

# Light energy partitioning in photosystems I and II during development of *Nothofagus nitida* growing under different light environments in the Chilean evergreen temperate rain forest

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**Abstract** *Nothofagus nitida* (Phil.) Krasser (Nothofagaceae) regenerates under the shade. Nonetheless, older seedlings are commonly found at full sun. We tested the hypothesis that light capture and photochemical and non-photochemical energy dissipation of both photosystems PSI and PSII adjust with ontogeny and brighter environment. Light energy partitioning in both photosystems was studied in seedlings of different developmental stages (small 9.7 cm, tall 36 cm) under contrasting light environments (8–200 and 1,800–2,043  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ ) in the Chilean evergreen temperate forest. Higher  $A_{\text{max}}$ , dark respiration, and light compensation and saturation points in sun seedlings of both developmental stages were accompanied by higher rates of electron transport. These seedlings also showed a high fraction of open PSII reaction centres and similar non-photochemical quenching at high-light in both photosystems, showing no effect of developmental stage in these parameters. Conversely, light capture, total thermal dissipation after photoinhibition, active down-

regulation of antenna efficiency, and state transitions were higher in smaller seedlings than in taller ones. These changes maintain photostasis, preventing photodamage, while favouring a more oxidized quinone pool. There is an independent effect of seedling development and light acclimation on this transition from shade to sun during early ontogeny. This transition reflects short-term responses of the photosynthetic apparatus to light and longer term responses that depend on seedling developmental stage.

**Keywords** PSI quantum yield · State transitions · NPQ components · Shade-sun phenotypic transition

## Introduction

The establishment of *Nothofagus nitida* (Phil.) Krasser (Nothofagaceae) seedlings naturally occurs under the shade in the Chilean temperate forest (Donoso 1995). At later developmental stages it becomes a canopy emergent species. In intensively thinned forests, regeneration begins in shady microsites composed of small vegetation and fallen trees. Nevertheless, it is common to find older seedlings and saplings growing at full sun light with a variety of other sun species such as *Drimys winteri* J.R. et G. Forst., *Embothrium coccineum* J.R. et G. Forst and *Eucryphia cordifolia* Cav. (Veblen et al. 1996). There appears to be an early development-dependent habitat expansion towards more illuminated environments in *N. nitida* (Coopman et al. 2008). Rainforest evergreen species often change their light requirements throughout their ontogeny (Lusk et al. 2008; Poorter et al. 2005). One possible explanation for this transition is that as seedlings grow, the foliage is exposed to new light environments and new leaves develop under higher irradiance (Krause et al. 2001). Acclimation

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to high photosynthetic photon flux density (PPFD) usually results in higher light-saturated  $\text{CO}_2$  assimilation rate ( $A_{\text{max}}$ ), dark respiration rate ( $R_d$ ), and light compensation (LCP) and saturation (LSP) points (Larcher 2003; Craine and Reich 2005). In a previous growth chamber study, it was found that the light acclimation capacity of seedlings implies changes in the absorbed light energy use and that it depends on the interaction between developmental stage and light intensity (Coopman et al. 2008). There has been little research focused on changes in absorbed light energy partitioning and the development of photoprotective mechanisms during the early ontogenetic transition from shade acclimated small seedlings to high-light acclimated taller seedlings (Ishida et al. 2005; Coopman et al. 2008).

Effective regulation of light energy utilization is essential for survival under light-limiting conditions and for protection against damage by excess radiation. Optimum plant performance requires a balance in the rate of source versus sink processes. Photoacclimation to high-light attempts to balance the energy absorbed versus the energy either utilized through metabolism or dissipated via NPQ to avoid photodamage (Ensminger et al. 2006). In PSII, light energy partitioning can be assessed by chlorophyll *a* fluorescence estimating the quantum yields of photochemical [ $\Phi(\text{II})$ ] and yields of intrinsic [ $\Phi(\text{NO})$ ] and down-regulation [ $\Phi(\text{NPQ})$ ] components of non-photochemical thermal dissipation as described by Kramer et al. (2004). According to Walters and Horton (1991), NPQ can be divided into three components; the major and most rapidly relaxed is the pH-dependent high-energy quenching ( $\text{NPQ}_f$ ), which involves changes in the PSII antenna proteins and the synthesis of the xanthophyll zeaxanthin from violaxanthin (Niyogi et al. 2005). Thus,  $\text{NPQ}_f$  is a measure of harmless thermal energy dissipation. A second component called state transition (qT) occurs when LHCI is preferentially excited over LHCII. It involves the reversible phosphorylation of light harvesting proteins which migrate from PSII to PSI antenna complex (Haldrup et al. 2001) or implies the movement of LHCI-PSI towards PSII-LHCII balancing the distribution of absorbed light between the two photosystems (Tikkanen et al. 2008). The third and slower relaxing component is related to photoinhibition ( $\text{NPQ}_s$ ) (Walters and Horton 1991). NPQ and its components have been successfully used to explore differences in photoprotection and photoinhibition at the phenotype level (Demmig-Adams et al. 1995). PSI energy conversion efficiency can be assessed by the redox state of the PSI reaction centre (P700), via its induced increased broad band in absorbance at 810–830 nm caused by the  $\text{P700}^+$  cation-radical (Harbinson et al. 1989; Klughammer and Schreiber 1994). According to the saturation method developed by Klughammer and Schreiber (1994), PSI quantum yield can be divided into quantum yield of

photochemical energy conversion [ $\Phi(\text{I})$ ], quantum yield of non-photochemical energy dissipation due to donor side limitation [ $\Phi(\text{ND})$ ], and dissipation due to acceptor-side limitation [ $\Phi(\text{NA})$ ]. In this way, we can distinguish two populations of PSI reaction centres with P700 in the non-oxidized form. These two populations may be either photochemically active or acceptor-side limited. PSI photochemical activity in excess light is more stable than PSII photochemistry in shade and light leaves of several neotropical forest species (Barth et al. 2001). Quantum yields of PSII and PSI are closely linked by the non-cyclic electron transport chain. Much of the regulation of PSII occurs via non-photochemical quenching; any further imbalance between PSII and PSI is absorbed by a change in the photochemical yield of PSII and is manifested by a change in qL (Harbinson et al. 1989; Ensminger et al. 2006).

Several ontogenetic changes of trees have been reported between seedlings and saplings in relation to allometric variables (Sack and Grubb 2003; Lusk 2004; Niinemets 2006), leaf anatomy and morphology (Ishida et al. 2005; Kenzo et al. 2006), gas exchange and water use efficiency (Grulke and Retzlaff 2001; Niinemets 2002), and light absorbed use energy balance (Ishida et al. 2005; Coopman et al. 2008). Nevertheless, the mechanism that mediates ontogenetic changes remains unclear. In many studies, it is not possible to distinguish between the size effects or environmental changes associated with ageing, much less the interactions among these factors (Bond 2000; Grulke and Retzlaff 2001; Ishida et al. 2005). We used an evergreen temperate rain forest 10 years after a clear cutting was done to investigate the influence of light environment and seedling height on this progressive shift from low-light acclimated small seedlings to high-light acclimated taller seedlings. We tested the hypothesis that light capture and photochemical and non-photochemical energy dissipation of both photosystems adjust with ontogeny and brighter environment so that as development progresses there is a functional shade-to-sun transition of the photosynthetic apparatus. This statement was addressed by quantifying the development of photoprotective mechanisms and the changes in absorbed light energy partitioning in both photosystems of seedlings of two different developmental stages growing in two contrasting light environments in the field.

## Materials and methods

### Study site

The study was carried out in February 2007 at Katalapi Park in the Quillaiepe coastal range, 10th Region, in South-Central Chile ( $41^\circ 31' 07.5''\text{S}$ ,  $72^\circ 45' 2.2''\text{W}$ ). This area has a

temperate maritime climate. Daily maximum and minimum air temperatures and relative humidity, total daily precipitation, and maximum PPFD were determined with a data logger LI 1400 (LI-COR Biosciences, Lincoln, NE, USA) using a LI 1400-104 sensor for air temperature and relative humidity, a LI 1400-106 for precipitation and LI 250 for PPFD.

#### Forest structure and light environments

The research area was subjected to clear cutting 10 years before the present study. There are some remnant *N. nitida* seed trees distributed approximately every 100 m. Since this species produces seeds and recruits yearly, there were *N. nitida* seedlings of different ages. At the present time, the regeneration stand is heterogeneously distributed in the area and is dominated by a variety of high-light tolerant species such as *Drimys winteri*, *Embothrium coccineum*, *Eucryphia cordifolia*, and *N. nitida*, where the tallest individuals reach about 4 m. This situation creates a high variation in light availability in the area. All *N. nitida* seedling heights were measured in two parcels of 30 × 15 m (450 m<sup>2</sup>). PPFD immediately above the apex of each seedling inside the parcels was measured five times at approximately 20 min intervals during the solar midday (12:00–14:00 h) using a LI-250 light meter (LI-COR Biosciences, Lincoln, NE, USA). With the previous information, two ranges of light environments, shade (0–200 μmol photons m<sup>-2</sup> s<sup>-1</sup>) and sun (1,800–2,000 μmol photons m<sup>-2</sup> s<sup>-1</sup>) and two ranges of seedling height (mean ± SD; small 9.7 ± 1.9 cm and tall 36 ± 5.4 cm) were defined as treatments (Fig. 2B). A total of 20 seedlings were sampled at random points along two transects laid out in the interior of either parcel. For each seedling height and light environment the nearest seedling to each sample point was located and tagged. We only included seedlings of healthy appearance growing at each light environment. Healthy appearance means without leaf chlorosis and with at least four new fully expanded leaves at the time of the measurements. This means that plants were growing normally. In addition, soil water availability, temperature, and general environmental factors were similar for tall and small plants. Therefore, there were no apparent differential stress factors affecting plants at the different developmental stages.

#### Relative chlorophyll density

Chlorophyll (Chl) density was measured with the chlorophyll meter (Minolta SPAD-502 meter, Minolta Camera CO., Osaka, Japan) as SPAD values. New fully expanded and light-exposed leaves near the apex of 15 seedlings from each treatment were evaluated. The SPAD-502 meter

provides reliable estimates of relative densities of leaf Chl (Markwell et al. 1995; Richardson et al. 2002).

#### Net photosynthesis

Net CO<sub>2</sub> assimilation at PPFDs ranging from 0 to 1,600 μmol photons m<sup>-2</sup> s<sup>-1</sup> was measured in attached leaves with an infrared gas analyzer (Ciras-2, PP systems, Hitchin, U.K.). CO<sub>2</sub> reference concentration was 360 ppm, with a flow rate of 200 mL min<sup>-1</sup>. Air relative humidity inside the leaf chamber was 75%. The temperature inside the leaf chamber was maintained at 15 ± 2°C. This value is within the range of the optimum temperature for photosynthesis in this species. LCP, LSP, maximal rate of net CO<sub>2</sub> assimilation ( $A_{\max}$ ), apparent quantum efficiency (AQE), and dark respiration ( $R_d$ ) were calculated with the Photosynthesis Assistant software 1.1 (Dundee Scientific, UK). All measurements were performed from 9:00 am to 13:00 pm. When small leaves did not cover the entire leaf cuvette surface (2.5 cm<sup>2</sup>), a digital photograph was taken from the leaf immediately after the measurement. The Sigma Scan Pro 5.0 software (SPSS, Inc) was used to estimate the actual leaf area from the photograph. Gas exchange values given by CIRAS-2 were corrected using the ratio cuvette area/actual leaf area as a correction factor.

#### Chlorophyll fluorescence and P700 measurements

Light response curves of chlorophyll *a* fluorescence and P700 assessment were performed simultaneously with a Dual-PAM-100 measuring system (Walz-Effeltrich, Germany) in detached new fully expanded leaves collected from the top of the crown. Leaves were immediately dark adapted for 30 min (to obtain open reaction centres). A saturating pulse was applied to obtain the maximal fluorescence ( $F_m$ ); then, leaves were exposed 5 min at each PPFD (0, 27, 58, 131, 221, 344, 435, 665, 1,033 and 1,957 μmol photons m<sup>-2</sup> s<sup>-1</sup>) in order to obtain steady state readings. All measurements were performed at 15°C. Temperature was controlled with a water jacket system placed around the Dual-D leaf holder and connected to a thermoregulated water bath. Leaf temperature was monitored with a fine wire K-type thermocouple (Sper Scientific, Ltd., USA). Recordings and calculations were performed with the Dual-PAM 1.7 data analyses and control software (Walz-Effeltrich, Germany). The saturation pulse method originally developed for chlorophyll fluorescence quenching analysis (Schreiber et al. 1986) was used for assessment of PSI quantum yield (Klughammer and Schreiber 1994). Electron transport rate of PSII (ETR II), yield of energy dissipation by down-regulation  $\Phi$ (NPQ) and the fraction of PSII centres in open state (qL) were calculated according to Kramer et al. (2004). Analysis of

PSI was based on a routine for assessment of the maximal P700 change ( $P_m$  determination), which involves preillumination with far-red light (FR) for 2 s and a saturation pulse that induces maximal P700 oxidation followed by full reduction. The  $P_m$  determination is analogous to minimal fluorescence ( $F_o$ ) and  $F_m$  determinations. The saturation pulse method originally developed for chlorophyll fluorescence quenching analysis (Schreiber et al. 1986) was used for assessment of PSI quantum yield according to Klughammer and Schreiber (1994). PSI quantum yield can be divided into quantum yield of photochemical energy conversion [ $\Phi(I)$ ], quantum yield of non-photochemical energy dissipation due to donor side limitation [ $\Phi(ND)$ ], and dissipation due to acceptor-side limitation [ $\Phi(NA)$ ].

#### Non-photochemical quenching components

Non-photochemical quenching components were determined in detached new fully expanded leaves. These measurements were performed using a pulse-amplitude modulated fluorimeter (FMS 2, Hansatech Instruments Ltd., UK). NPQ was resolved into the slow relaxing component of non-photochemical quenching (NPQ<sub>s</sub>) and the fast relaxing component of non-photochemical quenching (NPQ<sub>f</sub>) as described by Maxwell and Johnson (2000). The protocol used was the application of a photoinhibitory treatment of 2,000  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  of actinic light for 2 h provided by a LS2 white light source (Hansatech Instruments Ltd., UK) connected to LD2/3 electrode chamber attached to thermoregulated bath set at 4°C. Then, the actinic light was turned off and the 1 h dark recovery kinetics were analysed. During this time, saturating pulses to obtain  $F_m$  were applied. NPQ<sub>s</sub> =  $(F_m - F_m^r)/F_m^r$  and NPQ<sub>f</sub> =  $(F_m/F_m^r) - (F_m/F_m^r)$ .  $F_m^r$  (value of  $F_m$  that would have been attained if only slowly relaxing quenching had been present in the light) was obtained by extrapolation of the data points recorded towards the end of the relaxation back to the time when the actinic light was removed in a semilogarithmic plot of maximum fluorescence yield versus time.

#### State transitions

The relative capacity for state 1–2 transitions was estimated at 15°C as described in Lunde et al. (2000), using a FMS 2 fluorimeter and a LD2/3 electrode chamber. Blue light (100  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  at the leaf surface), which favours PSII, was provided using a LS2 white light source (Hansatech Instruments Ltd., UK) equipped with a corning 4–86 blue filter. FR light ( $\lambda = 735 \text{ nm}$ ), which favours PSI, was provided as 214  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  at the leaf surface by the FMS 2 fluorimeter. Relative state transitions ( $F_r$ ) were calculated as  $F_r = [(F'_i - F_i) - (F'_{ii} - F_{ii})]/(F'_i - F_i)$ ,

where  $F'_i$  and  $F'_{ii}$  designate fluorescence in the absence of PSII light in states 1 and 2, respectively (Lunde et al. 2000).

#### Pigment determinations

Newly emerged and fully expanded leaves from the top of the crown were collected at midday on a sunny day and immediately frozen in liquid nitrogen. Pigment separation and quantitation were done by HPLC analysis as described in detail in Ivanov et al. (1995). Briefly, an LC-6A chromatographic system equipped with a SPA-6AV UV/Vis detector (Shimadzu Corporation, Tokyo, Japan) and a Waters YMC ODS-A column (5  $\mu\text{m}$  particle size 4.6  $\times$  250 mm) was used. The mobile phase consisted of a mixture of HPLC-grade methanol:dichloromethane (95:5, v/v). All measurements were performed under dim light at room temperature. Standards for neoxanthin (N), violaxanthin (V), antheraxanthin (A), zeaxanthin (Z) and lutein (L) were obtained from DHI Water and Environment (Hørsholm, Denmark) and were dissolved in 100% ethanol. The pigment concentrations were expressed on chlorophyll a basis. Total xanthophyll pool size (VAZ) was determined as:  $VAZ = A + V + Z$  and De-epoxidation state (DEPS) of the pigment pool was calculated as:

$$DEPS = \frac{Z + 0.5A}{V + A + Z},$$

where A antheraxanthin, V violaxanthin, and Z zeaxanthin.

#### Statistics

Reported values of all measurements correspond to the mean of five measurements made on five different plants. Data were subjected to a two-way ANOVA (where the factors were seedling height and midday PPFD). A Fischer LSD-test was used for all post hoc analysis. Two post hoc analyses were performed in order to determine the fluorescence and P700 assessment light curve parameters. A first post hoc analysis of light curves was conducted within each treatment and between PPFDs. A second post hoc analysis was done between treatments within each PPFD. Differences between the values were considered significant at  $P \leq 0.05$ . Before ANOVA, data were checked for normality and homogeneity of variances. All the statistical analyses were performed with STATISTICA v6.0 (Statsoft, Tulsa, OK, USA).

## Results

#### Climatic conditions

The 2 months prior to the experiment (December 2006–January 2007) were the warmest, driest, and brightest

months between April 2006 and February 2007 (Fig. 1A–D). The average maximal and minimal air temperatures during this period increased by about 10 and 5°C, respectively, relative to winter temperatures (Fig. 1A). Midday PPFD increased about 400% in this period relative to the winter period, reaching an average of 1,900  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  (Fig. 1B). Rainfall decreased to one-fourth in summer relative to winter, reaching a daily average of 4 mm (Fig. 1C). In this period, daily minimal relative humidity decreased about 35% relative to winter, reaching an average of 65%. Maximum daily relative humidity remained at approximately 95% at all times (Fig. 1D).

### Forest structure and light environments

The *N. nitida* seedlings–saplings heights found in the study site were from 6 to 380 cm, where individuals taller than 70 cm grew exclusively at full sun light (midday PPFD > 1,800  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ ) (Fig. 2A). Individuals smaller than 40 cm height were the most abundant and grew throughout the entire midday PPFD range. From this pool of individuals, two ranges of light environments, shade (midday PPFD: 8–200  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ ) and sun (midday PPFD: 1,800–2,043  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ ) and two ranges of seedling height (mean  $\pm$  SD; small  $9.7 \pm 1.9$  cm and tall  $36 \pm 5.4$  cm) were defined as treatments. Five plants were selected and tagged for each condition (Fig. 2B).

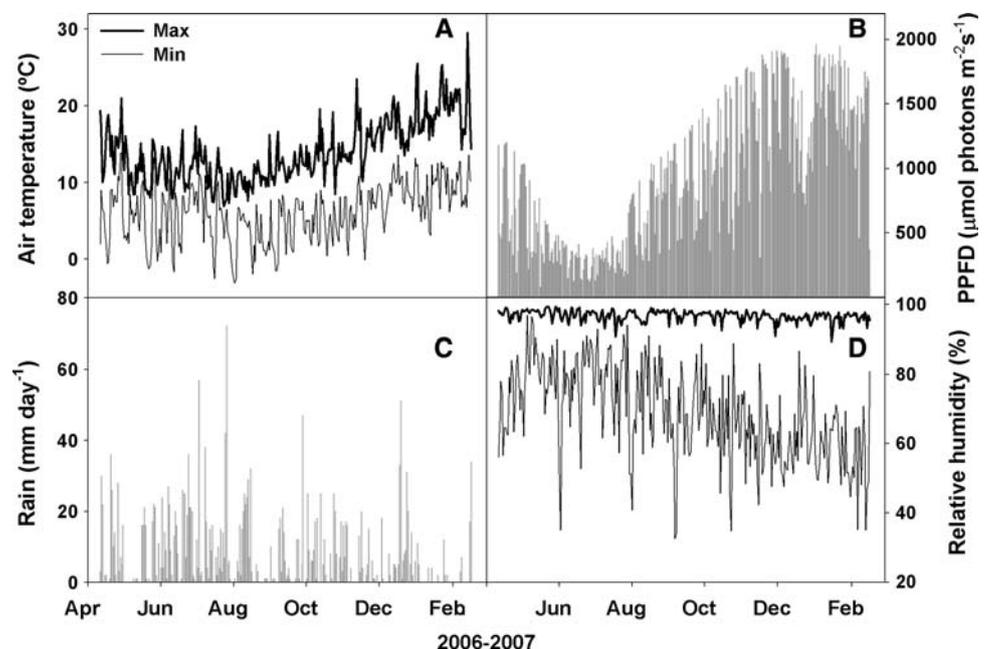
### Effect of light environment and developmental stage on relative chlorophyll density

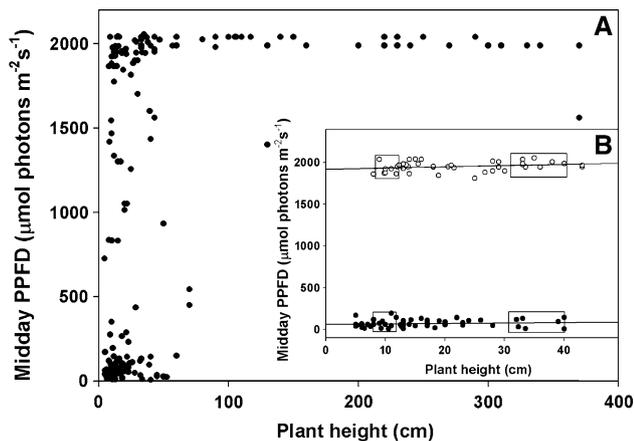
There was a non-significant interaction between light environment and seedling height on Chl density ( $P < 0.898$ ). Nevertheless, both factors significantly affected it. Independent of the light environment, small seedlings showed an 11% lower Chl density ( $P = 0.015$ ) than tall seedlings (Fig. 3B). Shade seedlings had 15% higher Chl density than sun seedlings ( $P < 0.001$ ) (Fig. 3A).

### Effect of light environment and developmental stage on light response curves of CO<sub>2</sub> assimilation

There were non-significant interactions between light environment and seedling height on CO<sub>2</sub> assimilation parameters obtained from light response curves (lowest  $P < 0.120$ ) (Fig. 4). Nevertheless, all parameters were independently affected by each factor. Sun seedlings exhibited 57% higher  $R_d$  than shade seedlings ( $P = 0.003$ ) (Fig. 4A). However, seedling height did not affect  $R_d$  ( $P = 0.891$ ). A 49.8% increase in LCP in sun seedlings was observed ( $P = 0.006$ ). Small seedlings exhibited a lower LCP than tall seedlings (48% with respect to tall seedlings) ( $P = 0.004$ ) (Fig. 4B).  $A_{\text{max}}$  increased 72% in sun seedlings relative to shade seedlings ( $P = 0.001$ ).  $A_{\text{max}}$  did not change with seedling height ( $P = 0.067$ ) (Fig. 4C). LSP increased 51% in sun seedlings ( $P < 0.001$ ) with

**Fig. 1** Annual course of air temperature, photosynthetic photon flux density (PPFD), rainfall and relative humidity in Katalapi Park (South-Central Chile). Measurements were made from April 2006 to February 2007. **A** Daily air maximum and minimum temperatures. **B** Maximum daily PPFD. **C** Daily total rainfall. **D** Daily maximum and minimum relative humidities





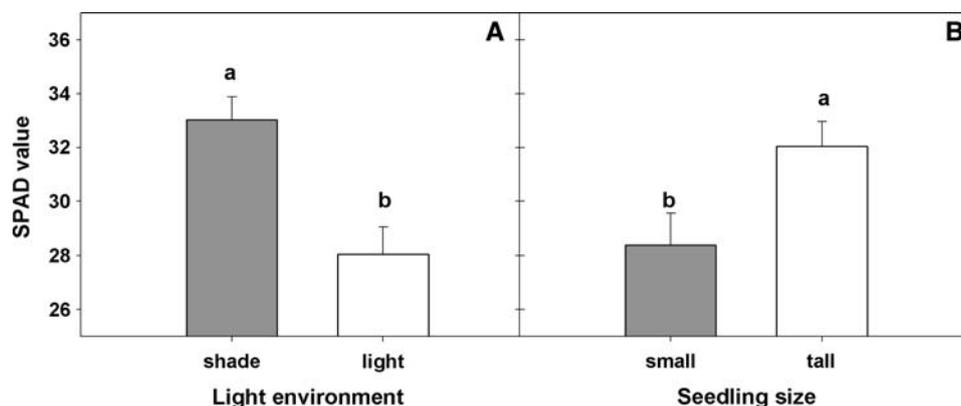
**Fig. 2** Forest structure and light environments of a *Nothofagus nitida* regeneration stand 10 years after clear cutting. All *N. nitida* seedling heights and PPFD during the solar midday (12:00–14:00 h) were measured in two parcels of 30 × 15 m (450 m<sup>2</sup>) located inside of the study area (A). Two ranges of light environment (shade 8–200 µmol m<sup>-2</sup> s<sup>-1</sup> and sun 1,800–2,043 µmol m<sup>-2</sup> s<sup>-1</sup>) and two ranges of seedling heights (mean ± SE; 9.7 ± 0.6 cm and 36 ± 1.7 cm) were defined as treatments (B). A total of 20 seedlings were sampled at random points along two transects laid out in the interior of either parcels

respect to shade seedlings. Non-significant differences in LSP between seedling heights were found (Fig. 4D). Sun seedlings showed 29% lower AQE than shade seedlings ( $P = 0.034$ ). Seedling height had no effect on AQE (Fig. 4E).

Effect of light environment and developmental stage on chlorophyll fluorescence and P700 light response curves

Light response curves were recorded up to a PPFD of 1,957 µmol photons m<sup>-2</sup> s<sup>-1</sup>. Since all the effects occurred below 400 µmol photons m<sup>-2</sup> s<sup>-1</sup>, light response

curves are shown up to this PPFD (Fig. 5A–F). There were non-significant effects of seedling height in almost all light response curve parameters. Conversely, all parameters obtained from the light response curves showed significant changes between seedlings grown at different light environment from 27 µmol photons m<sup>-2</sup> s<sup>-1</sup> upwards (Fig. 5A–F). Sun seedlings had 39 and 120% higher ETR(II) initial slope and maximum ETR(II) than shade seedlings, respectively (Fig. 5A). ETR(II) light saturation point of shade seedlings was 42 µmol photons m<sup>-2</sup> s<sup>-1</sup>. In small and tall sun seedlings these parameters were 94 and 176 µmol photons m<sup>-2</sup> s<sup>-1</sup>, respectively. Sun seedlings showed a significant increase of 28 and 109% in ETR(I) initial slope and maximum ETR(I) relative to shade seedlings, respectively. ETR(I) light saturation point increased from 42 µmol photons m<sup>-2</sup> s<sup>-1</sup> in shade seedlings to 176 µmol photons m<sup>-2</sup> s<sup>-1</sup> in light seedlings (Fig. 5B). The NPQ initial slope of shade seedlings was significantly higher (62%) than in sun seedlings. There were no differences in maximum ΦNPQ in all treatments. ΦNPQ light saturation point of small and tall shade seedlings was 176 µmol photons m<sup>-2</sup> s<sup>-1</sup>, while in sun seedlings it increased up to 390 µmol photons m<sup>-2</sup> s<sup>-1</sup> (Fig. 5C). There were no differences in maximum Φ(ND) between seedling heights and light environments. An 80% higher Φ(ND) initial slope was found in shade seedlings than in sun seedlings. Φ(ND) light saturation point of sun seedlings (390 µmol photons m<sup>-2</sup> s<sup>-1</sup>) was higher than in shade seedlings (282 µmol photons m<sup>-2</sup> s<sup>-1</sup>) (Fig. 5D). All seedlings regardless of developmental stage and light environment showed a slight increase in the acceptor-side limitation Φ(NA) at irradiances lower than 27 µmol photons m<sup>-2</sup> s<sup>-1</sup> reaching an average value of 0.05. Above this radiation, Φ(NA) was approximately zero (data not shown). Shade seedlings showed a significant decrease of 33 and 170% in qL initial slope and minimum qL relative



**Fig. 3** Relative chlorophyll density (SPAD value) of *Nothofagus nitida* individuals: **A** growing in two ranges of light environment (shade 8–200 µmol m<sup>-2</sup> s<sup>-1</sup> and sun 1,800–2,043 µmol m<sup>-2</sup> s<sup>-1</sup>) and **B** two ranges of seedling height (mean ± SD; small

9.7 ± 1.9 cm and tall 36 ± 5.4 cm) in an evergreen forest regeneration stand. Different letters on top of the bars (mean ± SE) indicate significant differences between light environment and seedling size evaluated with Fischer LSD-test ( $n = 5$ ;  $P \leq 0.05$ )

**Fig. 4** Light response parameters of *Nothofagus nitida* individuals measured in two parcels of  $30 \times 15$  m ( $450$  m<sup>2</sup>) located inside of the study area. **A** Dark respiration ( $R_d$ ), **B** light compensation point ( $LCP$ ), **C** maximal net photosynthetic rate  $A_{max}$ , **D** light saturation point ( $LSP$ ) and **E** maximum quantum yield of photosynthesis ( $AQE$ ) of *Nothofagus nitida* seedlings growing in two ranges of light environment (shade  $8$ – $200$   $\mu\text{mol m}^{-2} \text{s}^{-1}$  and sun  $1,800$ – $2,043$   $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) and two ranges of seedling height (mean  $\pm$  SD; small  $9.7 \pm 1.9$  cm and tall  $36 \pm 5.4$  cm). Different letters on top of the bars (mean  $\pm$  SE) indicate statistically significant differences between light environment and seedling height evaluated with Fischer LSD-test ( $n = 5$ ;  $P \leq 0.05$ )

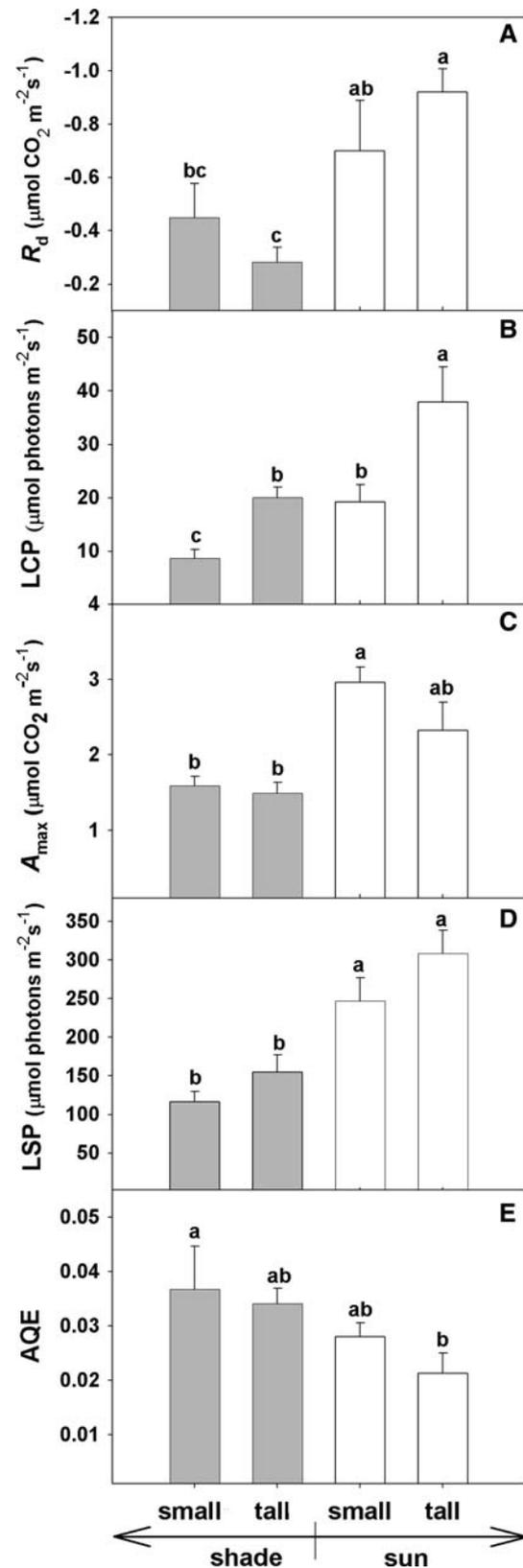
to sun seedlings, respectively.  $q_L$  light saturation point was  $282$   $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  in shade seedlings compared to  $390$   $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  in sun seedlings (Fig. 5E). The initial slope of  $\Phi(I)/\Phi(II)$  was 47% higher in shade seedlings than in sun seedlings. There were non-significant differences in the light saturation point ( $42$   $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ ) and maximum values for  $\Phi(I)/\Phi(II)$  between treatments (Fig. 5F).

#### Effect of light environment and developmental stage on non-photochemical quenching components

There were non-significant interactions between light environment and seedling height with respect to NPQ components (lowest  $P < 0.443$ ). A 22% higher NPQ was found in sun seedlings than shade seedlings ( $P = 0.006$ ) (Fig. 6A). Independent of light environment, small seedlings showed a 26% higher NPQ than the tall seedlings ( $P = 0.002$ ) (Fig. 6B). Small seedlings showed 43% higher NPQ<sub>f</sub> than tall seedlings ( $P < 0.001$ ) independent of light environment. Shade seedlings showed 32% higher NPQ<sub>f</sub> than sun seedlings ( $P = 0.003$ ). NPQ<sub>s</sub> was only affected by light environment ( $P < 0.001$ ). Sun seedlings exhibited 125% higher NPQ<sub>s</sub> than shade seedlings (Fig. 6). There were non-significant interactions between light environment and seedling height on  $F_r$  ( $P = 0.067$ ) (Fig. 7). A higher capacity for state transitions (521%) was observed in shade seedlings than in sun seedlings ( $P < 0.001$ ). A 53% higher  $F_r$  was found in small seedlings than in tall seedlings growing in the shade ( $P = 0.023$ ). This was not the case in sun seedlings.

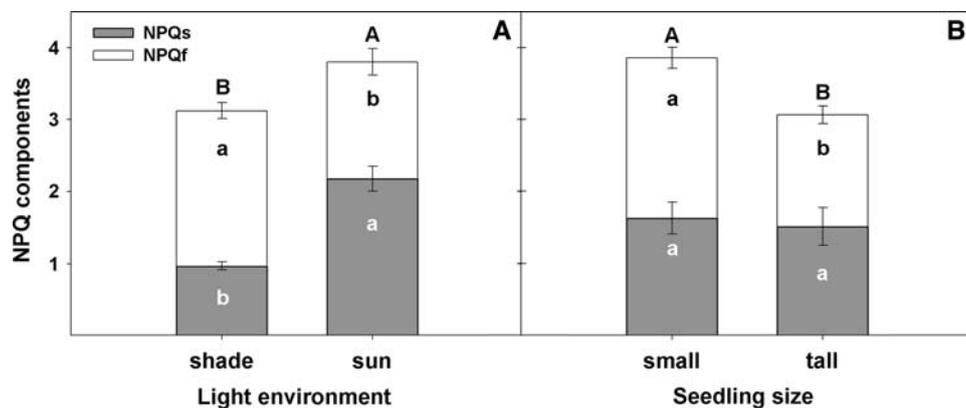
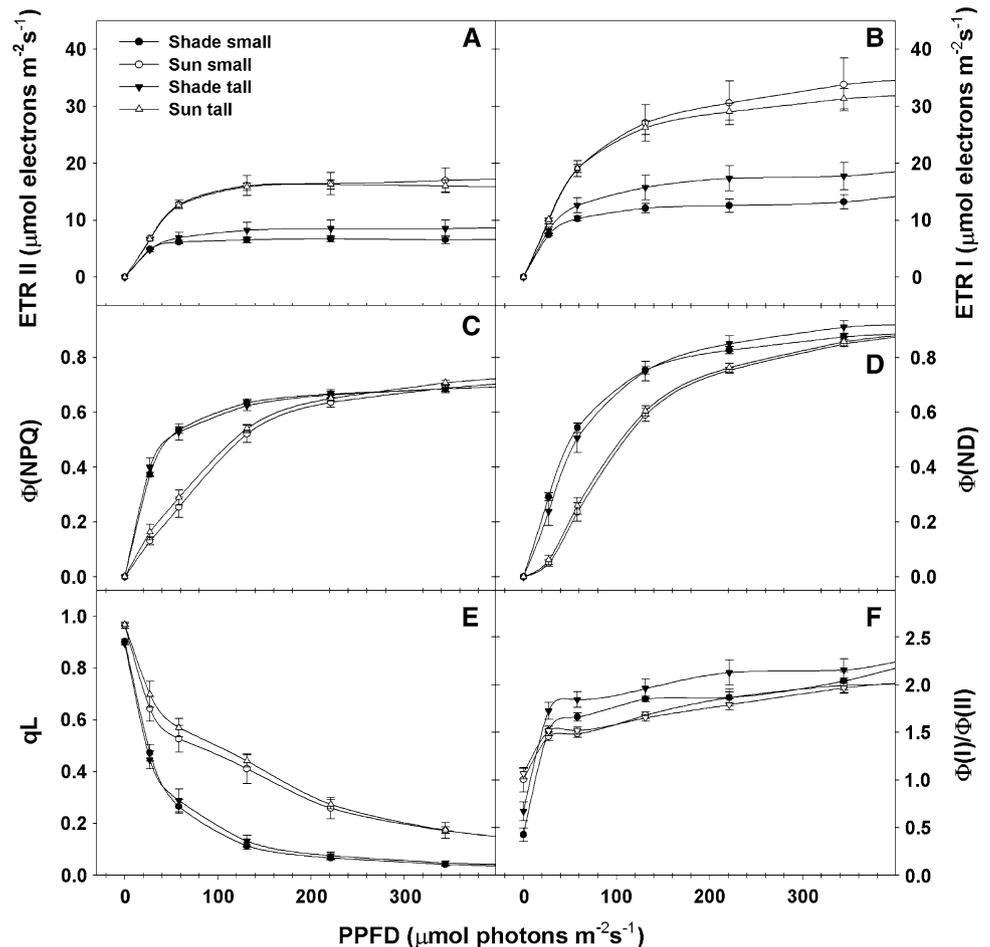
#### Effect of light environment and developmental stage in carotenoid pigments

There were significant interactions between seedling size and light environment on violaxanthin and zeaxanthin content ( $P < 0.05$ ). Both pigment contents decreased with seedling size only in the sun environment ( $P < 0.01$ ) (Fig. 8). Antheraxanthin content was higher in sun seedlings ( $P < 0.001$ ) but seedling size had no effect ( $P = 0.06$ ). There was a significant interaction between



seedling size and light environment on VAZ ( $P = 0.006$ ). VAZ was higher in small seedlings growing in the sun ( $P = 0.01$ ) (Fig. 8). There were no differences in DEPS

**Fig. 5** Chlorophyll fluorescence and P700 light response curve of *Nothofagus nitida* individuals growing in two ranges of light environment (shade 8–200  $\mu\text{mol m}^{-2} \text{s}^{-1}$  and sun 1,800–2,043  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) and two ranges of seedling height (mean  $\pm$  SD; small 9.7  $\pm$  1.9 cm and tall 36  $\pm$  5.4 cm) in an evergreen forest regeneration stand. **A** Electron transport rate through PSII and **B** PSI, **C** quantum yield of regulated energy dissipation, **D** non-photochemical quantum yield of PSI due to a donor side limitation, **E** relative redox state of PSII and **F** relative effective quantum yields of PSII and PSI in response to increased actinic light intensity. Each data point is the mean  $\pm$  SE. Statistically significant differences between light environment and seedling height were evaluated with Fischer LSD-test ( $n = 5$ ;  $P \leq 0.05$ )

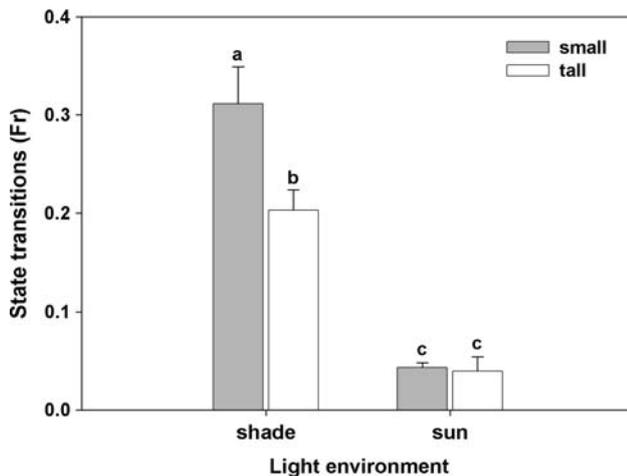


**Fig. 6** Fast and slow non-photochemical quenching components ( $\text{NPQ}_f$  and  $\text{NPQ}_s$ , respectively) of *Nothofagus nitida* individuals: **A** growing in two ranges of light environment (shade 8–200  $\mu\text{mol m}^{-2} \text{s}^{-1}$  and sun 1,800–2,043  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) and **B** two ranges of seedling height (mean  $\pm$  SD; small 9.7  $\pm$  1.9 cm and tall 36  $\pm$  5.4 cm) in an evergreen forest regeneration stand. All measurements were performed on a dark adapted, fully expanded new leaf

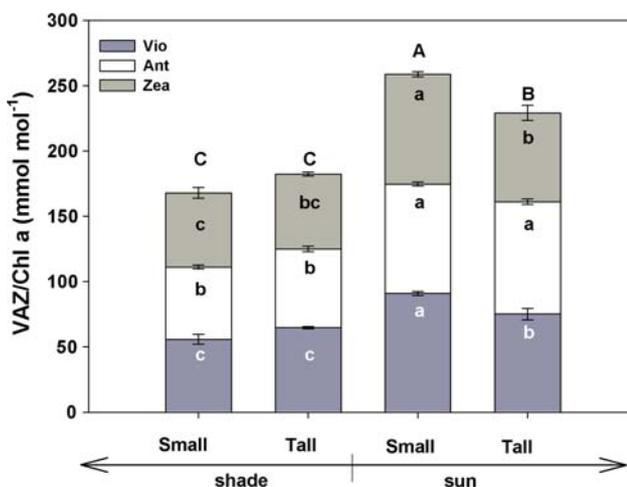
after photoinhibitory conditions (2,000  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  at 4°C for 2 h). Different lower case letters below the top of the bars (mean  $\pm$  SE) indicate statistically significant differences between light environment and seedling height NPQ components. Upper case letters indicate the same for total NPQ. Both evaluated with Fischer LSD-test ( $n = 5$ ;  $P \leq 0.05$ )

between treatments exhibiting an average of 50% de-epoxidation of the xanthophyll pool. Lutein content was affected independently by seedling size ( $P = 0.03$ ) and

light environment ( $P < 0.001$ ). Sun seedlings showed a 30.4% higher lutein content than shade seedlings (Fig. 8A). Lutein content of tall seedlings was 9.1% lower than in



**Fig. 7** Changes in the capacity for state transitions ( $F_r$ ) of *Nothofagus nitida* individuals growing in two ranges of light environment (shade  $8\text{--}200 \mu\text{mol m}^{-2} \text{s}^{-1}$  and sun  $1,800\text{--}2,043 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) and two ranges of seedling height (mean  $\pm$  SD; small  $9.7 \pm 1.9$  cm and tall  $36 \pm 5.4$  cm) in an evergreen forest regeneration stand. STs were estimated from fluorescence measurements at  $15^\circ\text{C}$  on a dark adapted, fully expanded new leaves. The leaves were exposed to either light favouring PSII (blue) or light favouring PSI (far-red). Different letters on top of the bars (mean  $\pm$  SE) indicate significant differences evaluated with Fischer LSD-test ( $n = 5$ ;  $P \leq 0.05$ )



**Fig. 8** Relative concentration of violaxanthin, antheraxanthin and zeaxanthin ( $\text{mmol mol}^{-1} \text{Chl a}$ ) of *Nothofagus nitida* individuals growing in two ranges of light environment (shade  $8\text{--}200 \mu\text{mol m}^{-2} \text{s}^{-1}$  and sun  $1,800\text{--}2,043 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) and two ranges of seedling height (mean  $\pm$  SD; small  $9.7 \pm 1.9$  cm and tall  $36 \pm 5.4$  cm) in an evergreen forest regeneration stand. Different lower case letters below the top of the bars (mean  $\pm$  SE) indicate statistically significant differences of each pigment between treatments. Upper case letters indicate the same for total xanthophyll pool size. Both evaluated with Fischer LSD-test ( $n = 3$ ;  $P \leq 0.05$ ). Ant antheraxanthin, vio violaxanthin, Zea zeaxanthin

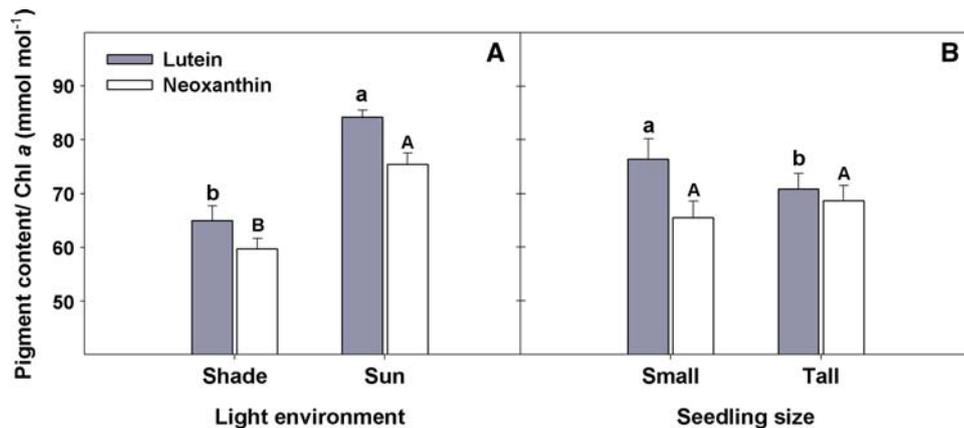
small seedlings (Fig. 8B). Neoxanthin content was higher in sun seedlings ( $P \leq 0.001$ ) but seedling size had no effect ( $P > 0.05$ ) (Fig. 9B).

## Discussion

The acclimation response of seedlings to sun and shade has been well studied. However, relatively little work has been conducted with respect to ontogenetic effects on the light acclimation capacity of seedlings. A typical plant response to high-light environment is a modification of light capture capacity by decreasing leaf Chl density. Chl turnover rate is controlled by excess excitation energy and can be adjusted to maintain a balance between degradation and synthesis (Lindahl et al. 1995). At the time of the experiment, the study site had been exposed to the annual maximum PPFD for more than 2 months (Fig. 1B). In this context, the lower leaf Chl density of small seedlings compared to more developed seedlings could be a photo-protective attribute of leaves growing at high-irradiance. These leaves have a reduced light capture capacity to avoid photodamage (Gilmore et al. 2003; Williams et al. 2003). Conversely, the higher Chl density found in shade seedlings responds to a light harvest maximization strategy (Larcher 2003). Thus, Chl density in seedlings depends both on light environment and developmental stage.

Light environment significantly affected all  $\text{CO}_2$  assimilation light response curve parameters. Only LCP increased with seedling development. The higher  $R_d$ , LCP, LSP,  $A_{\text{max}}$  and lower AQE, and Chl densities observed in sun seedlings reflect changes from shade to sun leaves that are indicative of photosynthetic light acclimation (Fig. 4) (Demmig and Winter 1988). Conversely, shade seedlings were unable to increase their carbon uptake at high irradiances.  $A_{\text{max}}$  is a dynamic parameter that can be altered during acclimation to different light environments through changes in enzyme activities and gene expression (Niyogi 1999). A rise of about 70% in  $A_{\text{max}}$  observed in sun seedlings indicates that even the ontogenetically younger *N. nitida* seedlings, about 9 cm height, were able to increase their photochemical light utilization through carbon fixation. Shade leaves usually present lower  $A_{\text{max}}$ ,  $R_d$ , and LSP than light acclimated leaves (Larcher 2003; Coopman et al. 2008). Changes in light requirements during ontogeny in the temperate evergreen Chilean rain forest have been reported (Lusk et al. 2008). The lower LCP showed by small seedlings growing in the shade is consistent with the distribution of seedlings according to their heights and light environment (Fig. 2A) and could allow a positive carbon balance in light limiting environments (Craine and Reich 2005). These results suggest that *N. nitida* seedlings at early stages of development optimize their metabolic balance to support successful regeneration in the low-light environment under the canopy.

Both photosystems operate sequentially in terms of linear electron transport. At steady state, the fluxes of electrons entering and leaving the electron transport chain



**Fig. 9** Relative concentration of **A** lutein and **B** neoxanthin ( $\text{mmol mol}^{-1}$  Chl a) of *Nothofagus nitida* individuals growing in two ranges of light environment (shade  $8\text{--}200 \mu\text{mol m}^{-2} \text{s}^{-1}$  and sun  $1,800\text{--}2,043 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) and two ranges of seedling height (mean  $\pm$  SE; small  $9.7 \pm 0.6$  cm and tall  $36 \pm 1.7$  cm) in an

evergreen forest regeneration stand. Different lower case letters on top of the bars (mean  $\pm$  SE) indicate significant differences for lutein (lower case) and neoxanthin (upper case) content between light environment and seedling size evaluated with Fischer LSD-test ( $n = 3$ ;  $P \leq 0.05$ )

must be balanced; any imbalance will result in compensatory increases or decreases in the quantum efficiencies of one or other system to restore this flux balance (Harbinson et al. 1989). The lower values of LSP of ETR(II) and lower maximum ETR(II) of shade seedlings compared to sun seedlings are consistent with the lower capacity of PSII effective quantum yield of photochemical energy conversion [ $\Phi(\text{II})$ ] of shade seedlings (data not shown). Consequently, shade seedlings at a low light range exhibited higher  $\Phi(\text{NPQ})$  than sun seedlings regardless of developmental stage (Fig. 5). Interestingly, at PPFD below the LSP of  $\Phi(\text{NPQ})$ , the higher initial slope of  $\Phi(\text{NPQ})$  from shade seedlings reflects a lower light requirement to develop additional thermal dissipation capacity. This higher light responsiveness of the heat dissipation system allows them to cope with the higher excess excitation energy indicated by a lower light level and higher over-reduction of PSII reaction centres (low qL) exhibited by the shade seedlings. The quinone pool appears to be the redox sensor within the photosynthetic electron transport chain in photoautotrophic cells (Allen and Nilsson 1997; Pfannschmidt 2003). Photochemical processes involved in maintaining a higher qL (which indicates a more oxidized state of the PQ pool) at higher irradiance in sun seedlings may play an essential role in photoprotection in *N. nitida* (Coopman et al. 2008). The higher excess of absorbed energy in shade seedlings was the consequence of bigger light harvesting capacity, as indicated by their higher relative Chl density. Thus, the partitioning of absorbed energy to photochemical and non-photochemical paths was primarily affected by light environment; in contrast, the light absorption capacity depends on both seedling developmental stage and environment.

The absence of acceptor-side limitation of ETR(I) implies a normal supply of oxidized NADP<sup>+</sup>, which could

indicate that there are no metabolic constraints by environmental stress (Cruz et al. 2005; Kim et al. 2005). The lower light intensity required to increase  $\Phi(\text{ND})$  in shade seedlings was concomitant with a lower LSP ETR(I) and lower maximum ETR(I). This was the result of the higher light sensitivity of the PSI quantum yield of photochemical energy conversion [ $\Phi(\text{I})$ ] (Data not shown). The higher  $\Phi(\text{ND})$  of shade seedlings was consistent with its higher  $\Phi(\text{NPQ})$  at low light, which limits charge separation at PSII and consequently  $\Phi(\text{I})$ . In addition, the higher maximum ETR(I) compared to maximum ETR(II) observed in light seedlings regardless of developmental stage could be indicative of electron donation to P700<sup>+</sup> from stromal components via the intersystem chain (Harbinson and Foyer 1991). Cyclic electron transport around PSI activated by high-light could also be contributing to increase ETR(I) (Clarke and Johnson 2001; Bukhov and Carpentier 2004; Johnson 2005). These results suggest that energy administration at the level of PSI depends on the light environment, regardless of the seedling developmental stage.

NPQ was resolved into three components: the  $\Delta\text{pH}$ -dependent quenching or fast relaxing component ( $\text{NPQ}_f$ ), the slowly relaxing component which is related to photo-damage ( $\text{NPQ}_s$ ), and state transitions ( $F_r$ ) (Niyogi 1999). Small seedlings independent of PPFD growing conditions showed greater capacity for total non-photochemical dissipation (NPQ) and  $\Delta\text{pH}$ -dependent high-energy quenching ( $\text{NPQ}_f$ ) after photoinhibitory treatment than more developed seedlings. The higher thermal dissipation capacity of smaller seedlings reflects a stronger antenna down-regulation than more developed seedlings. The higher total NPQ showed by small seedlings after photoinhibition could be a determinant for seedling establishment in forest understoreys, where a rapidly inducible short-term response to a

variable light environment represents a key factor (Larcher 2003). In addition, a higher NPQ was found in sun than in shade seedlings. A greater capacity for total non-photochemical dissipation by high-light-exposed leaves has been commonly reported and indicates light acclimation (Demmig-Adams and Adams 1992; Demmig-Adams et al. 1995; Ramalho et al. 2000; Walters 2005). Surprisingly, NPQ<sub>f</sub> was higher in shade seedlings and NPQ<sub>s</sub> was higher in sun seedlings. NPQ<sub>s</sub> was not affected by seedling developmental stage. This result differs from our own earlier growth chamber experiment, where high-light acclimated seedlings showed higher NPQ<sub>f</sub> and lower NPQ<sub>s</sub> than shade seedlings (Coopman et al. 2008). NPQ<sub>f</sub> has been shown to be important for seedling fitness under variable light conditions rather than for the induction of tolerance to high-light intensity (Kulheim et al. 2002). Additionally, a rapidly induced NPQ<sub>f</sub> capacity could be an adaptive feature that helps shade seedlings to cope with the highly dynamic irradiance regimes generated by forest canopies, where frequent short-term high-light exposures take place (Leakey et al. 2005). The higher NPQ<sub>s</sub> found in sun plants after photoinhibitory treatment at low temperature might be the consequence of a decrease in xanthophyll cycle activity, as evidenced by their lower NPQ<sub>f</sub>. It has been proposed that under low temperature the enzymatic conversion of violaxanthin to zeaxanthin within the xanthophyll cycle is thermodynamically restricted and additional reaction centre quenching can occur (Ivanov et al. 2003). Sun plants exhibited a higher pool of the xanthophyll cycle, lutein and neoxanthin (Figs. 8, 9). Nonetheless, under summer conditions the DEPS was similar in plants from both light environments. These results are consistent with previous results obtained under laboratory conditions which indicated that NPQ<sub>f</sub> has a better correlation with total xanthophyll pool than DEPS (Coopman et al. 2008). It is likely that sun seedlings are more affected by the low temperature of the photoinhibitory treatment because of their stronger dependence on the xanthophyll cycle for energy dissipation than shade plants (Demmig-Adams and Adams 1992).

Under severe stress conditions, the capacity of regulated thermal dissipation mechanisms can be overwhelmed and NPQ<sub>s</sub> occurs. NPQ<sub>s</sub> has been associated with prolonged exposure to high-light stress, sustained down-regulation of photochemical activity, and photo-damage (Werner et al. 2002). NPQ<sub>s</sub> usually refers to a situation in which photosynthetic efficiency remains low or partially irreversible after hours of darkness. Our results with respect to NPQ components indicate that as plants develop in the sun, they become more sensitive to photoinhibitory conditions. Thus, sudden photoinhibitory conditions, as it often occurs in spring of the study site, may be a limiting factor for the shade-to-sun transition in seedlings of *N. nitida*.

State transition ( $F_r$ ) is a dynamic mechanism that enables plants to respond rapidly to changes in illumination (Lunde et al. 2000). State transition involves the activation of a protein kinase, which phosphorylates a fraction of LHCII leading to a redistribution of excitation energy between LHCII and LHCI (Haldrup et al. 2001; Mullineaux and Emlyn-Jones 2005; Tikkanen et al. 2008). We found an ontogenetic decrease in the state transitions capacity only in the shade environment, where small seedlings of *N. nitida* showed the highest  $F_r$ . The abrupt  $F_r$  reduction in light seedlings agrees with a previous study that proposes that state transitions are a way to maximize the efficiency of utilization of absorbed light energy under conditions when light is strongly limiting for growth (Mullineaux and Emlyn-Jones 2005). Considering this, small shade seedlings of *N. nitida* appear to be well prepared to live in shady environments. Shade seedlings, regardless of developmental stage, showed greater capacities for short-term balancing the absorbed excitation energy between photosystems than sun seedlings. A well-balanced PSII/PSI excitation could increase linear and cyclic electron transport (Tikkanen et al. 2008). The additional ATP generated through the cyclic electron transport could be used in the photosynthetic carbon fixation reactions. Additionally, this could provide the necessary ATP for D1 protein turnover (Wollman 2001) and the  $\Delta$ pH gradient for NPQ<sub>f</sub> induction (Cornic et al. 2000; Johnson 2005). The possible role of cyclic electron transport in the developmental adjustments of *N. nitida* seedlings should be further explored. For example, small shade seedlings could use this dynamic mechanism in the forest understorey where extremely rapid and pronounced sunflecks occur (Leakey et al. 2005). Our field results corroborate previous growth chamber findings in which the highest capacity for state transitions was obtained in small plants of *N. nitida* subjected to a low light acclimation treatment (Coopman et al. 2008). Thus, there is a reduction in the capacity for state transitions during the early ontogenetic development of shade *N. nitida* seedlings. Sun seedlings have a much lower  $F_r$  capacity than shade seedlings and do not show a reduction in  $F_r$  during development. This is consistent with the higher level of NPQ<sub>s</sub> found in these seedlings with respect to shade ones.

In summary, small seedlings have a more efficient photosynthetic apparatus to cope with limiting instead of excessive light than tall seedlings. There is a differential effect of ontogeny and light environment on photosynthetic parameters. Ontogeny affected light capture parameters in both photosystems, such as Chl density and state transitions, while light environment affected all the light energy dissipation parameters. With the exception of state transitions, there was an independent effect of seedling ontogeny and light acclimation on this phenotypic transition from

shade-to-sun seedlings. It is concluded that the shade-to-sun transition that occurs in *N. nitida* seedlings reflects short-term responses of the photosynthetic apparatus to light environment and longer term responses that depend on the developmental stage of the seedling.

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