlings are found to be favoured by thinning under cool-moist conditions. However, under higher temperature and reduced water availability—conditions that are prognosticated in the near future—thinning reduces N uptake and plant N concentration and, thus, impairs N balance and growth of beech regeneration.

Keywords *Fagus sylvatica* · ^{15}N tracer · N balance · N content · Growth

Introduction

Forest management strategies which aim at transforming conifer-dominated monocultures to species-rich mixed forest stands, are promoted by governments and practitioners in central Europe (Tarp et al. 2000). Such strategies may induce positive responses of forest ecosystems to climate change and are, therefore, increasingly included in climate models (IPCC 2001). The support of the natural regeneration of European beech (*Fagus sylvatica* L.), the most important deciduous tree species of the potential natural vegetation in central Europe (Ellenberg 1992), is one of the priorities of forest policy (Dertz 1996; Ministerium für Ländlichen Raum, Ernährung, Landwirtschaft und Forsten in Baden Württemberg 1997).

A common silvicultural method to improve natural regeneration is thinning of the closed forest canopy by means of selective felling. Thinning improves abiotic conditions (light intensity, nutrient and water availability, temperature; e.g. Aussenac 2000; Mizunaga 2000; Thibodeau et al. 2000) for tree seedlings and, thus, results in increased nutrient uptake and growth. Consistent with this, European beech is favoured by gap formation (Johnson et al. 1997), although it is a late colonising species, capable of persisting as small tree for longer periods in the forest understorey. However, under unfavourable climatic conditions, such as dry periods during the growing season, thinning may also reduce soil water availability in forests (Čermak et al. 1993). In general, European beech is sensitive to drought and its geographic distribution is limited by water availability (Ellenberg 1992). Owing to this sensibility, thinning resulted in reduced transpiration and stomatal conductance of adult beech trees (Čermak et al. 1993; Geßler et al. 2001) and beech seedlings (Fotelli et al. 2003) under dry conditions.

Beside its direct effects on water balance of trees, water shortage was also found to impair pedospheric N uptake by beech seedlings under controlled conditions (Fotelli et al. 2002b). The reduction of N uptake was intensified when beech shared finite N resources with other co-existing plants (Fotelli et al. 2002b). Nitrogen is—next to carbon, oxygen and hydrogen—the element with the highest abundance in plants (Marschner 1995).

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Since N is a growth limiting factor in natural forest ecosystems not exposed to excess atmospheric N of anthropogenic origin (Rennenberg et al. 1998), changes in N uptake should influence the growth patterns of young seedlings (Marschner 1995; Fotelli et al. 2002b).

The effects of reduced water availability and the varying interactions between water and N balance will be of major importance for the success of beech regeneration in the near future, since actual climate models for central Europe predict prolonged summer droughts (e.g. IPCC 1997, 2001). Among the ecosystems that will be most affected by drought are natural European beech stands growing on shallow, low water capacity-rendzina soils derived from limestone. These forest stands are common in central and southern Europe (Schwäbische and Fränkische Alb, French Jura, Swiss Jura, several areas in northern and central Greece).

Currently, additional information based on physiological studies is necessary in order to (1) reliably predict the progress of re-establishment of young beech on shallow, limestone-derived soils, and (2) evaluate management strategies, particularly the manipulation of stand density, in the light of climate change. A first approach showed that a thinning treatment (50–70% reduction of the basal area of a beech stand) resulted in changes in the concentration and composition of soluble N compounds in beech seedlings, which indicated a thinning-induced improvement of the plant internal N status at a cool-moist site, and an impairment at a dry-warm site (Fotelli et al. 2002a). With the present study we aimed at substantiating these preliminary findings.

For that reason, we assessed the effect of a silvicultural treatment under two different climatic conditions (warm, dry vs cold, moist) on the main aspects of N balance of beech seedlings: (1) on ^{15}N uptake and incorporation into leaves, stems and roots, (2) on total N concentrations in different tissues above and below ground, and (3) on biomass accumulation and partitioning.

Our initial hypothesis was that thinning increases N uptake and biomass of beech seedlings under cool, moist climatic conditions whereas it has the opposite effects under warm, dry conditions.

Materials and methods

Site characteristics

The experimental sites are located in southern Germany (longitude: 8°40′E; latitude: 48°00′N), about 100 km south-southwest from Stuttgart in a low mountain range (Schwäbische Alb, 740–760 m a.s.l.). Mean annual regional air temperature measured at a climate station of the DWD (Deutscher Wetterdienst, Offenbach, Germany), about 4 km from the experimental sites, is about 6.6°C, and mean temperature during the growing season (May–October) about 11.5°C. Average annual precipitation is 856 mm with monthly maxima in June and July and the sum of precipitation during the growing season (May–October) amounts to 410 mm. During the growing season of the year 2000 the sum of precipitation was about the average, but mean air temperature was higher than long-term averages (Table 1).

The experimental sites are located on the two opposite-exposed sides (not more than 1,000 m apart) of a narrow valley. One experimental site faces to north-east (NE) and the other to south-west (SW). Rainfall does not vary significantly across the valley (Geßler et al. 2001). The slope at the SW-exposed site is very steep (36–58%) and at the NE-exposed site precipitous (NE: 58–100%). Soil profiles are characterized as Terra fusca-Rendzina derived from limestone (Weißjura beta and gamma series) and are shallow on both sites, averaging ca. 0.25 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 between 20% and 45% (volumetric basis) rocks and stones (>63 mm diameter) in the top 0.20 m of the soil, rising to 80% below 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 surface organic layer and 7.5 at 0.60 m depth.

On both sites beech (*Fagus sylvatica* L.) is the dominant species making up >90% of the total basal area of adult trees. The average age of the adult beech trees is 70–80 years. The difference in aspects (NE or SW) produces a difference in radiation interception at the canopy level. The maximum daily radiation above the canopy of adult trees on the NE site amounts to 79% and 47% of the radiation available on the SW site in July and October, respectively (Geßler et al. 2001). On the forest floor (1.5 m above ground) PAR during the growing season did not vary greatly between the SW- and NE-exposed sites (Table 1; control plots). The differences in radiation at canopy level, however, result in higher air and soil

Table 1 Climate and soil characteristics of the forest stands used for the purposes of the present study, during the growing season (May–September) 2000. Climatic parameters including PAR were recorded at four stations ca. 1.5 m above the forest floor. Soil water potential was measured with tensiometers in a soil depth of 0.20 m (Source: Meteorological Institute, and Institute for Soil Science and Forest Nutrition, University of Freiburg). *NEC* north-east aspect, control; *NET* north-east aspect, treatment; *SWC* south-west aspect, control; *SWT* south-west aspect, treatment

Site exposure Treatment	NE		SW	
	C	T	C	T
Sum of throughfall[a] (mm)	382.00	447.99	409.94	442.49
Mean daily T_{soil} at 0.05 m depth[b] (°C)	13.03	14.28	13.91	15.06
Mean daily T_{soil} at 0.10 m depth[b] (°C)	12.91	14.07	13.73	14.84
Mean daytime Rh[b] (%)	74.30	75.05	72.26	70.85
Mean midday T_{air} [b] (°C)	17.13	17.62	18.32	19.16
Mean daily T_{air} [b] (°C)	13.29	13.21	13.34	13.49
Mean daylight PAR[b] (μmol m^{-2} s^{-1})	25.69	121.69	20.33	176.09
Mean daily soil water potential[b] (MPa)	–0.019	–0.005	–0.033	–0.022

[a] Sum of throughfall (mm) refers to the amount of precipitation passing through the canopy of adult trees and reaching the forest floor

[b] All means shown are averages over the entire growing season 2000; the values of T_{soil}, and soil water potential are averages of mean daily values (24 h), while the values of RH are averages of mean daytime values (measured from 0800 hours until 2000 hours). The values of T_{air} are averages of mean midday temperatures, measured from 1200 hours until 1730 hours. The values of PAR are averages of mean PAR, measured during daylight

Table 2 Mean daily soil water potential and concentrations of nitrate in the soil (mean of organic layer at depth 0 m and mineral soil at depth 0.20 m), averaged over the months the field trials were conducted during the growing season 2000 (May, July, and September). Soil water potential was measured with tensiometers in a soil depth of 0.20 m. The values of soil water potential shown are means of two samples (Source: Institute for Soil Science and Forest Nutrition, University of Freiburg). The values of nitrate concentration shown are means of three samples ± S.E. (Source: Institut für Meteorologie und Klimaforschung-Atmosphärische Umweltforschung (IMK-IFU), Forschungszentrum Karlsruhe). *NEC* north-east aspect, control; *NET* north-east aspect, treatment; *SWC* south-west aspect, control; *SWT* south-west aspect, treatment

Time Site and treatment	May				July				September			
	NEC	NET	SWC	SWT	NEC	NET	SWC	SWT	NEC	NET	SWC	SWT
Soil water potential (MPa)	–0.015	–0.004	–0.037	–0.029	–0.025	–0.004	–0.037	–0.046	–0.018	–0.004	–0.040	–0.040
Mean NO_3^- (μg N g^{-1} d.w.)	5.73 (1.01)	14.97 (2.34)	6.55 (0.88)	3.57 (0.89)	1.37 (0.42)	2.18 (0.79)	4.44 (0.90)	2.49 (1.28)	1.74 (1.10)	5.18 (2.70)	7.28 (2.62)	3.53 (1.13)

temperatures and lower water availability for the vegetation on the SW site (Table 1). According to retrospective analyses of meteorological data (Geßler et al. 2001), as well as of growth and water status of adult beech trees, the SW-exposed site has permanently lower water availability and higher air temperatures than the NE-exposed site. This difference in soil water availability between the two sites was further verified by the values of soil water potential during the 2000 growing season (Table 2).

On each site, a silvicultural treatment (thinning) was established in March 1999. The experimental design consisted of two blocks, each containing a single plot (each approx. 0.53 ha in size) of the silvicultural treatment plus a control plot (Geßler et al. 2001). The total basal area (BA) of the mature beech trees was reduced from ca. 27 m^2 ha^{-1} on the NE site and 20 m^2 ha^{-1} on the SW site (controls), to 10 m^2 ha^{-1} on both sites. Furthermore, the leaf area index [LAI (m^2 m^{-2})] of the stand was reduced as consequence of thinning from 5.16 to 1.68, and from 5.12 to 2.12, on the NE and the SW site, respectively. One year after thinning, the density of understorey vegetation, other than natural regeneration of beech, increased in the thinned stands, compared to the controls (ca. 25% on the NE site, and ca. 8% on the SW site; Paul and Reif, personal communication). Thinning resulted, as expected, in increased amount of through-fall, i.e. precipitation reaching the forest floor (Table 1). Moreover, PAR was increased at the forest floor in the thinned stands on both NE and SW sites and was markedly higher on the SW site (Table 1). This enhancement in radiation caused daily mean temperatures of air and soil in the thinned stands to be consistently higher than in the control stands (Table 1). Thinning also resulted in higher concentrations of nitrate in soil on the NE-exposed site and lower concentrations on the SW-exposed site, throughout the growing season (Table 2).

^{15}N application

In general, preferential uptake of ammonium by roots of other woody plants (Plassard et al. 1991; Kronzucker et al. 1996) and of beech (Geßler et al. 1998a) is reported. Ammonium availability at the studied field site is, however, relatively low (E. Hildebrand, personal communication), since the soil pH >5.5 causes high nitrification rates. Therefore, a labelled $NH_4{}^{15}NO_3$ (98% enrichment) solution was applied in the field on 30 April 2000, ca. 1 week before bud-break. In each treatments (thinning, control) on both aspects (NE, SW) 24 non-neighbouring beech seedlings of the natural regeneration were selected for ^{15}N-application. The seedlings were between 2 and 3 years old and were representative for each site. In order to ensure slow and uniform tracer application, small tubes (diameter 0.005 m), forming a circle of a ca. 0.15 m radius, were placed around the selected beech seedlings, as well as any neighbouring vegetation grown within this circle. From each tube-circle tracer solution was dripped onto the soil with a rate of 2 l/h through three equally-distanced applicators. The amount of 29.7 mg $NH_4{}^{15}NO_3$ (420 mg/m^2 soil surface) was applied within each circle in order to increase mineral soil nitrogen content by not more than 10%. This calculation was based on the mean nitrate (Table 3; 4.92 μg N g^{-1} soil d.w.) and ammonium (3.20 μg N g^{-1} soil d.w.; H. Papen and R. Gasche, personal communication) contents of the topsoil of both aspects and treatments averaged over the growing season and on the mean soil density (0.96 g soil d.w. cm^{-3}; S. Augustin, personal comm.). Depth of the topsoil is on average 0.25 m (see above), and a mean volumetric stone and rock content of 25% was assumed for computation.

Table 3 Plant species comprising the two functional groups of vegetation surrounding the studied beech seedlings. All neighbouring vegetation was harvested during each field trial from a circle of ca. 0.15 m radius around each studied beech seedling. The functional group *woody vegetation* consisted of tree seedlings and shrub species. Mainly perennial herbs, with the exception of *I. parviflora* (annual) made up the group of "herbaceous vegetation". On average, 1–2 woody plants and 1–4 herbaceous plants were present within each ca. 0.15 m-radius-circle around the studied beech seedlings

Woody vegetation	Herbaceous vegetation
Acer platanoides L.	*Anemone nemorosa* L.
Acer pseudoplatanus L.	*Asarum europeum* L.
Cornus sanguinea L.	*Fragaria vesca* L.
Fraxinus excelsior L.	*Galium odoratum* (L.) Scop.
Prunus avium L.	*Impatiens parviflora* Dc.
Rubus fruticosus Agg.	*Mycelis muralis* (L.) Dumort
Rubus idaeus L.	*Oxalis acetosela* L.
Sambucus nigra L.	*Paris quadrifolia* L.
Sambucus racemosa L.	*Viola hirta* L.
Ulmus glabra Huds.	*Viola mirabilis* L.
	Viola reichenbachiana Jordan Ex Boreau
	Urtica dioica L.

Plant and soil material

Plant material of beech seedlings, as well as of numerous understorey species present at the field site, was collected once 2 weeks before ^{15}N tracer application, to determine natural δ^{15}N abundance of all tissues. Thereafter, plant material was selected at three dates within the growing season in 2000: in May after bud-break, in July in mid-summer, and in September at the end of the growing season. At each date, 6 beech seedlings per aspect and treatment were randomly selected among the 24 labelled ones and harvested. In addition, all plants present within a radius of 0.15 m around each beech seedling were also harvested.

Since beech seedlings were randomly selected they were surrounded by different neighbouring species at each date of harvest. Moreover, the composition of herbaceous species changed during the growing season. For that reason, the species from the adjacent vegetation were divided into two functional groups, i.e. woody plants (other than beech) and herbs (Table 3). Grouping criteria were total dry biomass (woody plants: 3–4.6 g; herbs: 0.2–0.8 g) and leaf transpiration rates at 09:00 a.m. (woody plants: 1.1–1.4 mmol m^{-2} s^{-1}; herbs: 1.5–2.1 mmol m^{-2} s^{-1}), measured at the beginning of the growing season. The entire plants were harvested at each date. Roots of all species were collected from a soil depth between 0 and 0.25 m. Given that extensive parts of parent rock material dominated the soil below 0.25 m, the entire root biomass of understorey species is assumed to be restricted to the soil layer taken into consideration.

The plant material of the beech seedlings was divided into leaves, roots and stem. All these plant parts together with the harvested neighbouring plants were oven dried (3 days, 65°C).

Soil samples were collected as mixed samples from a depth range of 0–0.25 m (a) immediately before tracer application, for the determination of natural soil $\delta^{15}N$ signatures, and (b) at the three harvest dates (May, July September). All soil samples were oven dried for 3 days at 65°C.

Determination of soil $\delta^{15}N$, plant ^{15}N uptake, and total N contents

For determination of ^{15}N abundance (atom % or $\delta^{15}N$) and total N, oven-dried soil and plant samples were homogenised and aliquots of 1–2 mg were transferred into tin capsules (Type A; Thermo Quest, Egelsbach, Germany). They were injected into an elemental analyser (NA 2500; CE Instruments, Milan, Italy) coupled with a Conflo II-Interface (Finnigan MAT, Bremen, Germany) to an isotope ratio mass spectrometer (Delta Plus; Finnigan MAT, Bremen, Germany). Specific ^{15}N incorporation (μmol g^{-1}) was calculated using the following equation:

$$^{15}\text{Nincorporation} = \frac{(^{15}N_t - {}^{15}N_c) \cdot [N] \cdot 10^4}{MW} \quad (1)$$

where $^{15}N_t$ and $^{15}N_c$ is the ^{15}N abundance (atom %) in ^{15}N treated and control plants (before ^{15}N application), [N] is the total N concentration (%N g^{-1} dry weight), and MW is the molecular weight of ^{15}N (g mol^{-1}). The calculation of total ^{15}N incorporation per plant was based on the specific ^{15}N incorporation and the respective plant biomass. The cumulative amount of ^{15}N taken up was determined for three time intervals, beginning at date of ^{15}N tracer application (April) and ending with plant harvest in May, July and September.

Data analysis

All statistical analyses were carried out using SPSS 10.05 (SPSS, USA). The effects of aspect (NE, SW) and treatment (thinning, control) on the different parameters were assessed for each date (May, July, September) separately, using a 2-way ANOVA procedure. Statistically significant differences among the four combinations of treatments (NE-thinned, NE-control, SW-thinned, SW-control) at each date were assessed with a one-way ANOVA procedure and the use of the Tukey HSD post-hoc test.

Results

Above- and below-ground biomass

Figure 1 shows the seasonal patterns of above- and below-ground dry biomass of beech seedlings, as affected by aspect and thinning treatment. In May, significantly higher above-ground biomass of beech seedlings on the NE site—particularly in the NE-thinned stand—compared to the SW was observed. In July, the highest above-ground biomass was also found in seedlings of the NE-thinned stand. In September, clear-cut differences among seedlings grown in the thinned and in the control stand were observed on each aspect. Above-ground biomass was comparable between both control treatments. Compared to the control stand above-ground biomass was significantly higher in the thinning treatment on the NE aspect, whereas it was lower in the same treatment on the SW aspect.

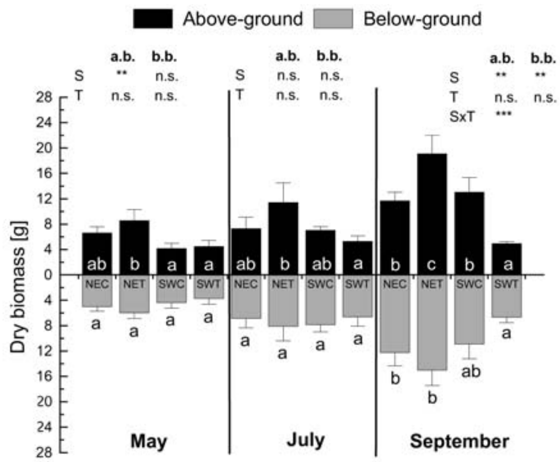

Fig. 1 Total dry biomass and its partitioning above (*black bars*) and below ground (*light grey bars*) in European beech seedlings grown in forest stands subjected to thinning (*T*) and in control (*C*) stands at two opposite exposed sites (NE vs SW). *, ** *or* *** correspond to site (*S*) or thinning (*T*) effects on a 95%, 99% or 99.9% significance level for above-ground (*a.b.*) and below-ground biomass (*b.b.*). Statistically significant differences among the four combinations of treatments at each time point (May, July, September) are indicated with letters (*white letters* above-ground biomass, *black letters* below-ground biomass). Two means are different at a 95% level of significance when they share no common letter. Values shown are means (+SE) of 6 seedlings

Patterns of below-ground biomass were comparable to above-ground biomass. However, significant differences in the root biomass of beech seedlings among aspects and treatments were detected only in September.

^{15}N Uptake

Analysis of $\delta^{15}N$ signature of the soil showed that ca. 15 days after tracer application $\delta^{15}N$ increased from ca. 10 to 30–35‰ (Fig. 2). $\delta^{15}N$ remained at about the same level for the next 2 months without differences between aspects and silvicultural treatments and decreased again to 12–16‰ (SW-exposed site) and 22–27‰ (NE-exposed site) until September.

The cumulative specific ^{15}N uptake (μmol ^{15}N g^{-1} d.w.) of beech seedlings and the cumulative ^{15}N uptake per plant, from the time of N application in April until May, July and September are given in Fig. 3a, b. Until May, no significant effects of site and/or thinning were observed on the specific ^{15}N uptake rate (Fig. 3a). This

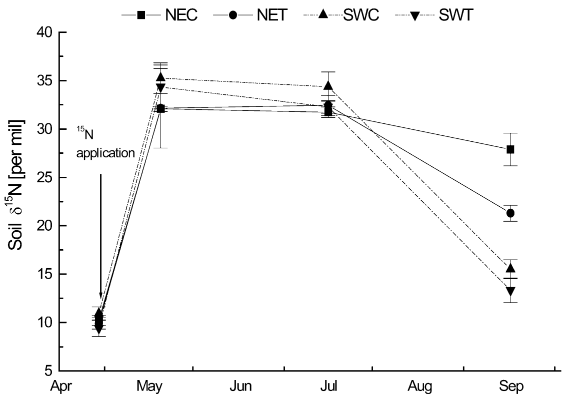

Fig. 2 Patterns of ^{15}N abundance in the soil. Soil samples were obtained (1) before the application of a ^{15}N-enriched solution, in order to provide the level of ^{15}N natural abundance, and (2) during each field trial as mixed sample from a soil depth between 0 and 0.15 m. The values shown are means (+SE) of 6 samples at each time point

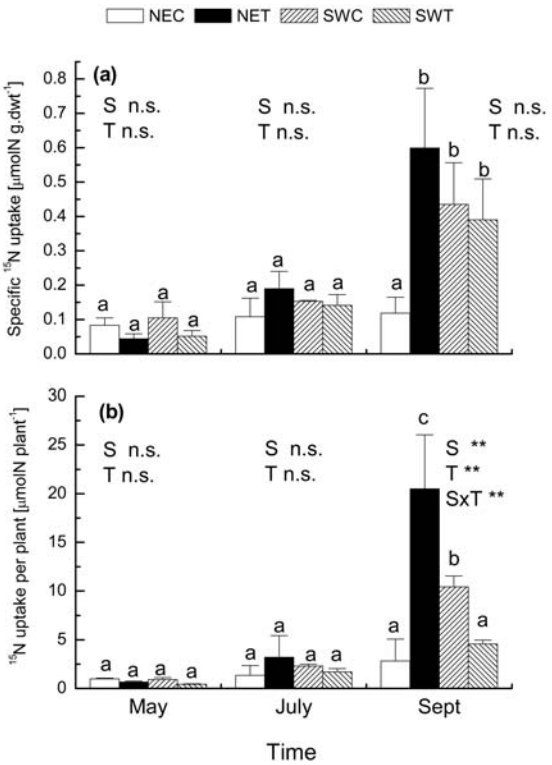

Fig. 3 Cumulative ^{15}N uptake of European beech seedlings grown in forest stands subjected to thinning (*T*) and in control (*C*) stands at two opposite exposed sites (NE vs SW) on a g dry weight (**a**) and a per plant (**b**) basis. ^{15}N tracer application was carried out on 30 April 2000 and the accumulation of ^{15}N in the biomass of beech seedlings was determined in May, July, and September. *, ** *or* *** correspond to site (*S*) or thinning (*T*) effects on a 95%, 99% or 99.9% significance level. Statistically significant differences among the four combinations of treatments at each point of time (May, July, September) are indicated with letters. Two means are different at a 95% level of significance when they share no common letter. Values shown are means (+SE) of 6 seedlings

pattern did not change in July. The cumulative amount of ^{15}N taken up until July increased—as compared to May—for all sites and treatments. Neither aspect nor treatment had a significant effect on specific ^{15}N uptake until September. However, compared to July, the amount of ^{15}N taken up was drastically higher in both, thinned and control stands on the SW aspect and in the NE-thinned stand. During the entire growing season the highest specific ^{15}N uptake was observed for seedlings in the thinned stand on the NE aspect, whereas a ca. 5-fold lower uptake was detected in the NE-control stand.

On a per plant basis, low ^{15}N accumulation was observed until May and July with no significant effects of site and thinning (Fig. 3b). Between July and September, a 6.4- and a 4.6-fold increase in cumulative ^{15}N uptake per plant was observed in the NE-thinned stand and in the SW-control stand. Moreover, in September thinning resulted in a significantly higher ^{15}N accumulation per plant on the NE aspect and in a significantly lower one on the SW aspect.

Incorporation of the ^{15}N into beech seedlings and neighbouring understorey vegetation

Figure 4 shows the ^{15}N incorporation into total plant biomass that was located within a 0.15 m radius around each beech seedling studied, where the ^{15}N tracer had been applied. In addition, the relative partitioning of this amount of ^{15}N between beech seedlings and the two functional groups of neighbouring vegetation (i.e. woody plants, other than beech, and herbs) and the biomass distribution between the groups is shown. In May, between 35 and 70% of ^{15}N accumulated in plant biomass was found in beech seedlings whereas they contributed to between 60 and 88% of plant biomass. In woody plants different from beech and in herbaceous vegetation ca. 13–56% and 4–18% of ^{15}N was accumulated. In July, the relative importance of herbaceous vegetation for ^{15}N accumulation increased and resulted in incorporation of ca. 40% of ^{15}N in all treatments. Thus, the share of herbaceous species in ^{15}N accumulation was higher than their relative contribution to biomass (10–27%). Beech and woody species contributed 32–55% and 5–30% to total ^{15}N incorporation in biomass. The portion of ^{15}N accumulated in herbs decreased to 1–5% in September—comparable to their contribution to biomass (1–6%) and the relative accumulation in beech increased. Generally, the main part (65–90%) of ^{15}N accumulated in plant biomass throughout the growing season (April–September) was found in beech seedlings.

Between 0.2–1.2% of the ^{15}N applied to the soil in April was recovered in plant material at the end of the growing season.

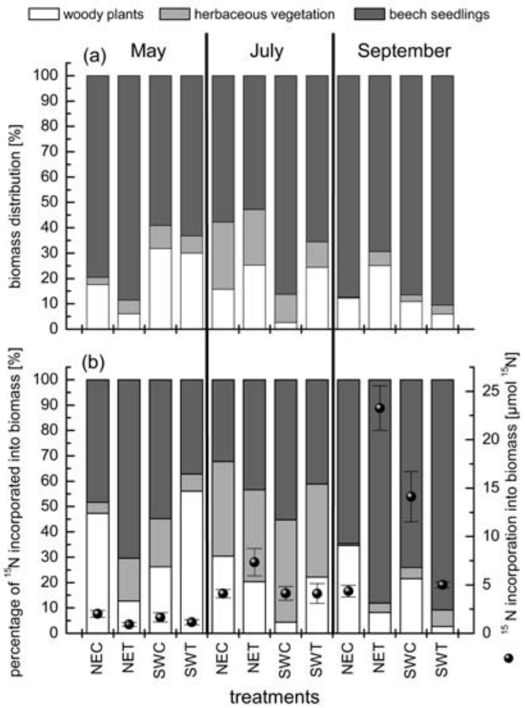

Fig. 4 Biomass distribution between functional groups (**a**), ^{15}N incorporation (●) into the total plant biomass and relative partitioning of this amount of ^{15}N among beech seedlings and neighbouring vegetation, in May, July, and September (**b**). The beech seedlings were located in forest stands subjected to thinning (*T*) and in control (*C*) stands on two opposite exposed aspects (NE vs SW). The neighbouring vegetation was divided into two functional groups i.e. woody species, other than beech, and herbs. Values shown are means of 6 beech seedlings, and 6–12 woody and 6–24 herbaceous neighbouring plants

^{15}N Partitioning between above- and below-ground biomass

Figure 5 shows the relative partitioning of ^{15}N in aboveground biomass (leaves and stems) and in roots of beech seedlings. The effect of site and thinning on ^{15}N partitioning between up- and below-ground parts was comparable during the entire growing season. Seedlings grown on the SW aspect tended to incorporate more ^{15}N below ground, compared to those grown on the NE aspect. Moreover, thinning resulted in higher incorporation of ^{15}N in the roots on the NE aspect, while the opposite effect was observed on the SW aspect.

In May, most of the ^{15}N incorporated in above ground tissues was found in the stems on the NE and in the leaves on the SW aspect. Compared to July, in September a higher relative proportion of ^{15}N taken up was recovered in the stems independent of site and aspect.

Total N concentration

Total N concentration in leaves, roots and stems was mainly affected by aspect at the beginning of the growing

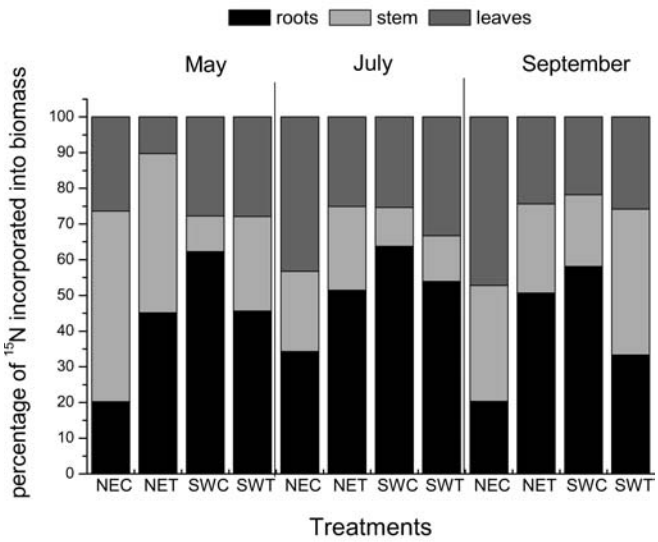

Fig. 5 Relative partitioning of the cumulative amount of ^{15}N incorporated in beech seedlings' biomass between ^{15}N application in 30 April 2000 and May, July, and September, between above- and belowground biomass. The seedlings were grown in forest stands subjected to thinning (*T*) and in control (*C*) stands at two opposite exposed sites (NE vs SW). Values shown are means of 6 seedlings per site and treatment

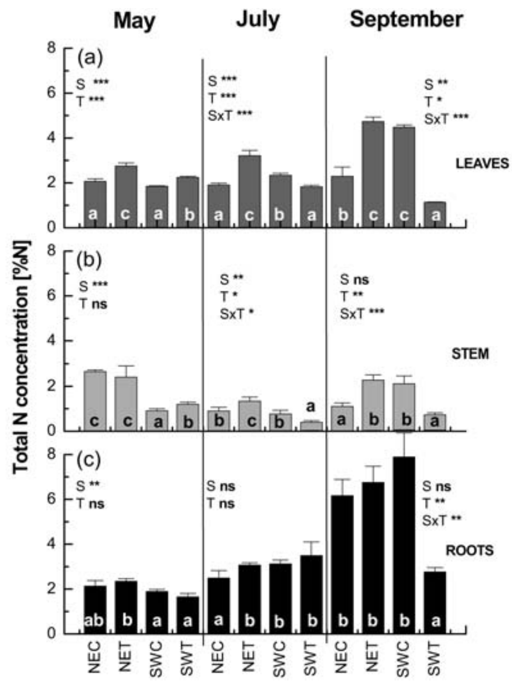

Fig. 6 Total N concentrations in leaves (**a**), stems (**b**) and roots (**c**) of European beech seedlings grown in forest stands subjected to thinning (*T*) and in control (*C*) stands at two opposite exposed sites (NE vs SW). Samples were obtained in May, July and September 2000. *, ** *or* *** correspond to site (*S*) or thinning (*T*) effects on a 95%, 99% or 99.9% significance level. Statistically significant differences among the four combinations of treatments at each time point (May, July, September) are indicated with letters. Two means are different at a 95% level of significance when they share no common letter. Values shown are means (+SE) of 6 seedlings

season in May (Fig. 6) with significantly lower values on the SW compared to the NE aspect. In July, the thinning treatment affected aboveground (leaf and stem) total N concentration in different ways depending on aspects. On the NE aspect thinning resulted in significantly increased, on the SW aspect in significantly decreased total N concentrations. In September, this same pattern was observed not only for leaves and stems but also for roots.

Discussion

The site studied is regarded as a model site for assessing climate change effects on beech

We investigated the effects of thinning and different local climatic conditions (air and soil temperature, radiation, water availability), as modulated by differences in exposure (SW vs NE), on biomass, total N contents, and pedospheric ^{15}N uptake of beech seedlings. Previous studies revealed that the SW-exposed site received more radiation, leading to higher air and soil temperatures (Table 1), and had lower soil water storage capacity, compared to the NE site (Geßler et al. 2001). Due to these environmental conditions, the water status of young and adult beech trees on the SW aspect was unfavourable during periods of low rainfall in summer, whereas water supply was sufficient throughout the growing season on the NE aspect (Geßler et al. 2001; Fotelli et al. 2003). Since temperatures, as well as the frequency and duration of summer droughts are expected to increase in central Europe in future (IPCC 2001), the experimental sites are regarded as suitable for assessing climate change effects on the natural regeneration of beech (Fotelli et al. 2002a).

Biomass accumulation of beech seedlings is increased by thinning on the NE and decreased on the SW aspect

On the cooler and moist NE site, thinning resulted in significantly increased total biomass of beech seedlings in September, at the end of the growing season (Fig. 1). This response can be attributed to the higher radiation and, thus, higher C assimilation, higher air and soil temperatures (Table 1) under the thinned forest canopy, and is in agreement with numerous studies performed with beech seedlings (e.g. Madsen 1995; Gemmel et al. 1996; Madsen et al. 1997; Welander and Ottoson 1997). In contrast, on the warmer and drier SW aspect biomass accumulation of beech seedlings in September was significantly lower in the thinned stand, compared to the control (Fig. 1). This indicates that opening of the canopy on the SW site worsened growth conditions for seedlings, as already shown for adult beech at the same sites by Geßler et al. (2001). Thinning—which in general increases soil water availability per plant (e.g. Breda et al. 1995)—can reduce soil water availability in the uppermost soil layers of already dry sites. As a result vitality of beech can be reduced during dry periods (e.g. Čermak et al. 1993). Reduced water availability was found to substantially decrease growth of beech seedlings under controlled conditions (Fotelli et al. 2001) and in the field (Madsen et al. 1997; Lof 2000).

^{15}N uptake shows aspect and treatment-dependent patterns comparable to biomass

^{15}N tracer application revealed that ^{15}N uptake per plant by beech seedlings on the NE aspect was steadily low in the control stand, while it was substantially increased in the thinned stand up to the end of the growing season (Fig. 3b). This increase was even higher than the respective biomass increase (Fig. 1) and is, therefore, partially attributed to enhanced specific ^{15}N uptake (Fig. 3a). Consistent to the pattern of biomass, ^{15}N uptake per plant by beech seedlings on the SW site was lower in the thinned stand, compared to the control, particularly at the end of the growing season (Fig. 3b). This decrease was, however, mostly a consequence of reduced growth in the thinned stand, as indicated by comparable specific ^{15}N uptake between seedlings of the control and the treatment (Fig. 3a).

^{15}N uptake rates were representative for total N uptake of the different functional groups

The natural soil $\delta^{15}N$ of 10‰ (Fig. 2) is rather high but is within the range of $\delta^{15}N$ of soil described in literature (e.g. Hopkins et al. 1998). Comparable $\delta^{15}N$ values (>7‰) were observed for example by Bauer et al. (2000) for the soil of a spruce ecosystem with comparably high soil pH.

The differences in ^{15}N uptake among treatments at the end of the growing season could not be attributed to differences in ^{15}N enrichment of total N in the soil (Fig. 2). On the SW site, ^{15}N uptake differed significantly between treatments, even though $\delta^{15}N$ of soil N was comparable. On the NE aspect, soil $\delta^{15}N$ was significantly lower in the thinned stand, compared to the control. However, ^{15}N accumulation in beech was 5 times higher in the thinned stand, compared to the control (Fig. 3). Consistent to other tracing experiments, only a very small part of the ^{15}N applied was recovered from plants, while a greater portion remained in the soil (Gebauer et al. 2000; Zeller et al. 2000). Hence, the strong decline in $\delta^{15}N$ of total soil N between July and September could not only be attributed to plant uptake, but most likely also to leaching of ^{15}N into ground water, denitrification processes (Rennenberg et al. 2001) and dilution of soil ^{15}N-nitrate by nitrification. Since we can not exclude, that such a dilution of soil ^{15}N-nitrate was different between sites and treatments, it is possible that also the ^{15}N-labelling of the plant available nitrate pools of the soil differed. In a previous study (Fotelli et al. 2002a) it was clearly shown that the contents of particular amino compounds in roots

(Glu) and leaves (Gln, Asn) are indicators for the N status of seedlings and reflect soil nitrate availability and uptake. Correlation analyses performed between specific cumulative ^{15}N uptake of beech seedlings from the different sites and treatments during the whole growing season (May–September; Fig. 3a) and the contents of the particular amino compounds in September (Data from Fotelli et al. 2002a) produced high correlation coefficients (Glu in roots: R =0.71; Gln in leaves: R =0.71, Asn in leaves: R =0.77). In addition, linear regression analysis between ^{15}N uptake on a plant basis (Fig. 3b) and total N accumulation calculated on the basis of biomass increase (Fig. 1) and total N content of beech biomass (Fig. 6) produced a high and significant R^2 (0.809; P <0.001) when all sites, treatments and times of measurement were included. Thus, there is reason to assume that soil N taken up by beech seedlings was comparably ^{15}N-labelled at different aspects and treatments during the growing season, and that—as a consequence—^{15}N uptake rates were representative for total N uptake of beech, woody species different from beech and herbaceous vegetation.

Herbaceous plants are strong competitors for soil N in summer

Plants of different species or functional groups have to share finite amounts of a resource with other species. A comparison of ^{15}N uptake between functional groups showed that herbaceous species were a substantial sink for ^{15}N only in July (Fig. 4). Then, their share in ^{15}N accumulation exceeded their relative contribution to biomass by the factor 1.5–4. It is assumed that herbaceous species are effective competitors for nitrogen in the understorey during mid-summer. However, owing to their [relative (Fig. 4) and absolute (data not shown)] biomass decrease until autumn, large proportions of the N incorporated are released again and available for other species. Woody plants different from beech were a substantial sink at the beginning of the growing season but the relative amount of ^{15}N accumulated in woody plants decreased during the growing season (Fig. 4). In September, when the most pronounced site and thinning effects on ^{15}N uptake by beech seedlings where observed (Fig. 3a, b), the major part of ^{15}N accumulated in plant biomass was taken up by beech seedlings (Fig. 4) independent of site or treatment.

Decreased ^{15}N uptake leads to a shift in ^{15}N partitioning between above- and below-ground tissues in beech

In the treatments where beech seedlings showed the highest ^{15}N uptake rates (NET, SWC; Fig. 3), most ^{15}N was incorporated into the roots (Fig. 5). However, this could not be attributed to a higher below-ground, compared to above-ground, biomass in beech seedlings of these treatments (Fig. 1). On the other hand, only between 20 and 34% of ^{15}N taken up was recovered in the roots of seedlings growing on the NE control site were ^{15}N uptake was lowest. Consistent to this pattern, is the finding of Fotelli et al. (2002a) that beech seedlings subjected to controlled low water supply incorporated a higher portion of ^{15}N to the shoots, when N uptake declined. This was attributed to decreased amounts of organic N re-allocated from the shoot to the roots via phloem transport when external N supply was low. The opposite finding—higher N allocation to roots with decreasing N supply—by Maillard et al. (2001) for pedunculate oak seedlings may be due to species-specific differences in strategies to cope with reduced N availability.

The higher relative amount of ^{15}N incorporated into leaves on the SW compared to the NE aspect in May (Fig. 5) may be due to the earlier leaf development on the warmer and drier aspect (Kirchgäßner 2002). The increased amounts of ^{15}N incorporated into the stems of seedlings from all aspects and treatments in September must be attributed to over-winter storage of ^{15}N-labelled compounds in the woody parts of the plants (Millard 1996).

Thinning leads to decreased N concentrations in the storage tissues of beech growing on the warm, dry site at the end of the growing season

The aspect-specific effect of thinning on total N concentrations in roots, stem and leaves in September (Fig. 6), is comparable to the effect on biomass and ^{15}N uptake (Figs. 1, 3). In autumn, there was a very strong decrease in N concentrations in stems and roots—the main over-winter N storage sites of beech (Millard 1996; Geßler et al. 1998b)—of seedlings from the SW site as a consequence of thinning. This observation indicates that thinning may have negative long-term consequences on the N status of beech seedlings under warmer, drier climatic conditions, since they depend to a great extent on stored N for new growth (Zeller et al. 2000; Dyckmans and Flessa 2001), particularly under unfavourable environmental conditions (Fotelli et al. 2002a).

Conclusions

The results of this study confirm our initial hypothesis and show that the effect of thinning on the N balance and growth of beech seedlings depends on the local climatic conditions. On cool, moist aspects, representative for the current climatic and edaphic conditions in beech forests in central Europe, thinning enhances growth, N uptake and N concentrations in beech, by increasing radiation and temperature. This response is consistent with published literature and long-term experience of forest practitioners. However, attention should be paid to the fact that thinning could impair beech regeneration under warmer and drier conditions, such as those expected in future (e.g. IPCC

2001), by further increasing temperature and decreasing water availability. These unfavourable growth conditions were pronounced, in our study, in terms of decreased N uptake, N concentration and growth of beech regeneration.

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References

Aussenac G (2000) Interactions between forest stands and microclimate: ecophysiological aspects and consequences for silviculture. Ann For Sci 57:287–301

Bauer GA, Gebauer G, Harrison AF, Hoegberg P, Hoegbom L, Schinkel H, Taylor AFS, Novak M, Buzek F, Harkeness D, Persson T, Schulze E-D (2000) Biotic and abiotic controls over ecosystem cycling of stable natural nitrogen, carbon and sulphur isotopes. In: Schulze E-D (ed) Carbon and nitrogen cycling in European forest ecosystems. Springer, Berlin Heidelberg New York, pp 189–214

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

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

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

Dyckmans J, Flessa H (2001) Influence of tree internal N status on uptake and translocation of C and N in beech: a dual ^{13}C and ^{15}N labeling approach. Tree Physiol 21:395–401

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

Fotelli MN, Geßler A, Peuke AD, Rennenberg H (2001) Drought affects the competition between *Fagus sylvatica* L. seedlings and an early successional species (*Rubus fruticosus*): growth, water status and $\delta^{13}C$ composition. New Phytol 151:427–435

Fotelli MN, Nahm M, 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: ^{15}N 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* L.) regeneration is indicative of recent environmental conditions within the forest understorey. New Phytol 159:229–244

Gebauer G, Zeller B, Schmidt G, May C, Buchmann N, Colin-Belgrand M, Dambrine E, Martin F, Schulze E-D, Bottner P (2000) The fate of ^{15}N-labelled nitrogen inputs to coniferous and broadleaf forests. In: Schulze E-D (ed) Carbon and nitrogen cycling in European forest ecosystems. Springer, Berlin Heidelberg New York, pp 144–170

Gemmel P, Nilsson U, Welander T (1996) Development of oak and beech seedlings planted under varying shelterwood densities and with different site preparation methods in southern Sweden. New For 12:141–161

Geßler A, Schneider S, von Sengbusch D, Weber P, Hanemann U, Huber C, Rothe A, Kreutzer K, Rennenberg H (1998a) 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, Schneider S, Weber P, Hanemann U, Rennenberg H (1998b) 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. New Phytol 138:385–399

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 beech (*Fagus sylvatica*). New Phytol 150:653–664

Hopkins DW, Wheatley RE, Robinson D (1998) Stable isotope studies of soil nitrogen In: Griffiths H (ed) Stable isotopes—Integration of biological, ecological and geochemical processes. Bios Scientific, Oxford, pp 75–88

IPCC (1997) The regional impacts of climate change: an assessment of vulnerability. In: Watson RT, Zinyowera MCA, Moss RH (eds) A Special Report of IPCC Working Group II. Cambridge University Press, Cambridge

IPCC (2001) Climate Change 2001: Impacts, adaptation and vulnerability. In: McCarthy JJ, Canziani OF, Leary NA, Dokken DJ, White KS (eds) Contribution of Working Group II to the Third Assessment report of the Intergovernmental Panel on Climate Change (IPCC). Cambridge University Press, Cambridge

Johnson JD, Tognetti R, Michelozzi M, Pinzautti S, Minotta G, Borgetti M (1997) Ecophysiological responses of *Fagus sylvatica* L. seedlings to changing light conditions. II. The interaction of light environment and soil fertility on seedling physiology. Physiol Plant 101:124–134

Kirchgäßner A (2002) Phänoklimatologie von Buchenwäldern im Südwesten der Schwäbischen Alb. PhD thesis, University of Freiburg

Kronzucker HJ, Siddiqi MY, Glass ADM (1996) Kinetics of NH_4^+ influx in spruce. Plant Physiol 110:773–779

Lof M (2000) Establishment and growth in seedlings of *Fagus sylvatica* and *Quercus robur*: influence of interference from herbaceous vegetation. Can J For Res 30:855–864

Madsen P (1995) Effects of soil water content, fertilization, light, weed competition and seedbed type on natural regeneration of beech (*Fagus sylvatica*). For Ecol Manage 72:251–264

Madsen P, Larsen JB, Skovsgaard JP (1997) Natural regeneration of beech (*Fagus sylvatica* L.) with respect to canopy density, soil moisture and soil carbon content. For Ecol Manage 97:95–105

Maillard P, Guehl J-M, Muller J-F, Gross P (2001) Interactive effects of elevated CO_2 concentration and nitrogen supply on partitioning of newly fixed ^{13}C and ^{15}N between shoot and roots of pedunculate oak seedlings (*Quercus robur*). Tree Physiol 21:163–172

Marschner H (1995) Mineral nutrition of higher plants, 2nd edn. Academic Press, London, pp. 3–6

Millard P (1996) Ecophysiology of internal cycling of nitrogen for tree growth. Z Pflanzenernaehr Bodenk 159:1–10

Ministerium für Ländlichen Raum, Ernährung, Landwirschaft und Forsten in Baden-Württemberg (ed) (1997) Richtlinien zur Jungbestandspflege. MLR, Baden-Württemberg, Stuttgart

Mizunaga H (2000) Prediction of PPFD variance at forest floor in a thinned Japanese cypress plantation. For Ecol Manage 126:309–319

Plassard C, Scheromm P, Moussain D, Salsac L (1991) Assimilation of mineral nitrogen and ion balance in the two partners of ectomycorrhizal symbiosis: data and hypothesis. Experimentia 47:340–349

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, Stoermer H, Weber P, Daum M, Papen H (2001) Competition of spruce trees for substrates of microbial N_2O-production and -emission in a forest ecosystem. J Appl Bot 75:101–106

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 N dynamics, microbial biomass, decomposition, and foliar nutrition. Can J For Res 30:229–238

Welander NT, Ottosson B (1997) Influence of photosynthetic photon flux density on growth and transpiration in seedlings of *Fagus sylvatica.* Tree Physiol 17:133–140

Zeller B, Colin M, Dambrine BE, Martin F, Bottner P (2000) Decomposition of ^{15}N-labelled beech litter and fate of nitrogen derived from litter in a beech forest. Oecologia 123:550–559