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Improvement of growth conditions and gas exchange of *Fagus sylvatica* L. seedlings planted below a recently thinned *Pinus sylvestris* L. stand

Abstract Gas exchange and growth of beech seedlings planted in the understory of a recently thinned pinewood were recorded for 2 years. Relative irradiance was assessed by hemispherical photographs taken just after the thinning. Predawn water potential (Ψ_{pd}), daily gas exchange and chlorophyll fluorescence were measured several times during the two growing seasons. Maximum values of photosynthesis (A_{max}) and stomatal conductance to water vapour (g_{wvmax}) were established from daily data. Maximum quantum efficiency of PS II was recorded at dawn by taking the variable to maximum chlorophyll fluorescence ratio on dark adapted leaves (F_v/F_m). In the middle of each summer, leaf nitrogen content and leaf mass per area were evaluated, and height growth and basal area increment were recorded at the end of the season. The thinning treatment removed half the trees and generated around 10% more available relative irradiance (GLF). This was followed by an increase in net photosynthesis at saturating PPFD (A_{sat}) and in maximum stomatal conductance to water vapour (g_{wvmax}). Moreover, specific leaf mass (SLM) and mass based nitrogen content (N_m) showed higher values for seedlings in the thinned stand. In both years, a positive relationship was established between the area based nitrogen content (N_a) and maximum net photosynthesis (A_{max}). In 1998, a year with a dry summer, seedlings suffered a significant drop in daily A_{max} irrespective of the thinning regime. This was a response to an increase in stomatal limitation to net photosynthesis, g_{wvmax} reaching the lowest value on dates with the highest drought. A lack of decrease of F_v/F_m confirmed the absence of significant non-stomatal limitation to A as a consequence of photoinhibition after opening the pinewood. A higher maximum quantum efficiency of open PS II centres (F_v/F_m) was registered in seedlings in the thinned stand. The significance of the differences between the treatments was stronger in the second year after thinning. In 1999, a year with frequent summer storms, water availability increased for seedlings growing under the thinned pinewood. Overall, the reduced pine overstory had a positive effect on physiological responses of beech seedlings, which was translated into improved seedling growth.

Keywords *Fagus sylvatica* · Gas exchange · Beech regeneration · Growth · Light

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Introduction

Differences in resource allocation (Mailly and Kimmins 1997), in physiological or morphological traits (Jurik 1986; Abrams and Kubiske 1990; Niinemets and Kull 1994) and in plant-level features such as crown architecture (Küppers 1989; Van Hees 1997; Valladares and Pearcy 1998) are produced in response to sun-shade acclimation of seedlings. Physiological changes are related to gas exchange and water relations and these vary according to the phenotypic plasticity of the species (Bazzaz 1979; Ellsworth and Reich 1992; Lei and Lechowicz 1997; Landhäusser and Lieffers 2001). Although light has been considered the most important factor that limits growth and survival of saplings in temperate natural environments (Pacala et al. 1994; Walters and Reich 1996; Chen 1997), other resources, such as water or nutrients, may be also important (Kloeppel et al. 1993; Abrams and Mostoller 1995; Walters and Reich 1997). This fact is relevant to forest dynamics as well as to stand management (Aussenac 2000), since seedlings grown in the understory may acclimatize differently to light increase after shelterwood thinning (Lieffers et al. 1993) or openings in mature stands (Abrams and Mostoller 1995; Naidu and DeLucia

1997). Besides light, other resources such as water availability may modulate coupling to the new environment. This question is very important in Mediterranean areas where plants in the understory must face not only shading but also summer drought. In a previous paper the decrease in water availability was noticeable at the end of the summer under two different forest overstories where beech seedlings had been previously out-planted (Aranda et al. 2002). Therefore, both factors may influence synergistically the physiology of the plants.

In the Iberian Peninsula natural beech stands are mainly restricted to the mountains in the north, with only a few populations remaining in the centre of the peninsula. In the latter areas, beech trees occur in a mixture with other more drought tolerant species such as *Quercus pyrenaica*, *Q. petraea* and *Pinus sylvestris*. The area is prone to dry periods during the growing season (Aranda 1998; Gil et al. 1999; Aranda et al. 2000). Therefore, survival and growth of either natural or artificial beech regeneration are mediated by drought avoidance/tolerance achieved through morphological/physiological acclimation.

The cover supplied by mature stands is necessary for the regeneration of shade-tolerant tree species like beech. Shading in the first years after germination or out-planting increases survival, since a low mortality of saplings of shade tolerant species has been reported in low light environments (Kobe et al. 1995). However, an increase in irradiance after the establishment of seedlings has a positive effect on their growth (Welander and Ottoson 1998; Collet et al. 2001).

Previous studies have considered the influence of the type of overstory on the physiological responses of planted beech seedlings underneath (Aranda et al. 2002) and of competitive vegetation on their successful establishment and growth (Löf 2000). Under high levels of available soil water, seedlings showed higher light-saturated photosynthesis (A_{sat}) on a leaf area basis as irradiance increased (Johnson et al. 1997; Aranda et al. 2002), a normal response to sun acclimation (Givnish 1988; Walters et al. 1993; Kloeppel et al. 1993). Nevertheless, drought stress was more intense in dry years at the end of summer in seedlings growing under higher irradiance (see Aranda et al. 2002). This was due to a greater decrease in soil water availability, which led to a drop in seedling predawn water potential. Although an increase in irradiance may improve beech seedling growth (Madsen 1994; Minotta and Pinzauti 1996; Van Hees 1997; Welander and Ottoson 1997; Collet et al. 2001), under natural conditions water stress must be taken also into account as a limiting factor. This may be modified together with light by changes in cover stocking. Therefore, planting beech seedlings below a mature pinewood and thinning the overstory some years after plant establishment, may be an option to accelerate transformation of pure pine plantations into mixed stands at the southern limit of beech distribution in Europe.

In a previous paper, changes in water relations of beech seedlings planted below a pine stand were analysed after the thinning of the overstory (Aranda et al. 2001a). In the present study, gas exchange data are presented and the following hypotheses are tested: (i) reduction in the overstory density leads to an increase in water or nutrient availability; (ii) the higher irradiance reaching the understory, following the cutting of pines, promotes a shade to sun acclimation of beech seedlings with an increase in gas exchange without any negative effect in terms of photoinhibition; and (iii) the overall effect of changes in understory environmental conditions is reflected in the growth of beech seedlings during the years after the pinewood thinning.

Materials and methods

In 1994, 1-year old beech seedlings were out-planted in a 50-year-old *Pinus sylvestris* plantation. The stand was located in the northwest of the Autonomous Community of Madrid (41°7′N 3°30′W) at 1,300 m elevation, with S-SE orientation and 15% slope. The pinewood density and basal area were 1,015 trees ha^{-1} and 55-m^2 ha^{-1}, respectively, with 18 m average dominant height. Beech seedlings were planted in rows at the midpoint between adjacent rows of pines.

In early March 1998, on a plot of 1,250 m^2 (thinned plot, T) alternate pine rows were removed leaving 50% of the pines. Another similar area in the pinewood, less than 50 m from the first one, was kept as control (plot C). After the felling, light conditions were evaluated with hemispherical photographs (Anderson 1964; Rich et al. 1993). These were taken over beech seedlings and processed with commercial HEMIVIEW software (Hemiview 2.1, Canopy Analysis Software, Delta-T Devices, UK). Photographs were taken at dawn without direct sun light. Relative irradiance was calculated from a gap index as a surrogate of canopy opening, which determines the proportion of global radiation relative to that in the open (global site factor, GSF).

During the growing seasons of 1998 and 1999, diurnal measurements of gas exchange were made on 4 days each year using an open-flow infrared gas analysis system (LCA-4, Analytical Development, Hertford, UK). Measurements were taken from early morning to afternoon on cloud-free days. Data were recorded for first flush leaves located in the mid-canopy. Seven beech seedlings were randomly chosen in every treatment and used for gas exchange measurements. The following day, predawn water potential (Ψ_{pd}) was measured in five seedlings per treatment (PMS 1000, Corvallis Instruments). Chlorophyll fluorescence was also registered in ten seedlings (PSM, Mark II, BioMonitor, Sweden). Maximum photochemical efficiency of PSII (Fv/Fm) was measured at dawn and midday in leaves after being submitted to half an hour dark adaptation period.

At the end of July in 1998 and 1999, two leaves per plant were sampled at the same mid-canopy position for nitrogen analysis by the Kjeldahl method. From the same leaves, one leaf disc was taken at the midpoint between the major vein and the blade edge. The discs were dried at 70°C for 48 h over 2 days and specific leaf mass (SLM) calculated as the ratio of dry mass to leaf disc area.

Photosynthetic response to photosynthetic photon flux density (PPFD) was described by a nonrectangular hyperbolic function fitted to the measured values of net carbon assimilation and PPFD (Landsberg 1986). Only data from measurements when VPD was <2.5 kPa, and on dates without water stress (Ψ_p >−0.2 MPa), were taken into account. This procedure excludes values affected by factors other than PPFD, such as stomatal closure in response to high drought or high evaporative demand. A Gauss-Newton non-

linear estimation routine was applied to the selected data set in order to estimate the parameters A_{sat} and α of the function:

$$A = \frac{\alpha \times PPFD \times A_{sat}}{(\alpha \times PPFD) + A_{sat}} \qquad (1)$$

where A_{sat} and α are respectively the light-saturated photosynthetic rate ($\mu mol\ CO_2\ m^{-2}\ s^{-1}$) and the apparent quantum yield efficiency (mol CO_2 / mol photons). Differences in the parameters in the model fitted to data for the treatments were established by comparison of 95% confidence intervals.

Maximum values of net photosynthesis (A_{max}) and stomatal conductance to water vapour (g_{wvmax}) were estimated as the average of maximum daily values of A and g_{wv} measured on each seedling selected for the gas exchange analysis. PPFD was recorded during gas exchange measurement by a quantum sensor mounted on the cuvette. Maximum values of photosynthetic photon flux density ($PPFD_{max}$) and vapour pressure deficit (VPD_{max}) were estimated for both treatments as the average of maximum daily values recorded.

Gas exchange parameters and water potentials were analysed with a repeated measurements ANOVA. Treatments and dates were taken as main factors. Growth at the end of the two years was analysed by ANCOVA, taking as covariant the height measured immediately before thinning. Data were log-transformed when necessary to meet the assumptions of the ANOVA. Differences between treatments within a date were established according to a Student's t-test ($P < 0.05$). Linear and logarithmic regression models were used to describe the relationship among physiological variables.

Results

Environmental conditions and water status of seedlings

The relative irradiance above the beech seedlings increased following thinning of the pinewood. This was evaluated by GSF and $PPFD_{max}$ measured at the time of gas exchange measurements (Table 1). The $PPFD_{max}$ was significantly lower in C though the PPFD pattern was very variable in both situations because of sunflecks of variable duration and intensity followed by periods of lasting dim irradiance, where PPFD rarely went beyond 100 $\mu mol\ m^{-2}\ s^{-1}$. Higher relative irradiance under thinned pinewood (T) was confirmed by GSF estimation as a measurement of canopy opening. Differences

between treatments in $PPFD_{max}$ were less marked in 1999 because 2 of the measurement days were partly overcast.

In both years, the VPD on sunny days was lower in C ($P < 0.05$). On overcast days differences between treatments were much less important. The general pattern of VPD for both treatments showed an increase in early morning to reach maximum values at midday (data not shown).

At the beginning of the growing season, in both years, water status was similar and relatively high ($\Psi_{pd} > -0.2$ MPa) independent of treatment. In 1998, a progressive drop was recorded in Ψ_{pd} by the end of July till early September in both C and T (Table 2). However, the initial decrease in water status was steeper in C seedlings since a significantly lower Ψ_{pd} was recorded on 29 July (-0.79 ± 0.05 MPa and -0.50 ± 0.09 MPa in C and T respectively). Differences between treatments were more pronounced in 1999, since seedlings in T showed significantly higher Ψ_{pd} on 29 July and 9 September.

Leaf gas exchange and chlorophyll fluorescence

Thinning the pine stand changed the A to PPFD response. In both years, there was a significantly higher light-saturated photosynthetic rate (A_{sat}) in T (Fig. 1), the magnitude of differences between treatments being larger in 1999, 2 years after thinning (Table 3). No significant differences between treatments were found in the apparent quantum yield (α), which ranged from 0.043 to 0.059 mol CO_2 (mol photons)$^{-1}$.

Daily A and g_{wv} were lower for seedlings growing in C at most times of day (Fig. 2). Maximum A and g_{wv} values were recorded before midday. Afterwards, the two parameters decreased in both treatments, more intensively on days of high water stress. Throughout the day, not only lower values for the parameters were reached in C, but also the decline in g_{wv} was steeper and more acute. Thus on 6 September 1999, stomatal closure was practically

Table 1 Maximum daily water vapour pressure deficit (VPD_{max}) and photosynthetic photon flux density ($PPFD_{max}$), and global site factor (GSF). Measurements were carried out on four different dates (for both 1998 and 1999)

Treatment	1998		1999		GSF (%)
	$PPFD_{max}$ ($\mu molm^{-2}\ s^{-1}$)	VPD_{max} (KPa)	$PPFD_{max}$ ($\mu molm^{-2}\ s^{-1}$)	VPD_{max} (kPa)	
C	646±81 a	2.26±0.10 a	534±83 a	2.15±0.11 a	0.298±0.016 a
T	1,227±111 b	2.63±0.11 b	899±107 b	2.37±0.15 b	0.386±0.014 b

Table 2 Water status of beech seedlings assessed by predawn water potential measurement (Ψ_{pd}) on three dates per year. Each value is the average (± SE) of five measurements (n=5). Differences between treatments are marked with a different letter ($P < 0.05$)

Treatment	1998			1999		
	10 June	29 July	2 September	11 June	29 July	9 September
C	−0.16±0.02 a	−0.79±0.05 b	−1.51±0.16 a	−0.21±0.03 a	−1.38±0.28 b	−1.27±0.21 b
T	−0.14±0.02 a	−0.50±0.09 a	−1.29±0.17 a	−0.17±0.03 a	−0.46±0.06 a	−0.37±0.04 a

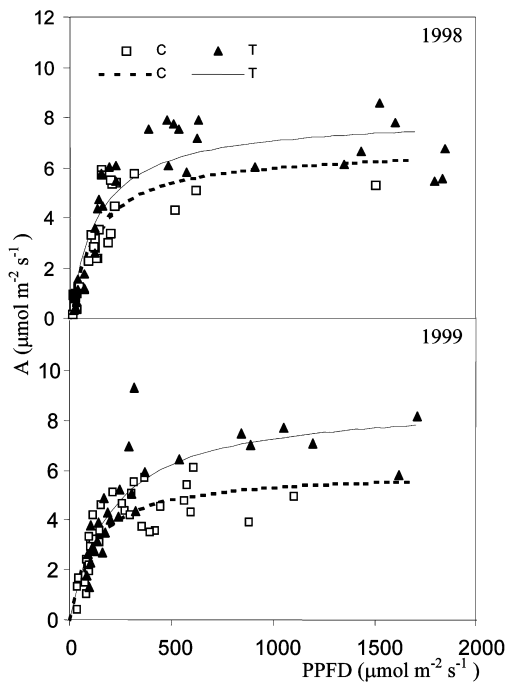**Fig. 1** Relationship between net photosynthesis (A, μmol m^{-2} s^{-1}) and photosynthetic photon flux density (*PPFD*, μmol m^{-2} s^{-1}) in beech seedlings growing under a pinewood thinned (*T*) and non-thinned (*C*). Data are from 1998 (*upper graph*) and 1999 (*lower graph*)

total in C whereas T plants maintained a relatively high stomatal conductance.

In both years, daily A_{max} values were higher ($P < 0.05$) for T seedlings on all measurement days (Fig. 3) and also closer to the asymptotic maximum rate (A_{sat}) estimated from the A-PPFD relationship (Fig. 1). They also showed a higher net CO_2 assimilation for the same g_{wv} when measurements were taken under saturating light (Fig. 4). Differences between treatments were stronger in 1999, when water stress in T seedlings was less than the previous year. There was a progressive seasonal increase in A_{max} and g_{wvmax} for T until early summer; afterwards, both decreased reaching the lowest values for the two treatments at the end of summer (Fig. 3). This was not so clear in 1999 in C, because at the end of June seedlings had already suffered from a more intense water stress than in the previous year at the same time. Water stress affected maximum A and g_{wv} in both treatments negatively so that A_{max} and g_{wvmax} decreased with Ψ_{pd} less than -0.5 MPa (Fig. 5). In spite of this, we observed in
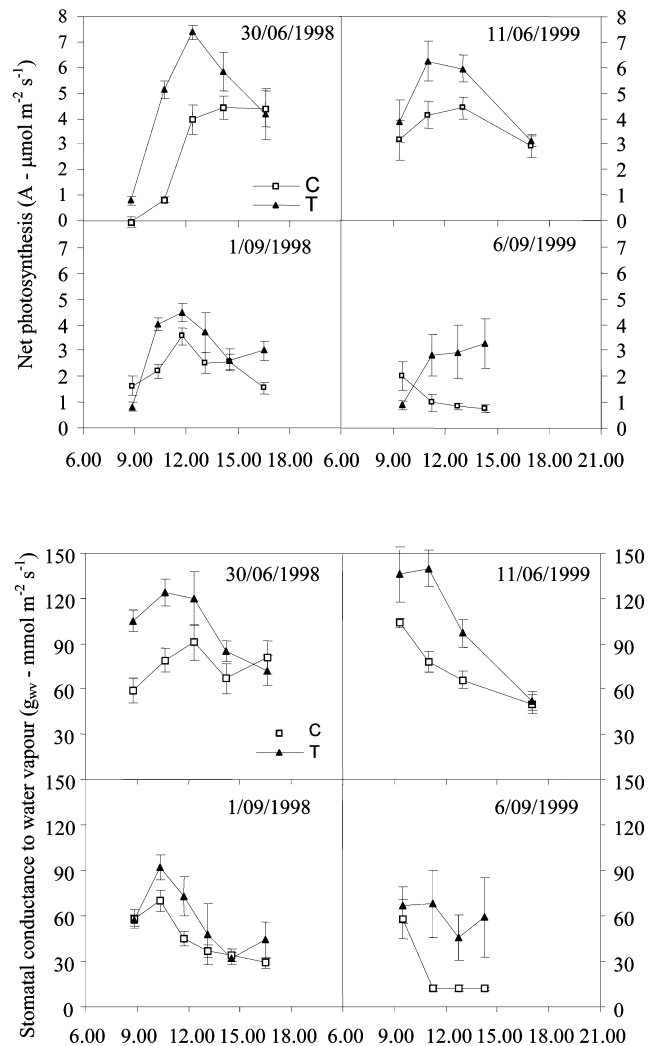

Fig. 2 Daily time course of net photosynthesis (*upper graph*) and stomatal conductance to water vapour (*lower graph*) on two dates in the beginning and late season during the growing season of 1998 and 1999. Seedlings were growing under two different light environments (*C* control plot, *T*, thinned plot). Each point is an average value (± SE, $n = 7$)

seedlings which had suffered water stress in mid summer of both years—this more intensively in C—that their Fv/Fm were always high showing values over 0.75 irrespective of treatment (Fig. 6). Differences between treatments in 1998 were only significant at the end of summer ($P < 0.05$), whereas in 1999 significantly higher values were found in T for all dates (Fig. 6). In 1998, Fv/Fm was lower on the first date of measurement than the following,

Table 3 Estimated parameters from the non-linear models of Fig. 1 in 1998 and 1999. Differences between treatments were marked with a different letter

Treatment	1998			1999		
	A_{max} (μmol m^{-2} s^{-1})	α (mol CO_2/ mol)	r^2	A_{max} (μmol m^{-2} s^{-1})	α (mol CO_2/mol)	r^2
C	6.78±0.78 a	0.051±0.009 a	0.87	5.91±0.46 a	0.051±0.009 a	0.69
T	8.02±0.46 a	0.059±0.010 a	0.83	8.73±0.72 b	0.043±0.007 a	0.72

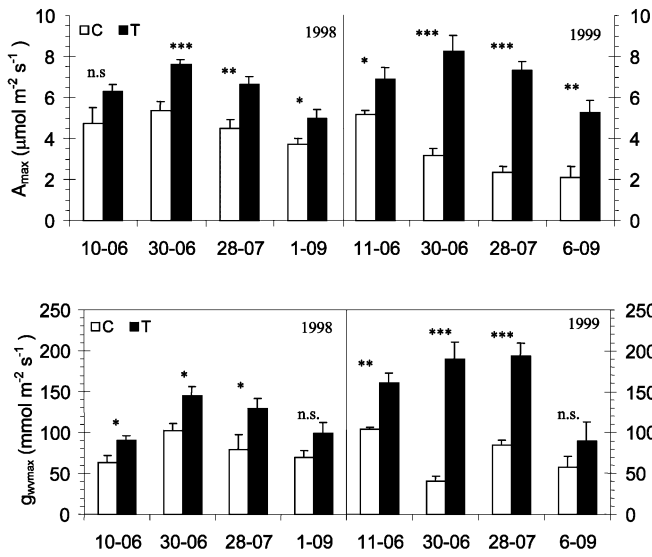**Fig. 3** Maximum daily net photosynthesis (A_{max} μmol m^{-2} s^{-1}) and stomatal conductance to water vapour (g_{wvmax} mmol m^{-2} s^{-1}) in beech seedlings. Standard error bars are shown. Different letters above bars denote significant differences between treatments (P <0.05)
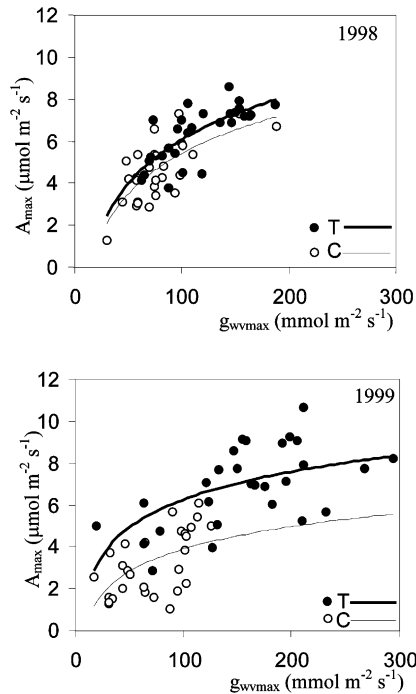

Fig. 4 Relationships between net photosynthesis (A μmol m^{-2} s^{-1}) and stomatal conductance to water vapour (g_{wv} mmol m^{-2} s^{-1}) in seedlings growing at control plot (*white points*) and thinned stand (*black points*). The regression equations are: seedlings growing in 1998 at control, A_{max}=2.76×ln(g_{wvmax})–7.32, r^2=0.51, P<0.0001, or under thinned stand, A_{max}=3.01×ln(g_{wvmax})–7.79, r^2=0.53, P<0.0001 (*upper graph*); the same relationships for 1999: control, A_{max}=1.55×ln(g_{wvmax})–3.23, r^2=0.29, P=0.0028 and thinned stand, A_{max}=1.92×ln(g_{wvmax})–1.92, r^2=0.32, P=0.0017 (*lower graph*)
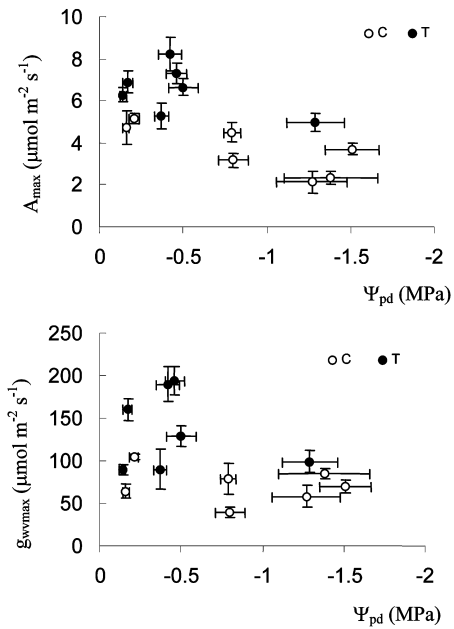

Fig. 5 Negative effect of water stress on maximum net photosynthesis (A μmol m^{-2} s^{-1}) and maximum stomatal conductance to water vapour (g_{wvmax} mmol m^{-2} s^{-1}). Symbols are as in previous figures

but the second year after pine thinning there was an interaction between measurement date and treatment. Thus, on 29 July, Fv/Fm tended to be higher than in the previous dates for T seedlings, but lower for C seedlings (P <0.05).

Nitrogen content and SLM

Leaf nitrogen content on a mass basis (N_m) was higher in 1998 (23.7 mg.g^{-1} ±0.6 overall data) than in 1999 (18.3 mg.g^{-1} ±0.4 overall data). In both years, N_m and SLM were higher for seedlings of T (Fig. 7). There was no year × treatment interaction (P >0.05). In both years, A_{max} was positively related with N_a (P <0.05) when data taken from both treatments were pooled together (Fig. 8).

Growth

Increasing relative irradiance (GSF) stimulated growth. Total height at the end of both years was higher in T seedlings (Fig. 9). Nevertheless, differences among treatments after covariance analysis were only significant (P<0.05) in 1999, total height values being 90.7 cm ±8.1 cm and 58.2 cm ±7.6 cm for T and C respectively. Differences in height were linked to the number of shoot flushes, which were higher in T seedlings (data not shown).

The T seedlings showed larger stem base diameters after the two growing seasons, reaching 13.32 mm ±0.52 mm at the end of 1999, a much higher value than

Fig. 6 Differences in predawn photochemical efficiency of PSII assessed by F_v/F_m measurement in dark adapted leaves of seedlings. Data are the means ± SE of ten seedlings for each treatment
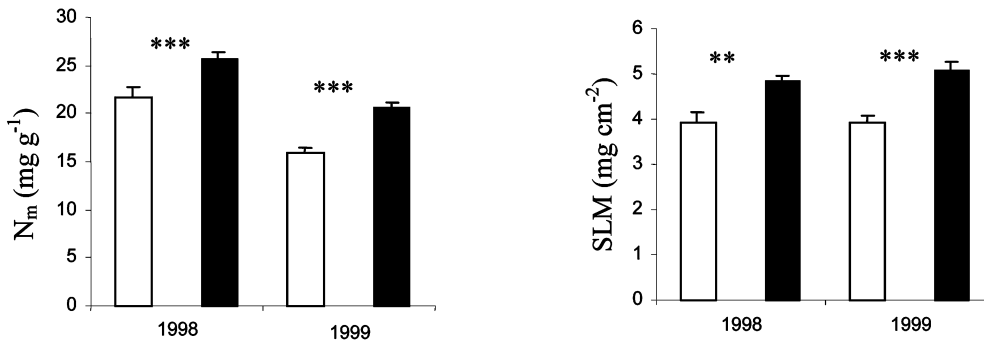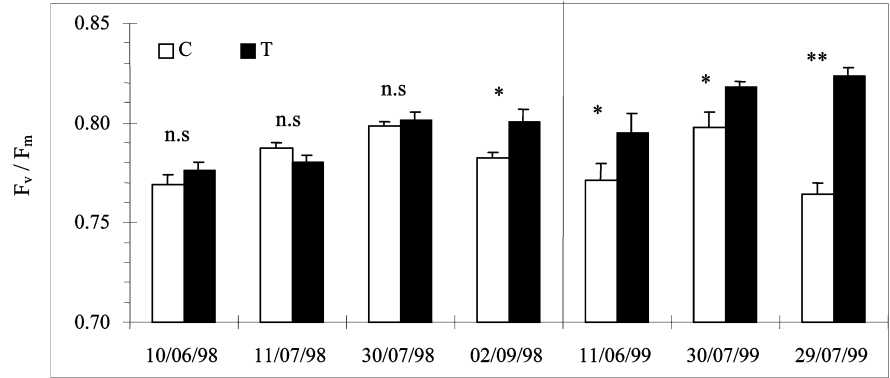

Fig. 7 Effects of treatments on specific leaf mass (*SLM* mg cm^{-2}) and nitrogen content on a mass basis (N_m mg g^{-1}) in 1998 and 1999. *Bars* with a different letter are means significantly different according to a Student's *t*-test (P <0.05). *White* and *black* bars denote seedlings from control and thinned stand respectively
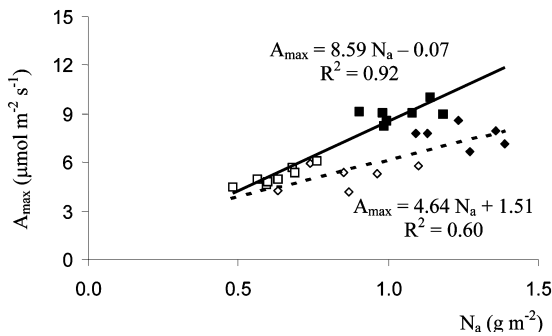

Fig. 8 Relationships between maximum leaf area based net photosynthesis (A_{max} μmol m^{-2} s^{-1}) and nitrogen content on a leaf-area basis (g m^{-2}) in 1998 (*squares*) and 1999 (*diamonds*) for control (*white points*) and treatment (*black points*) seedlings. Data were taken pooling maximum values from both treatments

$$A_{max} = 8.59\ N_a - 0.07$$
$$R^2 = 0.92$$

$$A_{max} = 4.64\ N_a + 1.51$$
$$R^2 = 0.60$$

the 8.56 mm ±0.36 mm stem base diameter measured in C seedlings.

Discussion

After pine felling there was an increase in irradiance, which led to the acclimation of beech seedlings growing in the understory (Tognetti et al. 1994; Johnson et al.
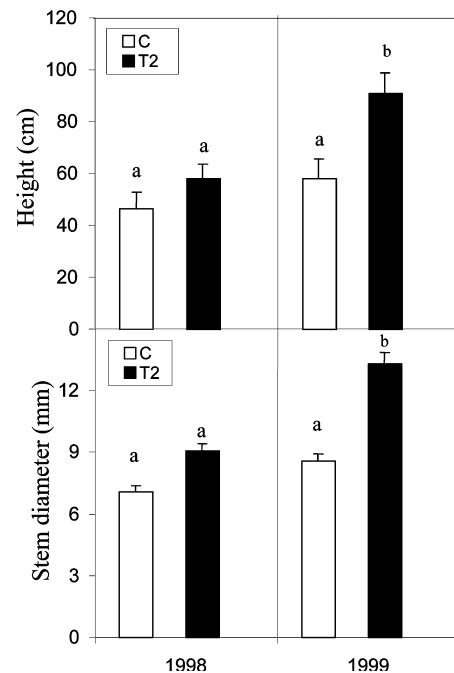
Fig. 9 Height (cm) and stem diameter (mm) of control (*white bars*) and treatment (*black bars*) beech seedlings at the end of the 2 years of the study. Significant differences between treatments are marked with a different letter (P <0.05)

1997). A higher light-saturated photosynthetic rate (A_{sat}) on a leaf area basis allowed an increased carbon gain at the leaf level. Apparent quantum yield (α) was on average around 0.05 mol CO_2 (mol photon)$^{-1}$. Similar values of α have been reported by Stickan and Zhang (1992) for the same species. Both are in the range found for other temperate species (Sullivan et al. 1996; Beaudet et al. 2000). Treatments were not significantly different, in concurrence with Björkman (1981) and reported more recently by Singsaas et al. (2001). However, differences in α between sun- and shade-leaves have been frequently reported by others (Boardmann 1977; Harley and Baldocchi 1995; Kubiske and Pregitzer 1996; Grassi and Minotta 2000). It is possible that the low N_m value in the control treatment, almost deficient in 1999, led to a decrease in the apparent quantum yield of shade-growing phenotypes (Boardman 1977; Björkman 1981). Differences among treatments in the A-PPFD response were more pronounced the second year after pine thinning, as previously reported for leaf anatomy (Aranda et al. 2001b). Furthermore, differences between treatments in A_{max} were higher in 1999. Development of photosynthetic competence may be a gradual process, reaching full acclimation to the new light environment the second year after thinning. Löf (2000) described a similar "carry-over" process in water consumption the first growing season after transfer of beech seedlings growing the previous year under drought conditions. Previous growth under shade conditions may limit the acclimation to increasing irradiance (Thiébaut et al. 1990; Kimura et al. 1998).

SLM and N_m were greater in seedlings growing in the thinned stand. The increase in SLM is probably linked to leaf anatomical changes (Larsen and Buch 1995). A higher lamina thickness and palisade mesophyll in leaves of seedlings growing under higher relative irradiance is commonly observed (e.g. Ziegenhagen and Kausch 1995; Aranda et al. 2001b). This increase in photosynthetic tissues per area was accompanied in the present study by higher nitrogen content expressed on a leaf-mass basis, both being highly correlated with the photosynthetic capacity (Field and Mooney 1986; Evans 1989; Reich et al. 1995).

Stimulation of light-saturated photosynthesis in T seedlings was accompanied by the enhanced water availability in 1999. Less competition for water and reduced rain interception after thinning could also improve water availability to beech seedlings. A similar response to the increase in resource availability has been described after perturbations such as wildfire (Fleck et al. 1995; Kruger and Reich 1997), harvests (Castell et al. 1994) or the opening of natural gaps (Ellsworth and Reich 1992; Bauhus and Bartsch 1995). In a previous paper, the positive effect of increasing irradiance on beech seedlings gas exchange was shown, when it takes place with concomitant high soil moisture availability (Aranda et al. 2002).

Higher net photosynthesis in T seedlings improved the capacity for osmotic adjustment (Aranda et al. 2001a).

This is linked to lower osmotic potential at full turgor in seedlings submitted to higher relative irradiance (Abrams 1986; Ellsworth and Reich 1992; Aranda et al. 2001a). Further, higher Ψ_{pd} was measured in seedlings under the thinned pine stand (Table 2) resulting in a better water status in terms of turgor at dawn (Aranda et al. 2001a). This might influence the higher g_{wvmax} value measured in T seedlings during mid-summer, which reduced the stomatal limitation to net photosynthesis. This reduction lasted the whole day (Fig. 2). Thus, seedlings growing under higher shade showed a very low daily g_{wv}, which was especially negative on the plant carbon balance at the end of summer. In a similar way, Abrams and Mostoller (1995), and more recently Valladares et al. (2002), have found higher negative impacts of shading on seedling carbon gain as a consequence of greater water stress in dry years. At present, together with the reduced stomatal limitation, it was observed that the enhancement of photosynthetic capacity of leaves in T seedlings appeared to be linked to acclimation to the new light environment. The decline in water stress in T seedlings in 1999 was possibly a consequence of the reduced competition from mature pine trees and a higher frequency of summer storms (see Aranda et al. 2001a for meteorological data).

Although beech is taken as one of the most late-successional and shade-tolerant species in Europe, it showed a positive response in photosynthetic behaviour after the increase of relative irradiance. This phenotypic plasticity is considered more characteristic of early successional species (Bazzaz 1979; Bazzaz and Carlson 1982; Strauss-Debenedetti and Bazzaz 1991). Acclimation to increased light was apparent the growing season after thinning the pine overstory. A reduced ability to acclimate following a sudden increase in irradiance has been noted for shade-tolerant species (Tognetti et al. 1994; Naidu and DeLucia 1997; Valladares et al. 2002). This observation indicates that successional status and shade tolerance may not be fully defined only by studies on carbon assimilation at the leaf level (Kitajima 1994). Overall carbon balance must be considered (Küppers 1994; Beaudet et al. 2000) together, in the long term, with the morphological and physiological adjustment in seedlings exposed to a change in light environment (Ellsworth and Reich 1992). In this sense, the increase in the photosynthetic capacity of T seedlings was linked to the increase in leaf nitrogen content and SLM.

This gradual, positive response to increasing light may be expected unless the inability to acclimate rapidly, puts seedling survival at risk. Furthermore, long-term survival during periods of suppression in natural stands (Kobe et al. 1995; Pacala et al. 1996) or in plantations established below shelter-woods (Gemmel et al. 1996; Chen 1997) may have a stronger influence on forest dynamics than rapid acclimation to high irradiances. In several studies, such as those by Ellsworth and Reich (1992) with *Acer saccharum*, another very shade-tolerant species, or by Welander and Ottoson (1997) with *Fagus sylvatica*, a similar conclusion has been suggested about the positive effect of a partial increase in relative irradiance on the

physiological and developmental response of shaded seedlings. Our results confirm the capacity of beech seedlings to accommodate to shading release by increasing gas exchange and growth, which agrees with the above-mentioned results. However, it must be emphasized that this response depends on the magnitude of the increase in relative irradiance (cf. Naidu and De Lucia 1998) and time of the season the change in light levels is generated. For instance, while in the present study a loss in maximum photochemical efficiency of PSII (Fv/Fm at dawn) was not observed (Fig. 6), Tognetti et al. (1998) showed an important decrease in Fv/Fm after the transfer of beech seedlings from low to high light. A similarly poor acclimation of beech seedlings to a sudden increase in light availability has been recently reported (Valladares and Pearcy 2002). In this study the acclimation response was measured in the short term and after leaf unfolding, with the plain development of leaf tissues under shade conditions. This would severely limit the potential for morphological or physiological acclimation to a new light environment (Kimura et al. 1998; Aranda et al. 2001b).

In the present research, the best behaviour was observed for a maximum relative irradiance around 40%, with a physiological response very similar to that observed in natural gaps (Johnson et al. 1997). It is possible that this irradiance constituted an optimum threshold for beech seedling growth because higher irradiance, together with an increase in water stress, might have a negative effect (Aranda et al. 2002). An increase in growth of beech seedlings in the understory after partial canopy opening of the overstory has been previously reported (Collet et al. 2001, 2002). Studies with beech seedlings grown under different degrees of shading have reached similar conclusions (Larsen and Buch 1995; Van Hees 1997; Welander and Ottoson 1997, 1998). Moreover, survival in the long-term of natural beech germinants which attain sapling size and increase the permanent seedling bank depends on a minimum relative irradiance threshold (Minotta and Pinzautti 1996; Emborg 1998; Szwagrzyk et al. 2001). Therefore, our results confirm those previously reported where a decrease in shading has positive consequences on successful growth (Grubb et al. 1996; Finzi and Canham 2000). However, water stress may be an important limitation to the positive effect of the relative irradiance increase due to its negative impact upon net carbon assimilation. Thus, independently of light availability, beech seedlings showed a reduction in net photosynthesis at the end of summer as a consequence of water stress. Both factors must be taken into account in areas where water shortage during summer months is frequent (Madsen 1994).

In conclusion, our results demonstrate that partial release of overstory competition improved growth conditions for beech seedlings previously planted in the understory. Long-term shading in areas of intense summer water stress may exacerbate mortality because of the antagonistic influence of both factors on seedling physiology. Partial reduction of the overstory competition minimises the negative effects of water shortage during summer months. Thinning of shelterwoods after planting beech seedlings in the understory may promote the successful introduction of beech into pine mature plantations. This would allow the transformation of pine stands in the long term to mixed conifer-broadleaved stands.

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