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ORIGINAL ARTICLE

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Carbohydrate storage, survival, and growth of two evergreen *Nothofagus* species in two contrasting light environments

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Abstract A number of traits have been attributed important roles in tolerance of shade by plants. Some explanations emphasize traits enhancing net carbon gain; others emphasize energy conservation traits such as storage of non-structural carbohydrates (NSC). To date, cross-species studies have provided mixed support for the role of NSC storage in low-light survival. We examined NSC status, survival, biomass, and growth of large seedlings of two evergreen species of differing shade tolerance (*Nothofagus nitida* and *N. dombeyi*) grown in deep shade and 50% light for two growing seasons. We expected to find higher NSC concentration in the more shade-tolerant *N. nitida* and since allocation to storage involves sacrificing growth, higher growth rate in the shade-intolerant *N. dombeyi*. NSC concentration of both species was >twofold higher in 50% light than in deep shade, and in roots and stems did not differ significantly between species in either environment. NSC contents per plant were also similar between dead and living plants in deep shade. *N. dombeyi* outgrew *N. nitida* in 50% light, while this pattern was reversed in deep shade. Survival in deep shade was not correlated with NSC concentration. Leaf mass fraction was similar between species in 50% light, but lower in *N. dombeyi* in deep shade. Results provide little evidence of a link between carbohydrate storage and low-light survival in *Nothofagus* species, and support the view that understorey survival is primarily a function of net carbon gain. Patterns of variation in NSC concentration of the temperate species we studied are likely dominated by more important influences than adaptation to shade, such as limitation of growth or adaptation to cold stress.

Keywords Allocation · Carbon gain · Non-structural carbohydrates · Shade tolerance · Temperate forest

Introduction

A central question in the physiological ecology of forest succession is whether tolerance of shade has more to do with carbon gain or carbon conservation (Valladares and Niinemets 2008). Early explanations emphasized traits likely to enhance whole-plant carbon gain in low light, such as development of a large ratio of leaf area to biomass through high allocation to foliage production, and low respiration rates and light compensation points (Bazzaz 1979; Givnish 1988). Some studies of young seedlings have lent little support to this concept of shade tolerance (Kitajima 1994; Walters and Reich 1999), although other studies of larger seedlings and saplings are more supportive (e.g., King 1991; Lusk 2002; Baltzer and Thomas 2007).

Another view of shade tolerance emphasizes storage and defensive traits favoring energy conservation (Kitajima 1994; Kobe 1997; Canham et al. 1999; Walters and Reich 1999). Non-structural carbohydrates (NSC) storage is potentially valuable in buffering against temporary resource deprivations: reserves could be mobilized to meet maintenance costs during periods of heavy shading, or to replace leaves lost to herbivores (Chapin et al. 1990; Kitajima 1994; Kobe 1997; Canham et al. 1999). Allocation to storage necessarily involves sacrificing growth and forgoing opportunities for increasing

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whole-plant carbon gain by deploying new leaves (Kobe 1997; Canham et al. 1999). If shade-tolerant species allocate more carbohydrate to storage than light-demanding species in all light environments, they might also be expected to grow slower than light-demanders, even in deep shade. To date, cross-species studies have provided mixed support for the hypothesis of carbon storage as a mechanism of shade tolerance. Support has been forthcoming in two major studies of tropical species (Myers and Kitajima 2007; Poorter and Kitajima 2007), but lacking in three studies of temperate trees (Canham et al. 1999; Machado and Reich 2006; Lusk and Piper 2007). The reasons for this lack of consensus are not clear, although few studies have taken into account phylogenetic relationships which could influence variation in NSC storage.

Although intraspecific variation in NSC content seems relevant to the hypothesis of carbon storage as a shade tolerance mechanism, it has received little attention. If carbohydrate storage is an important mechanism of shade tolerance, then we might also expect to find intraspecific variation in survival to be correlated with NSC concentration ([NSC]) (Canham et al. 1999; Gleason and Ares 2004). Furthermore, if NSC storage has an adaptive function in tolerance of shade, we might expect species differences in [NSC] to be more evident in shade than in high light, but few studies have measured [NSC] in more than one light environment. Kobe (1997) found that deciduous species of contrasting shade tolerance differed in [NSC] in 2% light, but not in 28% light. In a comparison between a mid-successional tropical species and a late-successional associate, however, Marenco et al. (2001) found a bigger difference in [NSC] in full sunlight than in 13% sunlight. Depending on the nature of its adaptive value, [NSC] could potentially vary in a number of ways across species and light environments (Fig. 1).

Here we report growth, survival, biomass, and NSC storage of large seedlings of two evergreen *Nothofagus* species of differing shade tolerance, grown in two contrasting light treatments. If survival in low-light is more a matter of carbon conservation than carbon gain, we would expect that (1) in low-light, mid-tolerant *N. nitida* would have higher NSC storage, but lower growth than intolerant *N. dombeyi*, and (2) low-light survival would correlate positively with NSC reserves in both species. We also examined the conservativeness of the patterns of carbon allocation to storage and growth under contrasting light environments.

Methods

Species and site studied

The study was carried out between August 2004 and March 2006 in the Reserva Costera Valdiviana, in the coastal range of southern Chile. The two species used were *Nothofagus dombeyi* (Mirb.) Blume and *N. nitida* (Phil.) Krasser. *N. dombeyi* is well documented as a shade-intolerant species establishing mainly in early successional stages and in large canopy openings (Veblen et al. 1980; Lusk and Kelly 2003) while *N. nitida* is regarded as mid-tolerant, with seedlings often present under the forest canopy (Donoso 1981; Christie and Armesto 2003).

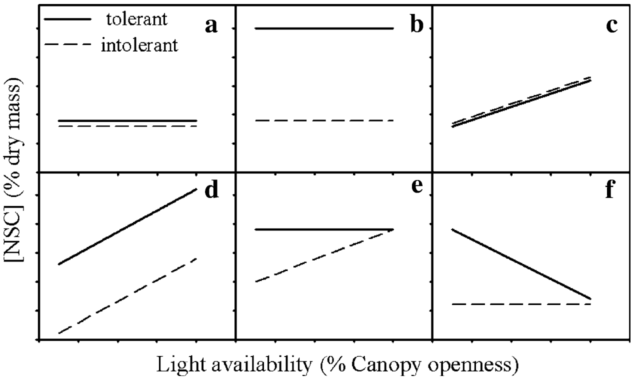

Fig. 1 Alternative models of the possible influences of species and light environment on non-structural carbohydrate concentration ([NSC]). **a** Neither species nor light environment affects [NSC]. **b** [NSC] is influenced by species, but not light environment, with tolerant species always allocating a larger proportional of carbohydrates to storage than intolerant species (fixed trade off between growth and storage). **c** [NSC] is influenced by light environment, but not by species. Carbohydrates are accumulated in high light environments as a mechanism to tolerate stress (i.e., cold, drought) or as consequence of growth limitations. **d** Species and light environment both influence [NSC], without interaction between factors. The growth-storage tradeoff is observed in species comparisons, but all species accumulate more NSC in high light. **e–f** Species and light environment both influence [NSC], with interaction between factors. In the former, shade-tolerant species have a fixed strategy of high allocation to storage, whereas light-demanders increase their [NSC] in high light. In the latter, shade-tolerant species allocate heavily to storage only in low-light, whereas light-demanders have a relatively fixed strategy

The seedlings of the two species were obtained from separate stands on the seaward (west-facing) slopes of the range. The *N. dombeyi* stand was located at 80 m a.s.l. (39°58′02″S, 73°33′39″W), and the *N. nitida* stand at about 350 m a.s.l. (39°59′40″S, 73°34′12″W). Mean annual temperature is 10°C and mean annual precipitation is 2,100 mm, with a marked winter maximum (Almeyda and Sáez 1958) and frequent summer short drought (Woda et al. 2006). Around 45 plants for each high- and low-light treatments were chosen from open (30–45% canopy openness) and shaded (< 5% canopy openness) situations, respectively. Seedlings were around 3 years old and 18–28 cm tall, with average initial height slightly greater in *N. dombeyi* ($P < 0.001$), but similar between light environments ($P = 0.263$). Plants were carefully excavated and transplanted to 5-l pots containing a 1:1 mixture of soil from both sites.

Experimental design

The experiment was installed in a cleared site of a second-growth forest located at an intermediate elevation between the two seedling provenances (200 m a.s.l.).

Plants from open sites were assigned to a high light treatment equivalent to 50% open sky-light (hereafter the “50% light” treatment). Understorey plants were initially acclimated to a 3% open sky-light environment before imposing the shade treatment, equivalent to 1% light. Each light treatment was replicated in three blocks. As the block effect was not significant for any of the variables, data from different blocks were assembled. Light treatments were provided with neutral shade cloth. From the beginning of the experiment until December 2005 pots received water from natural precipitation, while between December 2005 (at the time that the 3% light level was changed to 1% light) and the end of the experiment plants were constantly well watered. Plants surviving until December 2005 and submitted to 1% light amounted to 34 *N. dombeyi* and 40 *N. nitida*.

At the end of the experiment, relative growth rate, NSC concentration, total biomass, leaf mass fraction, and NSC pools were calculated in surviving plants from the low-light treatment. All of the 12 surviving plants of *N. dombeyi* were analyzed, while 20 of the 28 surviving plants of *N. nitida* were selected for analyses. These parameters were also measured for 29 *N. nitida* and 25 *N. dombeyi* seedlings from the 50% high light treatment. NSC storage and leaf mass fraction were also measured in plants that died at 1% light close to the end of the experiment (during February–March 2006), in order to examine the relative contributions of carbon reserves and biomass distribution to interspecific variation in survival. These plants amounted to seven *N. dombeyi* and 12 *N. nitida*.

Survival, growth, and biomass

Seedling survival was recorded at 2–5 month intervals from August 2004 to March 2006. Seedlings were considered alive when at least one green leaf or bud was present. At the end of the experiment, surviving plants were measured and then harvested. Growth was calculated as the yearly proportional change in height for the last growing season (cm cm^{-1} year^{-1}) as follows: relative growth rate in height ($\mathrm{RGR_H}$) = ($\ln h_2 - \ln h_1$) (Evans 1972), where h_1 and h_2 are the heights of a seedling before and after the last growing season of the experiment. After removal of soil from roots, complete seedlings were cooked at 600 W for 90 s in a microwave oven to denature enzymes (Popp et al. 1996) and then separated into leaves, stems, and roots. Stem fraction included the main stem and also all branches. Each biomass fraction was dried to a constant mass at 70°C. Dry mass of each organ was recorded for each plant and then added to obtain the total biomass per plant and the fraction of leaves mass. Samples were then ground to a fine powder and used for NSC analyses.

NSC analyses

Samples were analyzed for total soluble sugars and starch using ethanol and perchloric acid (Hansen and Moller 1975). Total soluble sugars were extracted from tissue in 86% v/v ethanol at 80°C for 1 h. The supernatant was collected after centrifugation and the concentration of total soluble sugars was determined spectrophotometrically at a wavelength of 520 nm by the Resorcinol method (Roe 1934) using sucrose as standard. Starch was extracted from the ethanol insoluble fraction by agitating for 1 h with 35% v/v perchloric acid (Sutton et al. 1981). This method of extraction can yield starch values higher than those estimated by more accurate enzyme methods (Rose et al. 1991), probably as a result of hydrolysis of some cell wall components. However, this is not a major problem for our study, as we were interested primarily in the relative concentrations of different species, size classes and organs. The protocol for starch determination in the extract was similar to that used for sugars, but using glucose as standard. Starch and soluble sugars in each plant organ were added together to determine total non-structural carbohydrate concentration [NSC] in mg g^{-1} dry mass. Total pools of NSC were calculated for each organ by multiplying the biomass by the [NSC] of that organ.

Statistics

Survival proportions in each light treatment were compared between species by the *z*-test (Sokal and Rohlf 1995). Two-way ANOVA was used to test the effect of the species and light treatment on growth. The concentration of NSC among dead plants at extreme shade, plants alive at extreme shade, and plants at 50% light, was also compared by a two-way ANOVA. Log-transformations were used to normalize data and to homogenize variances, when necessary. When log-transformations were not enough to achieve ANOVA assumptions, rank-based ANOVA was applied and pairwise comparisons analyzed with Dunn’s test (Sokal and Rohlf 1995). The relationships of both biomass and carbohydrate level with $\mathrm{RGR_H}$ in each light treatment were evaluated by correlation analyses, using Spearman coefficient. All these analyses were performed with Sigma Stat 2.0 software (SPSS, Chicago, IL). The relationships of biomass and carbohydrate parameters with survival in 1% light were assessed by logistic regression using JMP Statistical Software (SAS Institute, Cary, NC, USA).

Results

Survival

A marked summer drought in March 2005 affected our experiment. Total precipitation during this month was 28 mm, compared to an historical average of 70 mm. As survival of both species was severely affected by the drought, we analyzed only data recorded until January

2005 and after December 2005, when watering was provided. In 50% light, survival of both species to January 2005 was 100%. In 3% light, survival to January 2005 was 88% for *N. nitida* and 77% for *N. dombeyi*, without differences between species ($P = 0.292$). When watering was regularly provided and shade lowered to 1% light (December 2005–March 2006) survival differed between species ($P = 0.006$), being 70% in *N. nitida* and 35% in *N. dombeyi* (Fig. 2).

In 1% light, survival of *N. nitida* was not correlated with any biomass or storage parameter, while survival of *N. dombeyi* was marginally negatively associated with $\mathrm{NSC}_{(\mathrm{root+stem})}$ pool (Table 1). Survival of neither species was related to $\mathrm{RGR}_{\mathrm{H}}$ or to initial height.

Growth and biomass

Species reversed their $\mathrm{RGR}_{\mathrm{H}}$ ranks between the two light treatments (Fig. 3). Growth of *N. nitida* did not differ significantly between the two light environments, whereas *N. dombeyi* grew 78% slower at 1% light than at 50% light (Fig. 3). In the shade, *N. nitida* grew 66% faster than *N. dombeyi*, whereas in 50% light this pattern was reversed as *N. dombeyi* outgrew *N. nitida* by 18%. Total biomass in 50% light was similar between species and higher than in low light. Low-light total biomass at harvest was higher in *N. nitida* (Fig. 3), despite its initial height being slightly lower than that of *N. dombeyi*.

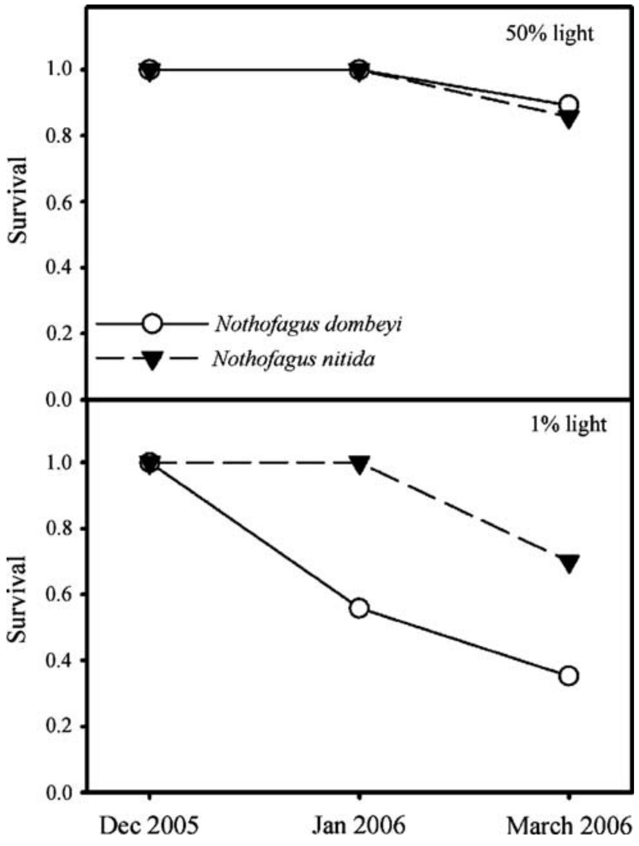

Fig. 2 Survival of large seedlings of two evergreen *Nothofagus* species growing in two light environments in the Coast Range, X Region, Chile (1% light: *N. dombeyi* $n = 34$, *N. nitida* $n = 40$; 50% light: *N. dombeyi* $n = 40$, *N. nitida* $n = 45$)

Leaf mass fraction of *N. nitida* was similar in the two light treatments and also between dead and live plants in 1% light (Fig. 4). In *N. dombeyi*, however, dead plants had lower leaf fraction than surviving seedlings in 1% light, while the latter had lower leaf fraction than plants in 50% light (Fig. 4). Faster leaf fall was observed in *N. dombeyi,* likely explaining the lower leaf mass fraction observed in this species (Fig. 4). No association was observed between NSC levels and $\mathrm{RGR}_{\mathrm{H}}$ (Table 2).

NSC levels

NSC concentration was much more strongly influenced by light environment than by species (Fig. 5, Table 3). NSC concentration of roots and stems was more than two-fold higher at 50% light than at 1% light, and a similar difference was observed in the NSC concentration of leaves. NSC concentration of leaves was slightly higher for *N. dombeyi*, but there were no other significant differences between species (Table 3, Fig. 5). Dead plants of both species had similar [NSC] to live plants (Fig. 5).

In general, NSC pool followed a similar pattern to NSC concentration (Fig. 5). In both species, NSC pool was similar between dead and alive plants at 1% light (Dunn's test $P > 0.05$) and lower at this light treatment than at 50% light (Dunn's test $P < 0.05$). Species only differed at 1% light for surviving plants, where the pool of roots and stems was higher in *N. nitida* ($P > 0.05$).

Discussion

Interspecific comparisons

Mid-tolerant *N. nitida* outgrew the light-demanding *N. dombeyi* in low light, suggesting that the former had a more positive low-light carbon balance (Fig. 3). Success in late-successional habitats is ultimately a question of low-light survival, rather than growth (Kobe et al. 1995), although the latter can nevertheless inform inferences about carbon balance and allocation. The literature shows varied influences of light environment on the comparative growth rates of species differing in shade tolerance. Studies including light levels approximating understorey environments under closed canopy (<2%) often show rank reversals that may not be detected in moderate shade (Walters and Reich 1996; Sack and Grubb 2001; Lin et al. 2002; Baltzer and Thomas 2007). However, the outcome of such comparisons can also be influenced by ontogenetic stage as well as light environment (Kneeshaw et al. 2006; Niinemets 2006). Crossovers in growth rate between species of contrasting shade tolerance along light gradients has been found

Table 1 Logistic regression of survival on biomass and storage variables of two Chilean evergreen *Nothofagus* species in shade

Variable	*Nothofagus nitida*		*Nothofagus dombeyi*	
	r	*P*	*r*	*P*
$[NSC]_{root+stem}$	(−0.03)	0.73	(0.09)	0.13
$NSC_{(root+stem)\ pool}$	(−0.0025)	0.74	(−0.14)	0.059
$[NSC]_{leaves}$	(0.02)	0.35	(−0.05)	0.7
$NSC_{leaves\ pool}$	(0.002)	0.76	(−0.08)	0.16
RGR_H	(−0.04)	0.2	(−0.14)	0.14
Initial height	(0.02)	0.38	(0.000)	0.92

Plants were grown at 3% light from July 2004 until December 2005, and at 1% light from December 2005 until the end of the experiment in March 2006

[NSC] concentration of non-structural carbohydrates, NSC_{pool} absolute amount of non-structural carbohydrates, RGR_H relative height growth rate

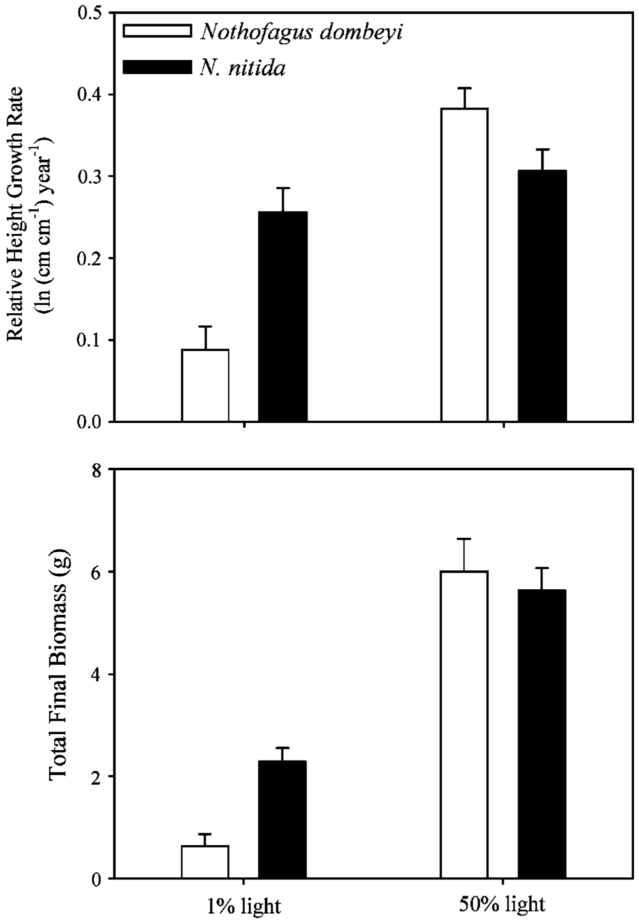

Fig. 3 Results of Anova. Relative height growth rate (RGR_H) of the last growing season and final biomass of large seedlings of two evergreen *Nothofagus* species, grown at deep shade and 50% light during two growing season (August 2004–March 2006) in the Coast Range, X Region, Chile. Plants at deep shade were grown at 3% light from August 2004 until December 2005, and at 1% light from December 2005 until the end of the experiment, in March 2006. Data in the plots are the average of survivors. *Error bars* correspond to errors standard. *Uppercase letters* indicate comparison between light environments; *lowercase letters* indicate comparison between species in each light environment

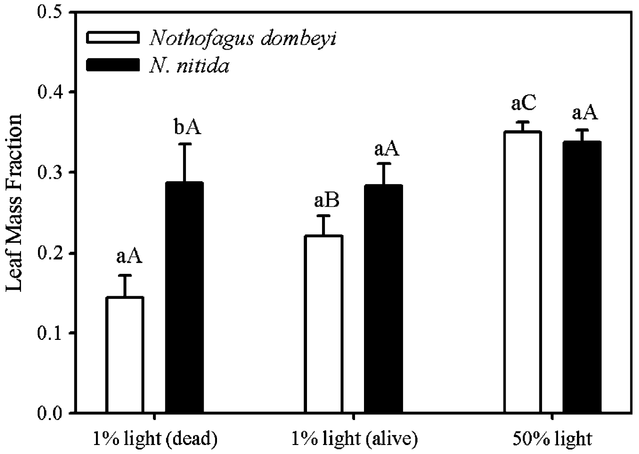

Fig. 4 Leaf mass fraction of large seedlings of two evergreen *Nothofagus* species, grown at extreme shade and 50% light during two growing season (August 2004–March 2006) in the Coast Range, X Region, Chile. Plants at extreme shade were grown at 3% light from August 2004 until December 2005, and at 1% light from December 2005 until the end of the experiment, in March 2006. *Uppercase letters* indicate comparison between light environments; *lowercase letters* indicate comparison between species in each light environment

frequently in saplings (Lin et al. 2002; Lusk 2004; Baltzer and Thomas 2007), but not in seedlings (Kitajima 1994; Poorter 1999; Walters and Reich 1999), reflecting the influence of ontogenetic changes in biomass distribution (Lusk 2004).

The difference in shade tolerance between *Nothofagus dombeyi* and *N. nitida* was not associated with differences in carbohydrate storage (Figs. 2, 3, 5). NSC concentrations in 1% light were similar between species and did not relate to survival in shade (Table 3). The NSC pool of roots and stems was larger in *N. nitida* than in *N. dombeyi*; however this result mainly reflects the higher total biomass of *N. nitida* at the end of the experiment, rather than any adaptive role of carbon reserves (Fig. 3, Table 1). The lack of an adaptive role of NSC storage in the shade tolerance of *N. nitida* is supported by the similarity of NSC levels between live and dead plants (Fig. 5). Our comparison of two very closely related species is consistent with other studies of temperate species that have also found little or no relationship between shade tolerance and tissue concentrations of NSC (DeLucia et al. 1998; Canham et al. 1999; Lusk and

Table 2 Correlation coefficients between RGR_H and biomass and storage parameters in two Chilean evergreen *Nothofagus* species in two light environments

Variable	*Nothofagus nitida*		*Nothofagus dombeyi*	
Deep shade	*r*	*P*	*r*	*P*
$[NSC]_{root+stem}$	(−0.02)	0.9	(−0.20)	0.61
$[NSC]_{leaves}$	(−0.10)	0.7	(−0.01)	0.97
$NSC_{(root+stem)\ pool}$	(−0.2)	0.42	(0.07)	0.85
$NSC_{leaf\ pool}$	(−0.25)	0.31	(0.06)	0.9
50% Light				
$[NSC]_{root+stem}$	(−0.19)	0.31	(−0.11)	0.61
$[NSC]_{leaves}$	(−0.01)	0.94	(−0.09)	0.65
$NSC_{(root+stem)\ pool}$	(0.04)	0.82	(−0.16)	0.45
$NSC_{leaf\ pool}$	(0.25)	0.18	(−0.3)	0.14

Deep shade plants were grown firstly at 3% light (from July 2004 until December 2005), and then at 1% light (from December 2005 until the end of the experiment in March 2006)

[NSC] concentration of non-structural carbohydrates, NSC_{pool} absolute amount of non-structural carbohydrates

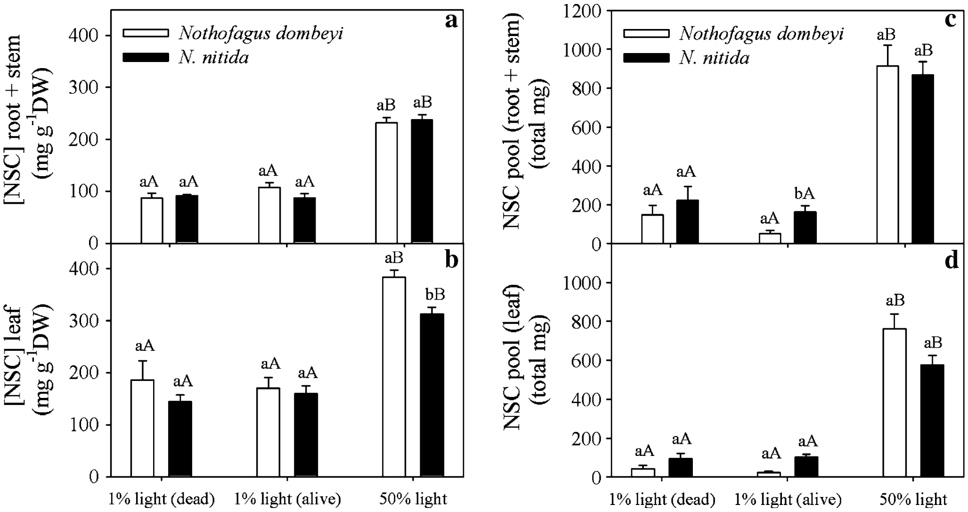

Fig. 5 Non-structural carbohydrates of large seedlings of two evergreen *Nothofagus* species grown in deep shade and 50% light for two growing seasons (August 2004–March 2006) in the Coast Range, X Region, Chile. **a**, **b** Concentration of non-structural carbohydrates ([NSC]); **c**, **d** total pools of non-structural carbohydrates. Plants at extreme shade were grown at 3% light from August 2004 until December 2005, and at 1% light from December 2005 until the end of the experiment, in March 2006. At 1% light, [NSC] and NSC pools are shown for both dead and alive plants. Data of plots correspond to average values, with *error bars* showing one standard error. *Uppercase letters* show comparison between light environments; *lowercase letters* show comparison between species in each light environment

Table 3 ANOVA examining effects of light treatment and species on non-structural carbohydrate concentrations ([NSC]) in roots and stems and in leaves of two Chilean evergreen *Nothofagus* species grown at two light conditions; 50% light and deep shade

Response variable	Effect	*df*	*F*-ratio	*P*-value
$[NSC]_{root+stem}$	Treatment	2	116.4	< 0.001
	Species	1	0.10	0.754
	Treatment × species	2	0.84	0.430
$[NSC]_{leaves}$	Treatment	2	90.10	< 0.001
	Species	1	7.32	0.008
	Treatment × species	2	1.85	0.163

Plants at deep shade were grown at 3% light from July 2004 until December 2006, and at 1% light from December 2005 until the end of the experiment in March 2006

Piper 2007, but see Kobe 1997). NSC storage as a mechanism of shade tolerance has been mainly demonstrated in tropical species (Myers and Kitajima 2007; Poorter and Kitajima 2007). This raises the question about the generality of carbon storage as a mechanism to tolerate shade and also as to whether selective pressures experienced in shaded environments differ between tropical and temperate forests. There is evidence that tropical species invest more in anti-herbivore defenses than their temperate relatives (Hallam and Read 2006), suggesting that the former are exposed to a higher risk of defoliation. The ability to replace leaves lost to herbivores might therefore be more important for understorey survival in tropical forests than in their temperate counterparts.

The growth and survival advantage of *N. nitida* in the shade was associated with a higher leaf mass fraction than that of *N. dombeyi* (Fig. 4). Although we did not measure leaf area ratio (total leaf area/plant dry mass; the product of specific leaf area and leaf mass fraction), the two species likely also differed in this parameter, which is a key determinant of low-light carbon gain (Lusk 2004; Pearcy et al. 2004). In a previous study, where seedlings of both species were grown in $<1\%$ full light, leaf area ratio was higher in *N. nitida* despite the higher specific leaf area of *N. dombeyi*; interspecific differences in leaf area ratio were caused by differences in leaf mass fraction (Lusk and Del Pozo 2002). The higher leaf mass fraction of *N. nitida* (Fig. 4) likely promoted higher whole-plant carbon gain and, therefore, higher survival. Two lines of evidence suggest that the lower leaf mass fraction of *N. dombeyi* was due to faster leaf loss rates, rather than differences in allocation to foliage. First, at harvest time, more shed leaves were observed in pots of *N. dombeyi* than in those of *N. nitida*. Second, a field study found that leaf lifespan was 23% shorter in *N. dombeyi* than in *N. nitida* (Piper and Lusk, unpublished). Similarly, a comparative study of large seedlings of eight Chilean rainforest evergreens found that species differences in low-light leaf mass fraction were associated with variation in leaf lifespan (Lusk 2002).

Intraspecific comparisons

Intraspecific variation in NSC concentrations and NSC pools was not correlated with low-light survival probability of either species (Table 1). In contrast, Canham et al. (1999) found that both total content and concentration of NSC were good predictors of intraspecific variation in low-light seedling survival of four deciduous species. Differences between the carbon allocation patterns of evergreen and deciduous species might explain the divergence of our results from those of Canham et al. (1999). Deciduous species allocate more biomass to roots than evergreen species during the seedling stage, (Walters and Reich 1999) consistent with higher investment in storage (Kobe 1997). The classical view of deciduous species having higher dependence on carbon reserves for spring has been challenging by Hoch et al. (2003), who demonstrated that the higher levels of carbon reserves in deciduous trees are not used during the leaf-out period. These studies suggest that allocation to storage might be a more important adaptation to shade in deciduous temperate species than in their evergreen counterparts.

The significance of the high [NSC] of both species in 50% light (Fig. 5) is unclear. It might mean that carbon storage has a more important adaptive function in open environments than in shade: open sites are expected to undergo lower minimal temperatures and stronger summer drought than the understorey (de Freitas and Enright 1995; Porte et al. 2004; Zang et al. 2005), and NSC reserves are involved in mechanisms protecting against such stresses in many species (Chapin et al. 1990), including in these *Nothofagus* spp. (Alberdi 1995; Reyes-Diaz et al. 2005). Alternatively, the relative NSC concentration of sun and shade plants could reflect differences in growth limitations between the two environments: NSC accumulates whenever growth is more limited than photosynthesis (Li et al. 2002; Körner 2003). Summer drought, winter water-logging and low soil fertility affect many sites in southern Chilean (Armesto et al. 1995), and these factors might constrain the growth rates of plants in high light, despite their high rates of C gain (Chapin et al. 1990). Since the soil of plant pots used in this study did not receive any fertilizer, the depletion of soil nutrients might have promoted NSC accumulation. The summer drought also likely contributed to NSC accumulation; our potted seedlings had access to only a small volume of soil, likely accentuating the effect of summer drought.

NSC levels of plants in the 1% light environment (Fig. 5, Table 3) were low compared with most values reported in the literature. The root and stem NSC concentrations of ca. 100 mg g^{-1} in these plants were considerably lower than those measured in large seedlings of six rainforest evergreens (including *N. dombeyi*) growing in 2–5% light (Lusk and Piper 2007), consistent with more acute carbon limitation in 1% light. The fact that NSC comprised about 10% of the dry weight of dead plants in this treatment might seem inconsistent with death by shade-induced carbon starvation. This might reflect over-estimation of NSC by our method: extraction with perchloric acid can yield starch values higher than those estimated by enzymatic methods (Rose et al. 1991), probably as a result of hydrolysis of some cell wall components. Alternatively, some fraction of non-structural carbohydrates are likely involved in other essential functions (Kozlowski 1992), and cannot be mobilized to meet energy needs.

Conclusions

In conclusion, our comparison of two closely related species of evergreen *Nothofagus* showed that a difference in shade tolerance was not associated with differences in carbohydrate storage. This result adds to evidence found by other studies of temperate species (Canham et al. 1999; Machado and Reich 2006; Lusk and Piper 2007), challenging the generality of carbon reserves as a mechanism of shade tolerance. However, the faster low-light growth of *N. nitida* was consistent with this species having a more favorable carbon balance than its more light-demanding associate *N. dombeyi*. Intraspecific variation in carbohydrate storage was not correlated with survival under low light, and [NSC] of both species were much higher in high light than in shade. Patterns of variation in [NSC] of the temperate species we studied are therefore probably dominated by factors other than adaptation to shade, such as limitation of growth in high light by drought, waterlogging, and low fertility, or adaptation to cold stress.

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