# Changes during early development in photosynthetic light acclimation capacity explain the shade to sun transition in *Nothofagus nitida*

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**Summary**   *Nothofagus nitida* (Phil.) Krasser, an emergent tree of the Chilean evergreen forest, regenerates under the canopy. Nonetheless, it is common to find older saplings in clear areas. We hypothesized that this transition from shade to sun during the early developmental stages is made possible by an ontogenetic increase in the light acclimation capacity of photosynthesis. To test our hypothesis, we studied photosynthetic performance and photoprotection in *N. nitida* saplings at different developmental stages corresponding with three different height classes (short: 16.2 cm; medium-height: 48.0 cm; and tall: 73.7 cm) grown under contrasting light conditions (photosynthetic photon flux (PPF) of 20, 300 or 600 $\mu$mol m$^{-2}$ s$^{-1}$) until newly expanded leaves had developed. Light-saturated $CO_2$ assimilation rate and light compensation and saturation points increased with increasing PPF. Medium-height and tall saplings acclimated to high light had higher electron transport rates and higher proportions of open Photosystem II reaction centers than shorter plants acclimated to high light. Short saplings showed higher thermal dissipation and contents of xanthophylls than taller saplings. Only medium-height and tall saplings acclimated to high light recovered after photoinhibition. State transitions were higher in short plants growing in low light, and decreased with plant height and growth irradiance. Thus, during development, *N. nitida* changes the balance of light energy utilization and photoprotective mechanisms, supporting a phenotypic transition from shade to sun during its early ontogeny.

*Keywords: non-photochemical quenching components, photoacclimation, photoprotection, plant ontogeny, state transition, xanthophylls.*

## Introduction

*Nothofagus nitida* (Phil.) Krasser is a canopy-emergent species of the Chilean evergreen forest, common to the wet sectors of the coastal mountain range. It is distributed from 40° to 47° S. *Nothofagus nitida* regenerates successfully under the canopy (with a photosynthetic photon flux (PPF) of up to 30 $\mu$mol m$^{-2}$ s$^{-1}$), behaving similarly to extremely shade-tolerant species, such as *Podocarpus nubigena* Lindl. and *Laureliopsis philippiana* (Looser) Schodde (Lusk 2004, Veblen et al. 1996). Nonetheless, it is common to find older saplings growing in open areas with *Drimys winteri* J. R. Forst. & G. Forst. and *Eucryphia cordifolia* Cav., two sun species (Donoso 1993, Donoso and Lara 1998). As *N. nitida* develops, it acquires the capacity to grow in a higher light environment. This transition from shade to sun occurs early during development. In previous research it was found that *N. nitida* seedlings have the photosynthetic response typical of shade plants, whereas adult trees have the photosynthetic response typical of sun plants (Reyes-Díaz, unpublished results). As plants grow and cross the first canopy layers composed of ferns and shrubs, they are exposed to progressively higher irradiances (Kira and Yoda 1989). Several species change their light requirements throughout ontogeny (Lusk et al. 2008), and it is common to find transitions within a species from shade to sun as they grow taller (Poorter et al. 2005).

The photosynthetic apparatus of green plants is highly prone to damage by various types of stress (Walters 2005). Plants adjust their photosynthetic apparatus to the irradiance experienced during growth (Krause et al. 2001, Larcher 2003). Acclimation to high irradiances usually results in higher light-saturated rate of $CO_2$ assimilation ($A_{max}$), dark respiration rate ($R_d$), and light compensation (LCP) and saturation (LSP) points (Lambers et al. 1998, Larcher 2003). Light energy absorbed by chlorophyll can be used to drive charge separation at the reaction center and electron transport along the photosynthetic electron transport chain. These reactions can be probed by fluorescence studies of a process called photochemical quenching (qL). The excess energy can be dissipated as heat at the Photosystem II (PSII) antenna by a non-photochemical quenching process (NPQ) (Maxwell and Johnson 2000, Kramer et al. 2004).

Non-photochemical quenching can be divided in three components; the major and most rapidly relaxed component is the pH-dependent high energy quenching ($NPQ_f$), which involves changes in the PSII antenna proteins, and conversion of violaxanthin to zeaxanthin (Horton and Ruban 2005, Niyogi et al. 2005). High energy quenching is essential for photoprotection because it is involved in thermal dissipation of excess absorbed energy. A second component called state transition (qT), occurs when light harvesting complex (LCH) II is preferentially excited over LHCI. It involves the reversible phosphorylation of light-harvesting proteins that migrate from PSII to the PSI antenna complex, balancing the distribution of absorbed light between the two photosystems (Haldrup et al. 2001). It is unclear if qT participates in photoprotection; however, there is evidence that qT is a mechanism to increase photosystem efficiency when light is limiting (Mullineaux and Emlyn-Jones 2005). The third and slower relaxing component ($NPQ_s$) is a sustained slowly reversible component related to photoprotection and photodamage, and occurs when the light energy absorbed by leaves exceeds both the capacity for energy utilization in photosynthesis and of the photoprotective mechanisms (Demmig-Adams and Adams 1992). Dark relaxation of $NPQ_s$ usually requires damage repair of PSII, such as D1 turnover (Melis 1991). The physiological role of $NPQ_s$ is unclear because the apparent damage that results in the disassembly or major reorganization of pigment–protein complexes of PSII may contribute to a reduction in light absorption and hence to photoprotection (Walters and Horton 1991, Horton et al. 1996, Müller et al. 2001). Both NPQ and its components have been successfully used to explore differences in photoprotection and photoinhibition at the phenotype level (Demmig-Adams et al. 1995, Niyogi 1999), and photoinhibition effects on forest succession have been widely studied (Ball et al. 1991, 1997, Mulkey and Pearcy 1992, Osmond 1994, Kitao et al. 2000 and Slot et al. 2005).

Plants acclimated to high irradiances are less susceptible to photoinhibition and photodamage than shade plants (Gray et al. 1996, Savitch et al. 2000). A common photoprotective mechanism of the photosynthetic apparatus is the active down-regulation of PSII efficiency. This process is regulated by a trans-thylakoid pH gradient that induces the interconversion of pigments in the xanthophyll cycle. The ability to rapidly de-epoxidize violaxanthin to zeaxanthin allows the plant to cope with changes in the light environment (Horton et al. 1996). Plants acclimated to high irradiances often have an elevated concentration of xanthophyll cycle pigments (VAZ, violaxanthin, antheraxanthin, zeaxanthin) (Demmig-Adams 1990, Demmig-Adams and Adams 1992, Krause et al. 2001). Lutein and neoxanthin contribute to the de-excitation of singlet chlorophyll (Niyogi 1999) and serve as an additional mechanism of energy dissipation (Matsubara et al. 2005).

Differences in allometric variables have been reported between seedlings and saplings in relation to traits conferring shade tolerance in trees (Sack and Grubb 2003, Lusk 2004, Niinemets 2006). Nevertheless, studies on variations in leaf photosynthetic traits related to high-light tolerance and its relationships with seedling and sapling heights are scarce. The aim of our study was to examine how irradiance and plant ontogeny interact to trigger the shade to sun transition of photosynthesis in *N. nitida*. We studied changes in photosynthetic performance and photoprotection after light acclimation in plants differing in height. We hypothesized that this shade to sun transition is made possible by an ontogenetic increase in the light acclimation capacity of photosynthesis.

## Materials and methods

### Plant material and treatments

Plants of three height classes (mean ± SE; short: $16.2 \pm 0.9$ cm; medium height: $48.0 \pm 1.4$ cm; and tall: $73.7 \pm 1.7$ cm) were collected in a *N. nitida* forest stand in Katalapi Park, located in Pichiquillaipe, X Region, in south central Chile (41°31′8″ S, 72°45′2″ W). A significant linear regression ($r^2 = 0.83$) was obtained between plant height and age, indicating that the selected plants covered a range of plant ages (Figure 1). All plants were carefully transplanted to black plastic bags filled with organic soil mixed with pine bark compost (1:1 v/v) and maintained outdoors beneath a shade cloth (80% solar interception) for about 3 months. Seedlings were irrigated twice a week and fertilized with $0.2$ g $l^{-1}$ of a commercial fertilizer solution (14,10,22 N,P,K plus Mg, S and chelated trace elements) every 3 weeks. Before the experiments, pots were placed in controlled-environment chambers and maintained for 1 month at 15 °C in a 10-h photoperiod, with a PPF of $50$ μmol $m^{-2}$ $s^{-1}$ at the top of the canopy. To study the light acclimation process, plants of the three sizes were maintained at 15 °C in controlled-environment chambers at a PPF of 20, 300 or $600$ μmol $m^{-2}$ $s^{-1}$ at the top of the canopy in a 15-h photoperiod, for 45 days until new leaves had developed and newly expanded in the light acclimation treatments. The light source was a mix of cool white fluorescent tubes, incandescent bulbs and halogen lamps with water filters to maintain the leaf temperature between 16 and 21 °C.
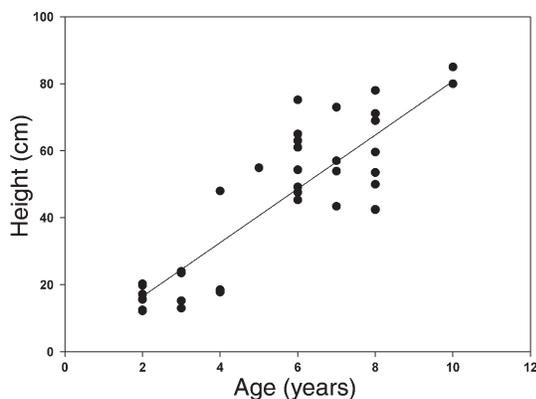


Figure 1. Relationship between plant height and age in *Nothofagus nitida* plants collected in the evergreen Chilean temperate rain forest. Plant age was determined by counting tree rings on discs taken from 2 cm height on the trunk ($n = 30$). Plant height = $-2.210 + (8.575 \times$ Age), $r^2 = 0.83$, $P < 0.001$.

*Net photosynthesis*

Net $CO_2$ assimilation at PPFs ranging from 0 to 1000 µmol $m^{-2} s^{-1}$ was measured in attached leaves with an infrared gas analyzer (Ciras-2, PP systems, Hitchin, U.K). The $CO_2$ reference concentration was 360 ppm, with a flow rate of 200 ml $min^{-1}$ and 75% external relative humidity. The temperature inside the leaf chamber was maintained at $15 \pm 2$ °C, which is within the optimum temperature range for photosynthesis in this species. Light compensation point, LSP, $A_{max}$ and $R_d$ were calculated with the Photosynthesis Assistant software 1.1 (Dundee Scientific, Dundee, U.K.). All measurements were performed between 0900 and 1300 h. When small leaves did not cover the entire leaf cuvette surface (2.5 $cm^2$), a digital photograph of the leaf was taken immediately after the gas exchange measurement and its area estimated with the Sigma Scan Pro 5.0 software (SPSS, Chicago, IL). Measured gas exchange values were adjusted for the cuvette area/actual leaf area ratio.

*Chlorophyll fluorescence measurements*

Fluorescence signals were measured by a pulse-amplitude modulated fluorimeter (FMS 2, Hansatech Instruments, King's Lyn, U.K) in detached newly expanded leaves collected from the upper third of the foliated shoot. Leaves were dark adapted for 30 min. All measurements were performed at 15 °C controlled with an LD2/3 electrode chamber (Hansatech Instruments). Different light pulses (see below) were applied following standard routines programed in the machine. According to the terminology of Van Kooten and Snel (1990), minimal fluorescence ($F_o$) was determined by applying a weak modulated light (0.4 µmol $m^{-2} s^{-1}$) and maximal fluorescence ($F_m$) was induced by a short pulse (0.8 s) of saturating light (9000 µmol $m^{-2} s^{-1}$). After 10 s, an actinic light was turned on for 5 min to obtain fluorescence parameters during steady-state photosynthesis. Saturating pulses were applied after a steady-state of photosynthesis was reached to determine maximal fluorescence in the light ($F_m'$) and steady-state fluorescence in the light ($F_s$). Finally, the actinic light was turned off and immediately a 2-s far-red (FR) pulse was applied to obtain minimal fluorescence after light-driven steady state ($F_o'$). The electron transport rate (ETR) was calculated according to Genty et al. (1989) as: ETR = 0.5($\phi$PSII)(PPF)0.84, where $\phi$PSII = $(F_m' - F_s)/F_m'$ is effective quantum yield of PSII, PPF is incident photosynthetic photon flux, the factor 0.5 assumes that the efficiency of the two photosystems is equal and that light is equally distributed between them and the factor 0.84 is the mean value of absorbance for green leaves (Demmig-Adams et al. 1987). No specific leaf absorbance measurement for *N. nitida* was carried out. The fraction of PSII centers in the open state (qL) was calculated as described by Kramer et al. (2004): qL = $((F_m' - F_s)/(F_m' - F_o'))(F_o'/F_s)$. Non-photochemical quenching was calculated as: NPQ = $(F_m - F_m')/F_m'$ (Bilger and Björkman 1990). Fluorescence measurements were performed at PPFs of 19, 103, 275, 577 and 998 µmol $m^{-2} s^{-1}$. Radiation at the leaf surface was calibrated with an LI-250 light meter (Li-Cor, Lincoln, NE).

*NPQ component determinations*

The NPQ components were determined in detached newly expanded leaves at 15 °C with an FMS 2 fluorimeter. The NPQ was resolved into $NPQ_s$ and $NPQ_f$ as described by Maxwell and Johnson (2000). Briefly, actinic light with a PPF of 20, 300 or 600 µmol $m^{-2} s^{-1}$ (hereafter referred to as before photoinhibitory treatment) and 2000 µmol $m^{-2} s^{-1}$ (hereafter referred to as the photoinhibitory treatment) was supplied for 2 h by an LS2 white light source (Hansatech Instruments) connected to an LD2/3 electrode chamber. The actinic light was then turned off and recovery kinetics were analyzed during a 1-h dark period. During the recovery period, saturating pulses of light were applied to obtain $F_m$. The NPQ components were calculated as: $NPQ_s = (F_m - F_m^r)/F_m^r$ and $NPQ_f = (F_m/F_m') - (F_m/F_m^r)$, where $F_m^r$ is the maximum fluorescence yield that would have been attained if there had been only slow quenching relaxation. We obtained $F_m^r$ by extrapolation of measurements recorded toward the end of relaxation back to the time when the actinic light was turned off in a semilogarithmic plot of maximum fluorescence yield versus time. To evaluate if the light acclimation treatments caused photochemical damage to the leaf, determinations of the NPQ components were also made before the 2000 µmol $m^{-2} s^{-1}$ treatment.

*State transition determinations*

The relative capacity for State 1–State 2 transitions was estimated at 15 °C as described by Lunde et al. (2000), with an FMS 2 fluorimeter and an LD2/3 electrode chamber. Blue light (100 µmol $m^{-2} s^{-1}$ at the leaf surface), which favors PSII, was provided by an LS2 white light source (Hansatech Instruments) equipped with a Corning 4-86 blue filter. Far-red light, which favors PSI, was provided as 214 µmol $m^{-2} s^{-1}$ ($\lambda$ = 735 nm) at the leaf surface by the FMS 2 fluorimeter. Relative state transitions (Fr) were calculated as Fr = $[(F_i' - F_i) - (F_{ii}' - F_{ii})]/(F_i' - F_i)$, where $F_i'$ and $F_{ii}'$ denote fluorescence in the absence of PSII light in State 1 and State 2, respectively (Lunde et al. 2000).

*Pigment determinations*

Newly expanded leaves at the top of the canopy were collected for pigment determinations at midday on Day 45 of the light acclimation treatments. Leaves (100 mg fresh mass) were ground to a fine powder with liquid nitrogen in a cold mortar. About 1 mg of $CaCO_3$ was added before grinding. Pigments were extracted in 100% (v/v) HPLC-grade acetone at 4 °C in dim light. The supernatant was filtered through a 0.22 µm syringe filter. These samples were stored at −80 °C in an $N_2$ atmosphere until analyzed. Before analysis, samples were homogenized in cold acetone and centrifuged at 3000 *g* for 5 min. This extraction was repeated twice. The resulting extract was dried in a stream of nitrogen and partitioned with 1:1 (v/v) petroleum ether (35–60°): methanol containing 10% of an aqueous 10% w/v NaCl solution. The methanolic hypophase was recovered and used immediately for pigment separation and quantification by HPLC analysis as described previously (Ivanov et al. 1995) on a chromatographic system LC-6A

equipped with a SPA-6AV UV/Vis detector (Shimadzu Corporation, Tokio, Japan) and a Waters YMC ODS-A column (5 μm particle size 4.6 × 250 mm). The flow rate for all separations was 0.5 ml min$^{-1}$, and all sample injections volumes were 20 μl. The solvent was HPLC-grade methanol:dichloromethane (95:5, v/v). All runs were performed in dim light at room temperature. Standards for neoxanthin (N), violaxanthin (V), antheraxanthin (A), zeaxanthin (Z) and lutein (L) were obtained from DHI Water & Environment (Hörsholm, Denmark) and were dissolved in 100% ethanol. The extinction coefficients for l g$^{-1}$ cm$^{-1}$ were: 224 for neoxanthin at 439 nm; 255 for lutein at 455 nm; 255 for violaxanthin at 443 nm; 235 for antheraxanthin at 446 nm; and 254 for zeaxanthin at 450 nm. Linearity of the peak-area (absorbance units × minutes) calibration against pigment concentration was $r^2 = 0.991$ for all pigment standards. The pigment concentrations were expressed on a chlorophyll a basis. Xanthophyll pool size (VAZ) was determined as: VAZ = A + V + Z and the de-epoxidation state (DEPS) of the pigment pool was calculated as: DEPS = (Z + 0.5A)/(V + A + Z).

*Statistical analysis*

Reported values correspond to the means of five measurements made on five plants. Data were subjected to a two-way analysis of variance (ANOVA) where the factors were plant height and light acclimation treatment. Before ANOVA, data were checked for normality and homogeneity of variances. To simplify the post hoc interpretation of the fluorescence light curves, the analysis was performed only at two independent fixed irradiances, 300 μmol m$^{-2}$ s$^{-1}$ (medium light acclimation treatment) and 1000 μmol m$^{-2}$ s$^{-1}$ (maximum irradiance during measurement), for each plant height. A Tukey HSD-test was used specifically for fluorescence light-curve post hoc analysis. For all other measurements, a Fischer LSD-test was used for post hoc analy-

sis. Differences between values were considered significant at $P = 0.05$.

## Results

*Light response curves of CO₂ assimilation*

A 114% increase in LCP in response to the highest light acclimation treatment (a PPF of 600 μmol m$^{-2}$ s$^{-1}$) was observed in short plants (Figure 2a). In contrast, LCPs in medium-height and tall plants did not differ significantly between light acclimation treatments. Within a light acclimation treatment, there were no differences in LCP between plants of different sizes (Figure 2a). The high light acclimation treatment significantly increased the LSP of plants in all height classes by about 160%. Plant size had no effect on LSP in any light treatment (Figure 2b). Dark respiration was unaffected by light treatment or plant height (Figure 2c). In response to the highest light acclimation treatment (a PPF of 600 μmol m$^{-2}$ s$^{-1}$), $A_{max}$ increased 59% in short plants and 116% in tall plants. Within light acclimation treatments, differences in $A_{max}$ between plant heights were not significant (Figure 2d).

*Chlorophyll fluorescence response curves*

Electron transport rate, qL and NPQ were significantly affected by the interaction between light acclimation and plant height ($P = 0.001$). The ETR of short plants remained almost constant at a PPF of 20 μmol m$^{-2}$ s$^{-1}$ in all acclimation treatments (Figure 3a). Medium-height and tall plants acclimated to a PPF of 600 μmol m$^{-2}$ s$^{-1}$ had a 250% higher ETR than plants acclimated to a PPF of 300 μmol m$^{-2}$ s$^{-1}$ at the high measurement PPFs (Figures 3b and 3c). Short plants exhib-
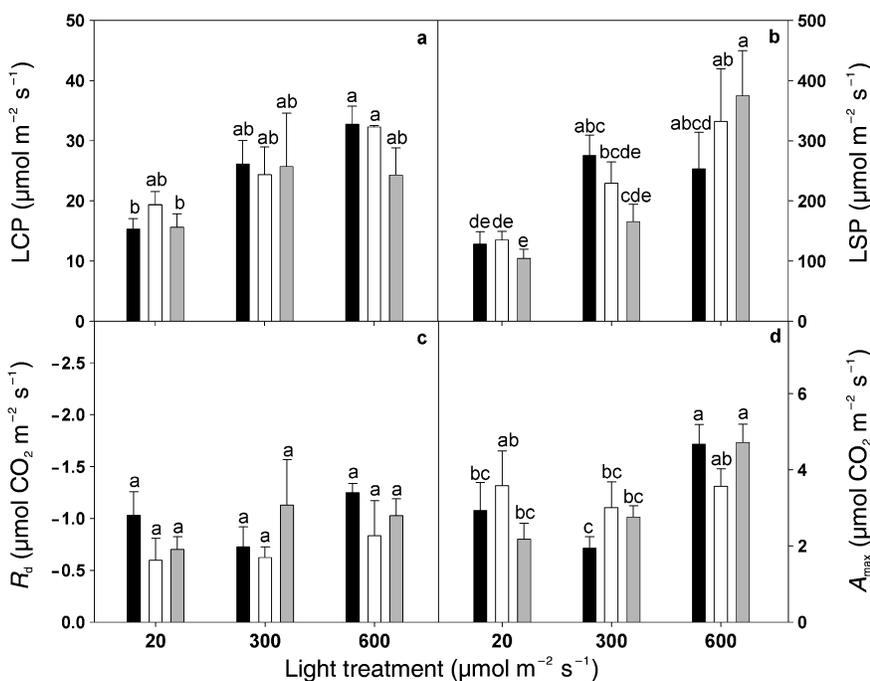
Figure 2. Light acclimation and plant size effects on parameters derived from photosynthetic light response curves in *Nothofagus nitida*: (a) light compensation point (LCP); (b) light saturation point (LSP); (c) dark respiration ($R_d$); and (d) maximal net photosynthetic rate ($A_{max}$). Before the measurements, plants of three height classes (short, 16.2 ± 0.9 (black bars); medium height, 48.0 ± 1.4 (white bars); and tall, 73.7 ± 1.7 (gray bars)) were acclimated for 45 days to a photosynthetic photon flux of 20, 300 or 600 μmol m$^{-2}$ s$^{-1}$. Different lowercase letters above the bars indicate statistically significant differences between light acclimation treatment and plant height, as evaluated by the Fischer LSD-test ($n = 5$, $P = 0.05$).

ited similar qL in all light acclimation treatments (Figure 3d). Generally, medium and tall plants acclimated to a PPF of 600 μmol m$^{-2}$ s$^{-1}$ had higher qL than medium-height and tall plants acclimated to the other light treatments, which showed a similar decrease in qL as actinic irradiance increased (Figures 3e and 3f). In short plants, qL was similar in all light acclimation treatments (Figure 3d). Non-photochemical quenching different significantly between light acclimation treatments only in short plants, increasing by 60 and 140% compared with the taller plants acclimated to a PPF of 300 or 600 μmol m$^{-2}$ s$^{-1}$, respectively (Figure 3g). In medium-height and tall plants, NPQ increased up to 300 μmol m$^{-2}$ s$^{-1}$ remaining relatively constant at higher measurement PPFs (Figure 3h and 3i).

*Non-photochemical quenching and its components under photoinhibitory conditions*

Before application of the photoinhibitory treatment, we evaluated NPQ and its components in short, medium-height and tall plants by exposure to actinic light at the same irradiance used for the light acclimation treatments. Plants grown at a PPF of 20 μmol m$^{-2}$ s$^{-1}$ had low NPQ values compared with plants grown at a PPF of 300 or 600 μmol m$^{-2}$ s$^{-1}$. In general, NPQ was independent of plant height (Figures 4a–c, dark bars). After application of the photoinhibitory treatment—2000 μmol m$^{-2}$ s$^{-1}$ for 2 h—medium-height and tall plants acclimated to a PPF of 20 μmol m$^{-2}$ s$^{-1}$ and short plants acclimated to a PPF of 300 μmol m$^{-2}$ s$^{-1}$ increased their NPQ significantly more than non-photoinhibited controls (Figures 4a and 4b), but no significant differences were observed between medium-height and tall plants acclimated to a PPF of 300 μmol m$^{-2}$ s$^{-1}$ (Figure 4b). Plants acclimated to a PPF of 600 μmol m$^{-2}$ s$^{-1}$ exhibited a lower NPQ than the non-photoinhibited controls (Fig-

ure 4c). The fast-relaxing component was predominant for all acclimation conditions and plant sizes, representing about 50, 75 or 92% of the NPQ values for plants acclimated to 20, 300 or 600 μmol m$^{-2}$ s$^{-1}$, respectively. The NPQ$_f$ increased with light acclimation treatment independently of plant size (Figures 4d–f). The slow-relaxing component represented about 42, 26 or 8% of total NPQ for plants acclimated to a PPF of 20, 300 or 600 μmol m$^{-2}$ s$^{-1}$, respectively (Figures 4g–i). After photoinhibition, NPQ components were evaluated only in medium-height (which had similar values of NPQ$_f$ and NPQ$_s$) and tall plants (95% NPQ$_f$ and 5% NPQ$_s$) acclimated to a PPF of 300 or 600 μmol m$^{-2}$ s$^{-1}$ (Figures 4d–i), because there was no significant $F_m$ recovery during the dark period in plants in the other treatments over the evaluation time (Figures 5a and 5b).

*Carotenoid pigments*

The light acclimation treatments resulted in significant increases in xanthophyll pool size in plants in all height classes ($P = 0.001$). The short plants acclimated to a PPF of 600 μmol m$^{-2}$ s$^{-1}$ had the highest VAZ increase (326%) (Figure 6a). In medium-height and tall plants acclimated to a PPF of 300 or 600 μmol m$^{-2}$ s$^{-1}$, VAZ concentrations increased by 104 and 178%, respectively. The de-epoxidation state of the xanthophyll pool increased (20%) significantly only in short plants in the highest light acclimation treatment (Figures 6a–c). A stronger quadratic relationship was found between NPQ$_f$ and VAZ ($r^2 = 0.83$, $P < 0.001$) than between NPQ$_f$ and DEPS ($r^2 = 0.36$, $P < 0.001$) (Figures 7a and 7b). Lutein concentration was about 100% higher in plants acclimated to a PPF of 300 or 600 μmol m$^{-2}$ s$^{-1}$ than in plants acclimated to a PPF of 20 μmol m$^{-2}$ s$^{-1}$ (Figure 8a). The highest lutein concentration was found in short plants acclimated to a PPF of 600 μmol m$^{-2}$
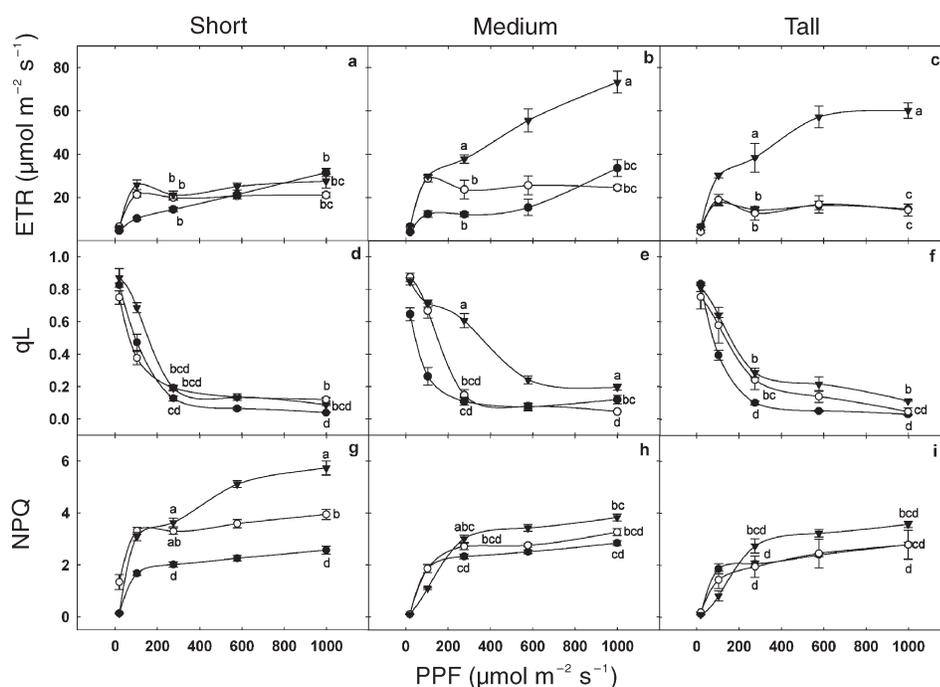


Figure 3. Electron transport rate (ETR), relative redox state of PSII (qL) and non-photochemical quenching (NPQ) of light-acclimated *Nothofagus nitida* plants of different heights in response to increased actinic irradiance. Before exposure to actinic light, plants of three height classes (short, 16.2 ± 0.9; medium height, 48.0 ± 1.4; and tall, 73.7 ± 1.7) were acclimated for 45 days to a photosynthetic photon flux of 20, 300 or 600 μmol m$^{-2}$ s$^{-1}$ (●, ○ or ▼, respectively). Within an actinic light irradiance, different lowercase letters indicate significance differences between light acclimation treatments based on a Tukey HSD test ($n = 5$, $P = 0.05$).
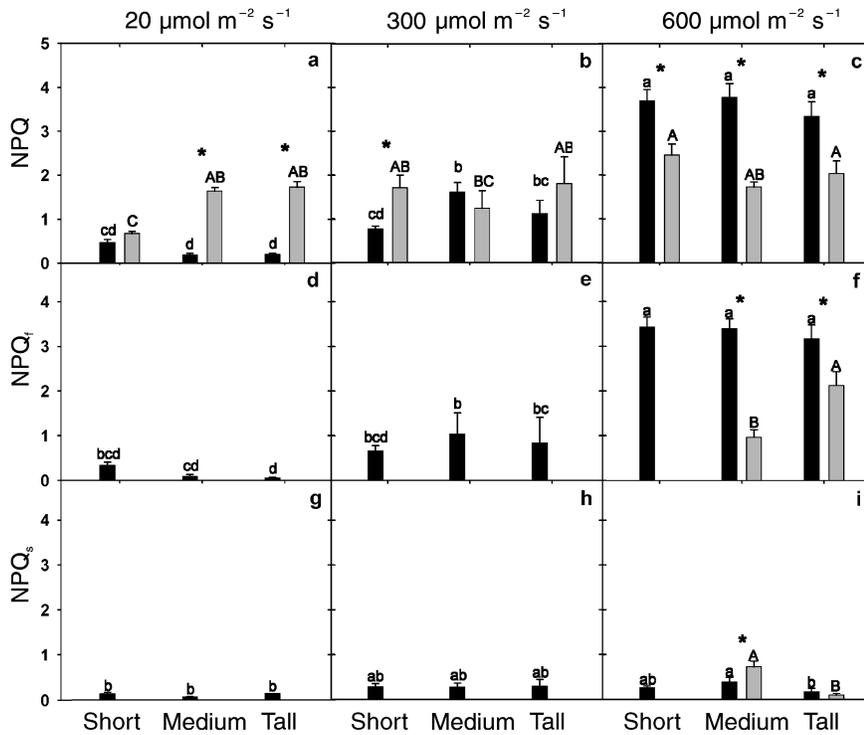
Figure 4. Responses of non-photochemical quenching (NPQ) and its components ($NPQ_f$ and $NPQ_s$) to a photoinhibitory treatment in light-acclimated *Nothofagus nitida* plants. Before application of the photoinhitory treatment (a photosynthetic photon flux (PPF) of 2000 µmol m$^{-2}$ s$^{-1}$ for 2 h), plants of three height classes (short, 16.2 ± 0.9; medium height, 48.0 ± 1.4; and tall, 73.7 ± 1.7) were acclimated for 45 days to a PPF of 20, 300 or 600 µmol m$^{-2}$ s$^{-1}$. All measurements were made in dark-adapted newly fully expanded leaves at 15 °C. Missing bars indicate that there was no recovery of $F_m$ in the dark (see Figure 5). Different lower- and uppercase letters above the bars indicate significant differences based on the Fischer LSD test ($n = 5$, $P = 0.05$) in NPQ, $NPQ_f$ and $NPQ_s$ before and after photoinhibition as a result of light acclimation treatment and plant height, respectively. An asterisk (*) indicates significant differences in NPQ, $NPQ_f$ and $NPQ_s$ before (black bars) and after (gray bars) the photoinhibition treatment based on a Student's *t*-test ($n = 5$, $P = 0.05$).

s$^{-1}$ (Figure 8a). There was a significant interaction between plant height and light acclimation treatment on neoxanthin concentration ($P = 0.001$) (Figure 8b), with short plants acclimated to a PPF of 600 µmol m$^{-2}$ s$^{-1}$ having the highest neoxanthin concentrations (Figure 8b).

*State transitions*

Among plant sizes and acclimation treatments, short plants acclimated to a PPF of 20 µmol m$^{-2}$ s$^{-1}$ had the highest capacity for Fr (about 0.8), with short plants acclimated to a PPF of 300

or 600 µmol m$^{-2}$ s$^{-1}$ having 51 and 90% lower Fr values, respectively (Figure 9). Medium-height and tall plants showed a similar tendency, but with lower Fr values than short plants acclimated to a PPF of 20 µmol m$^{-2}$ s$^{-1}$. There were no significant differences in Fr between medium-height and tall plants acclimated to a PPF of 300 or 600 µmol m$^{-2}$ s$^{-1}$ (Figure 9).

**Discussion**

The light acclimation treatments significantly affected param-
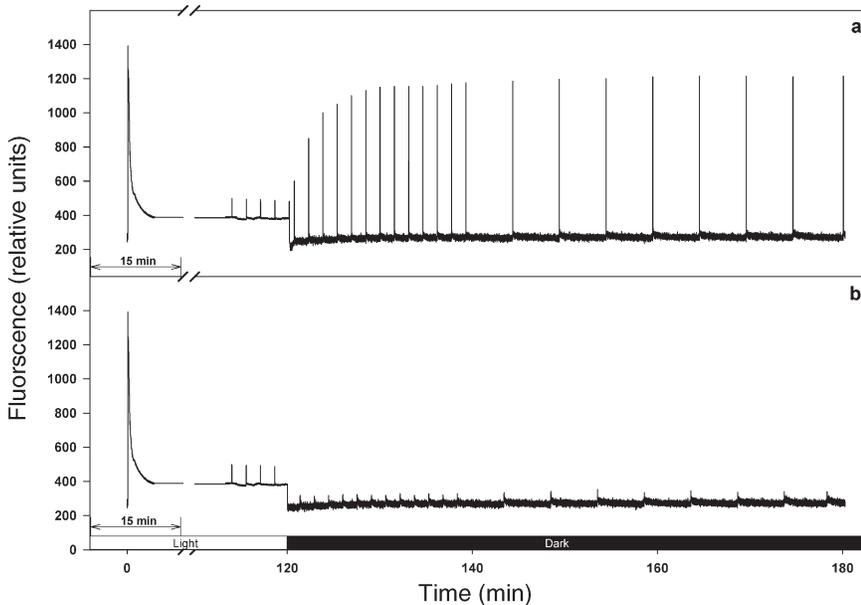


Figure 5. Typical fluorescence trace of dark recovery kinetics after photoinhibitory treatment (to a PPF of 2000 µmol m$^{-2}$ s$^{-1}$ at 15 °C by 2 h) in (a) medium-height and tall *Nothofagus nitida* plants acclimated to a photosynthetic photon flux (PPF) of 600 µmol m$^{-2}$ s$^{-1}$ and (b) short plants acclimated to a PPF of 20 µmol m$^{-2}$ s$^{-1}$. All measurements were performed on dark-adapted newly expanded leaves.
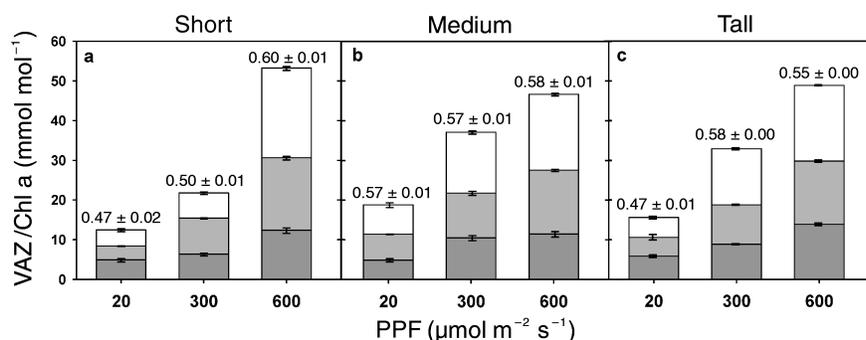
Figure 6. Concentrations of violaxanthin (dark gray), antheraxanthin (light gray) and zeaxanthin (white bars) (mmol mol$^{-1}$ Chl a) in *Nothofagus nitida* plants of different height classes (short, 16.2 ± 0.9; medium height, 48.0 ± 1.4; and tall, 73.7 ± 1.7) that were acclimated for 45 days to a photosynthetic photon flux (PPF) of 20, 300 or 600 µmol m$^{-2}$ s$^{-1}$. Standard errors (SE) are indicated on each bar ($n$ = 3), and the numbers (means ± SE) above the bars indicate the de-epoxidation state (DEPS) of the pigment pool calculated as: DEPS = (Z + 0.5A)/(V + A + Z), where: A = antheraxanthin; V = violaxanthin; and Z = zeaxanthin.

eters obtained from the light response curves of CO$_2$ assimilation. The higher LCPs, LSPs and $A_{max}$ in plants acclimated to a PPF of 600 µmol m$^{-2}$ s$^{-1}$ than of 300 or 20 µmol m$^{-2}$ s$^{-1}$ reflected the transition from shade to sun leaves that are indicative of photosynthetic light acclimation (Givnish 1988, Demmig and Winter 1988). Nevertheless, we found no effects of plant height on these parameters over the range of plant heights investigated (15 to 75 cm). Thus, an increase of more than 100% in $A_{max}$ was observed between plants acclimated to a PPF of 20 and 600 µmol m$^{-2}$ s$^{-1}$, independently of plant height, indicating that even short *N. nitida* plants grown in relatively high irradiances are capable of adjusting their carbon assimilation rates following high light exposure. Conversely, plants of all heights acclimated to a PPF of 20 or 300 µmol m$^{-2}$ s$^{-1}$ were unable to increase their carbon uptake when suddenly exposed to high irradiances, suggesting that regeneration of *N. nitida* forests could be limited by sudden large increases in irradiance providing insufficient time for photo-acclimation. Such a scenario could occur when plants acclimated to low irradiances in the forest understory are exposed to sun flecks or high PPFs as a result of sudden canopy gap formation.

Depending on growth conditions and ontogenetic stage, a particular irradiance may be more or less excessive for the photosynthetic apparatus of a given species. Thus, carbon fixation capacity and excess energy dissipation determine the degree of plant tolerance to high PPFs. We assessed both properties by fluorescence light curves in newly expanded leaves ac-

climated to different PPFs. Medium-height and tall plants acclimated to a PPF of 600 µmol m$^{-2}$ s$^{-1}$ were able to increase their ETRs in response to exposure to high incident radiation. A high capacity for light energy utilization implies a high potential to safely quench potentially damaging excitation energy via photosynthesis (Demmig and Winter 1988). An increased qL was detected in medium-height and tall plants acclimated to a PPF of 600 µmol m$^{-2}$ s$^{-1}$ in response to sudden exposure to high light, indicating that a high proportion of the absorbed energy was directed toward photochemistry and that this group of plants was able to maintain a low excitation pressure on PSII. The lower light saturation of NPQ in plants acclimated to a PPF of 20 or 300 µmol m$^{-2}$ s$^{-1}$ than in plants acclimated to a PPF of 600 µmol m$^{-2}$ s$^{-1}$ indicates that the degree of photoprotection by thermal dissipation of excess excitation energy could be insufficient at high irradiances in the field, and may lead to the formation of reactive oxygen species in these plants (Müller et al. 2001). However, in response to sudden exposure to high light, short plants grown at a PPF of 600 µmol m$^{-2}$ s$^{-1}$ increased their thermal dissipation capacity by about 30% over that of short plants grown under a PPF of 20 or 300 µmol m$^{-2}$ s$^{-1}$, indicating that, although short plants were unable to adjust their photochemical process to high light, the light acclimation treatment enhanced non-photochemical thermal dissipation, perhaps enabling the plants to cope with the stochastic events of increased excitation pressure. These results support the idea that both progressive exposure to
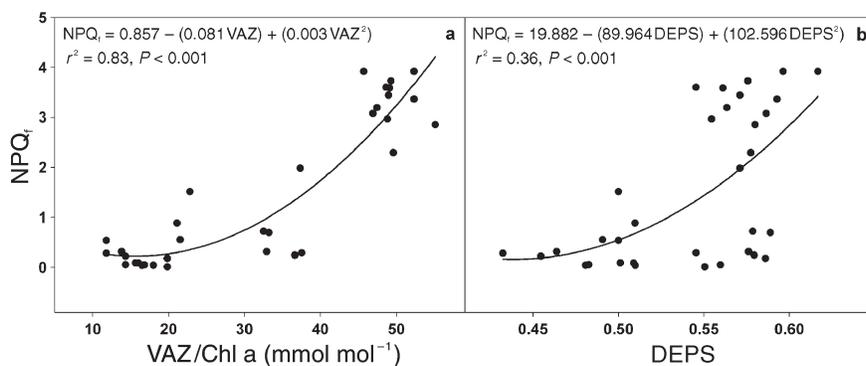


Figure 7. Fast non-photochemical component (NPQ$_f$) in response to xanthophyll pool sizes (VAZ = V + A + Z) and de-epoxidation state of the pigments pool (DEPS = (Z + 0.5A)/(V + A + Z)) measured in *Nothofagus nitida* plants of three height classes (short, 16.2 ± 0.9; medium height, 48.0 ± 1.4; and tall, 73.7 ± 1.7) acclimated for 45 days to a photosynthetic photon flux of 20, 300 or 600 µmol m$^{-2}$ s$^{-1}$. Abbreviations: A = antheraxanthin; V = violaxanthin; and Z = zeaxanthin.
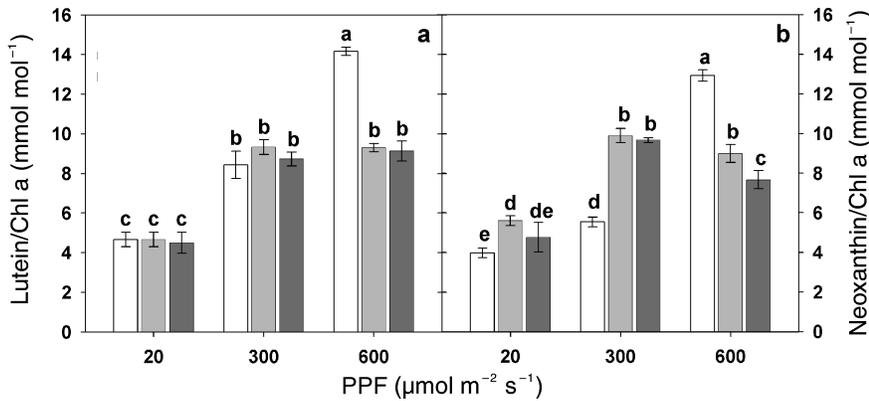
Figure 8. Concentrations of lutein and neoxanthin in *Nothofagus nitida* plants of different height classes (short, 16.2 ± 0.9 (white bars); medium height, 48.0 ± 1.4 (light gray bars); and tall, 73.7 ± 1.7 (dark gray bars)) that were acclimated for 45 days to a photosynthetic photon flux (PPF) of 20, 300 or 600 $\mu$mol m$^{-2}$ s$^{-1}$. Standard errors are indicated, and different lowercase letters above the bars indicate significant treatment differences based on the Fischer LSD-test ($n = 5$, $P = 0.05$).

increased irradiance and ontogenetic changes are responsible for the adjustment of the *N. nitida* photosynthetic apparatus during the early developmental stages.

Non-photochemical quenching has at least three components that can be resolved by studying their fluorescence dark relaxation kinetics (Walters and Horton 1991). We resolved NPQ into NPQ$_f$ and NPQ$_s$. The NPQ$_f$ is driven by $\Delta$pH and reflects thermal dissipation mediated by the xanthophyll cycle. The NPQ$_s$ is related to photodamage in plants exposed to photoinhibitory irradiances. Plants of all heights acclimated to a PPF of 600 $\mu$mol m$^{-2}$ s$^{-1}$ before the photoinhibitory treatment showed increased NPQ$_f$ (similar to NPQ) and low values of NPQ$_s$ (Figure 4) and qL, indicating that a PPF of 600 $\mu$mol m$^{-2}$ s$^{-1}$ was saturating, but that all plants were able to dissipate
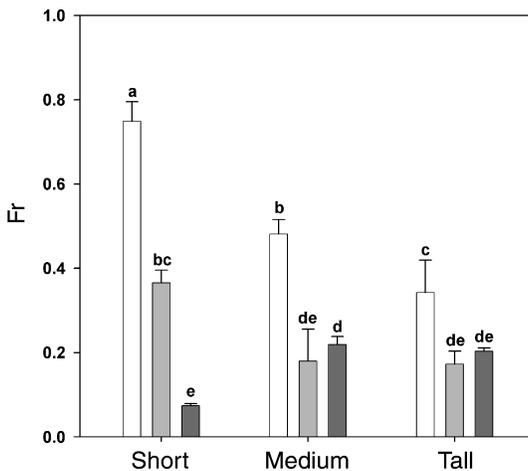


Figure 9. Changes in the capacity for state transitions (Fr) in newly expanded leaves of *Nothofagus nitida* plants of different height classes (short, 16.2 ± 0.9; medium height, 48.0 ± 1.4; and tall, 73.7 ± 1.7) that were acclimated for 45 days to a photosynthetic photon flux of 20, 300 or 600 $\mu$mol m$^{-2}$ s$^{-1}$ (white, light gray and dark gray bars, respectively). Values of Fr were estimated from fluorescence measurements at 15 °C. The newly expanded leaves that had developed in the light acclimation treatments were dark-adapted for 30 min before measurement and then exposed to either light favoring PSII (blue) or light favoring PSI (far-red). Standard errors are indicated, and different lowercase letters above the bars indicate significant treatment differences based on Fischer LSD-test ($n = 5$, $P = 0.05$).

the excess absorbed energy harmlessly, with minimal photodamage (measured as an increase in NPQ$_s$). The NPQ in plants acclimated to a PPF of 600 $\mu$mol m$^{-2}$ s$^{-1}$ decreased after severe photoinhibition (Figure 4c), probably implying partial impairment of xanthophyll cycle function, as reflected by the reduction in NPQ$_f$ observed after the photoinhibitory treatment (Figure 4f). The reduction in NPQ after photoinhibition was not observed in plants in the other light treatments. It is likely that the actinic light used to evaluate NPQ (irradiance with actinic light was the same as used for the light acclimation treatments) before applying the photoinhibitory treatment was insufficient to attain maximum thermal dissipation, especially in the short plants (Figure 4a). An increased capacity for non-photochemical dissipation by leaves developed at high PPFs had been observed previously (Demmig-Adams and Adams 1992) and indicates high light acclimation (Demmig-Adams et al. 1995, Ramalho et al. 2000, Walters 2005). The NPQ$_s$, which is analogous to chronic photoinhibition (photodamage), is slowly reversible. This component has been associated with prolonged exposure to light stress, sustained downregulation and photodamage (Werner et al. 2002, Horton and Ruban 2005), and usually describes a situation in which photosynthetic efficiency remains low after hours of darkness. The plants acclimated to a PPF of 20 or 300 $\mu$mol m$^{-2}$ s$^{-1}$ before application of the photoinhibitory treatment had significantly lower NPQ values than plants acclimated to a PPF of 600 $\mu$mol m$^{-2}$ s$^{-1}$, and they did not recover after a 1-h dark period, suggesting severe damage of PSII and indicating that the photoinhibitory treatment far exceeded the capacity of the plants for zeaxanthin conversion and thermal dissipation (Demmig-Adams et al. 1987). However, poor recovery was also observed in short plants acclimated to a PPF of 600 $\mu$mol m$^{-2}$ s$^{-1}$ that apparently retained an intact VAZ pool and de-epoxidation capacity (Figure 6a). It seems that the additional photochemical energy conversion observed in medium-height and tall plants that keeps the plastoquinone pool more oxidized at higher irradiances (Figures 3d–f) may play an essential role in photoprotection in *N. nitida*.

The high values of NPQ$_f$ obtained before photoinhibitory induction in plants of all sizes acclimated to a PPF of 600 $\mu$mol m$^{-2}$ s$^{-1}$ were consistent with the high VAZ and DEPS in these plants. Zúñiga et al. (2006) reported similar values of VAZ and

DEPS measured at midday in summer in *N. nitida* seedlings growing under light-exposed field conditions. In many plants growing under a variety of conditions, it has frequently been reported that the amount of zeaxanthin synthesized by the xanthophyll cycle is highly correlated with the amount of NPQ (Demmig-Adams 1990, Xu et al. 2000). We found a stronger quadratic relationship between $NPQ_f$ and VAZ ($r^2 = 0.83$) than between $NPQ_f$ and DEPS ($r^2 = 0.36$) (Figure 7), indicating that the amount of VAZ better explains the increase in thermal energy dissipation than the relative fraction of zeaxanthin in the pool. An important role of the lutein–lutein depoxide cycle in photoprotection during acclimation to high radiation in shade plants has been documented previously (García-Plazaola et al. 2004, Matsubara et al. 2005). Lutein and neo-xanthin concentrations were 67 and 44% higher in short *N. nitida* plants than in larger plants acclimated to a PPF of 600 µmol m$^{-2}$ s$^{-1}$. Among plant sizes and light acclimation treatments, the highest NPQ was observed in the same short plants (Figure 3g).

To optimize photosynthetic performance and avoid damage when exposed to excess light, plants balance excitation of the two photosystems (Haldrup et al. 2001) through state transitions (Fr), another non-photochemical quenching component. Among plant heights and light acclimation treatments, the highest capacity for Fr was in short plants acclimated to a PPF of 20 µmol m$^{-2}$ s$^{-1}$. The lower Fr values in plants acclimated to a PPF of 300 or 600 µmol m$^{-2}$ s$^{-1}$ compared with plants ac-climated to a PPF of 20 µmol m$^{-2}$ s$^{-1}$ agree with the findings of Mullineaux and Emlyn-Jones (2005) who propose that state transitions maximize the efficiency of utilization of absorbed light energy under conditions when growth is strongly light limited. Thus, short plants of *N. nitida* growing in a PPF of 20 µmol m$^{-2}$ s$^{-1}$ should be better adapted to grow in shady en-vironments. In addition, plants of all sizes acclimated to a PPF of 20 µmol m$^{-2}$ s$^{-1}$ showed a greater capacity to balance the absorbed excitation energy between photosystems in the short term than plants acclimated to higher irradiances. State transi-tions are a rapid mechanism that could reduce the rate of PSII photodamage by minimizing PSII antenna size (Wollman 2001), enabling short plants acclimated to low irradiances to balance the energy between photosystems in environments with rapid and pronounced fluctuations in excess light. Our re-sults indicate a shift in the capacity for state transitions among short, medium-height and tall plants of *N. nitida* that can be re-lated to the shade–sun transition of *N. nitida* plants previously observed in the field (Donoso 1993, Veblen et al. 1996, Reyes-Díaz, unpublished results) and to the finding that the flexibility of the response to a variable light environment rep-resents a key factor for seedling establishment in forest under-stories (Lambers et al. 1998, Larcher 2003).

The data presented confirm our hypothesis that the light ac-climation capacity of the photosynthetic apparatus depends on developmental stage and on light availability. A high $CO_2$ up-take capacity in the medium-height and tall plants acclimated to high light was accompanied by a high electron transport rate and little non-photochemical fluorescence quenching. The short plants were able to increase their heat dissipation follow-

ing acclimation to high light; however, in general, they had a more efficient photosynthetic apparatus to cope with limiting rather than excessive light compared with the taller plants. We observed increased susceptibility to photoinhibition in short plants acclimated to low light. Part of the increased tolerance to photoinhibition in the taller plants can be associated with structurally more robust foliage (Niinemets 2002). We ob-served several changes in the balance of photochemical energy conversion versus energy dissipation during early ontogenetic stages in *N. nitida*. Thus short seedlings naturally growing un-der several strata of the canopy had photosynthetic machinery that was efficient in a light-limited environment, as well as a basic capacity for dissipating short-term excess energy that is probably received naturally from sun flecks. As *N. nitida* plants grow, the foliage is exposed to new light environments and new leaves develop in higher irradiances, leading to in-creased carbon fixation and electron transport capacity. There-fore, under natural unperturbed conditions, there is an ongoing interaction between plant size and light acclimation in the phenotypic transition from shade to sun plant during the early ontogenetic stages. In the case of stochastic events resulting in gaps and permanently increased irradiances reaching *N. nitida* seedlings leaves, it is likely that only light-acclimated leaves survive.

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