

## Photosynthetic plasticity of *Nothofagus pumilio* seedlings to light intensity and soil moisture

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### Abstract

*Nothofagus* seedlings often survive and grow slowly for a long time in the shaded understory. This creates a seedling bank with a potential advantage in reestablishing canopy disturbances. To manage primary forests more effectively, it is important to understand the basis of plant regeneration ecophysiology, and their plasticity to changes in environmental factors. The objective was to evaluate the photosynthetic plasticity of *Nothofagus pumilio* seedlings to light intensity and soil moisture gradients; and to relate them with silvicultural prescriptions. Six treatments with three light intensities (4, 26 and 64% of the natural incident irradiance) and two soil moistures levels (40–60 and 80–100% soil capacity) were assayed under greenhouse controlled conditions. CO<sub>2</sub> gas exchanges were measured every month on seedlings growing in each condition. In the shaded treatments seedlings grow below their optimum photosynthetic potential (leaf light-saturated net photosynthesis rate of 5.1 μmol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup>) compared with the lighted treatments by improving their photosynthetic performance (8.3–8.4 μmol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup>). Seedling growing under low soil moisture conditions had higher leaf light-saturated net photosynthesis rate than plants grown under 80–100% soil water capacity (7.8 and 6.6 μmol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup>, respectively). When light (up to 150–200 μmol m<sup>-2</sup> s<sup>-1</sup>) and soil moisture (40–60% soil capacity) levels were favourable, seedling plants could exhibit their maximum photosynthetic capacity. If one of these factors became limiting, the plants reduced their photosynthetic rate, e.g. *N. pumilio* seedlings with enough light and high levels of soil moisture, probably decreased their growth and fine roots activity. For this, application of silviculture systems must take into account the changes in both factors (light and soil moisture) for maximize the growth potential in the natural regeneration. These findings must be combined with morphological variables at a whole-plant, shoot, crown and leaf levels to determine the optimum growth conditions.

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### 1. Introduction

Temperate forests of southern Chile and Argentina are recognized as one of the world's most pristine wilderness areas (Mittermeier et al., 2002; Rozzi et al., 2006). Consequently, the integration of effective forest management strategies and a greater understanding of forest regeneration in this area are of great importance for conservation. In these forests, seedlings of *Nothofagus* species often survive and grow slowly for long periods of time in the shaded understory (Rebertus and Veblen, 1993), creating a seedling bank (Cuevas and Arroyo, 1999) with

a potential advantage in reestablishing canopy disturbances after the opening of gaps due to windstorms, ice damage, timber harvesting or beaver engineering (Veblen, 1989; Rebertus and Veblen, 1993; Gutiérrez, 1994; Rebertus et al., 1997; Heinemann et al., 2000; Martínez Pastur et al., 2000, 2006; Anderson et al., 2006). However, while it is generally accepted that these forests regenerate in a gap dynamic from an established seedling bank, the eco-physiological factors that determine the success of seedling growth are much less known.

The current silvicultural prescriptions for *Nothofagus* forests are based mainly on opening the canopy to stimulate natural regeneration by modifying soil moisture and light availability at the understory level, e.g. shelterwood cuts (Martínez Pastur et al., 2000). Both factors are the most critical (Lieffers et al., 1999) that affect growth and survival of the understory seedlings of austral forests (Heinemann et al., 2000). After

Abbreviations: PPFD, photosynthetic photon flux density; S.D., standard deviation

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harvesting, the survival of the seedling bank and its rapid growth depends on the adaptation to the new micro-climatic conditions (Tognetti et al., 1998). If new silvicultural approaches intend to manage primary *Nothofagus* forests more effectively, it is important to understand the basis for interspecific differences in seedling growth (Messier et al., 1999). However, the success or failure of silvicultural systems depends on the functional responses of the species to different environmental conditions. Therefore, a balance between the new silvicultural systems that favor timber yield and the functional integrity of the forest ecosystem is needed (Messier et al., 1999).

Photosynthesis is one of the most important physiological parameter for all plant growth (Kramer and Kozlowski, 1979). Therefore, in this study the objective was to evaluate the photosynthetic plasticity of *Nothofagus pumilio* (Poepp. et Endl.) Krasser seedlings to light intensity and soil moisture gradients comparable to those environmental conditions created from silvicultural prescriptions in Tierra del Fuego Island. Seedling growing conditions are improved when the canopy is gradually opened, due to a higher light availability at understory level. However, when canopy is abruptly opened, higher effective rainfall reaches to the forest floor and consequently generates higher soil moistures, which could be unfavourable to the root development and seedling growth.

## 2. Methods

### 2.1. Plant material and growing conditions

Two-to-three-year-old *N. pumilio* seedlings of 6–7 cm in height were obtained from the understory in natural primary forests (54°06'S, 68°37'W). The seedlings were collected in stands with high canopy cover ( $94 \pm 5\%$  S.D. measured with a concave spherical crown densiometer, Forestry Suppliers, Jackson, MS, USA) at the beginning of the spring, during the first week of September before budburst occurs. Seedlings were transplanted into plastic pots with 14 cm diameter and 15 cm height, which were filled with a substrate of peat–sand–humic forest soil (1:1:1). The substrate corresponded to a clay loam soil (sand–silt–clay, 36–24–40%), with 7% organic matter, pH 4.99 and water field capacity of 81%. Field capacity was determined gravimetrically with the water content after 2 days of full soil moisture content.

Plants were grown in a greenhouse covered of 100  $\mu\text{m}$  of plastic commercial nylon to avoid the natural rainfall at Ushuaia city (Tierra del Fuego) (54°46'S, 68°12'W) under three light intensities (4, 26 and 64% of the natural incident irradiance) using one or two layers of commercial black cloth shade. The mean total solar irradiance over a broader spectrum during the summer was  $2085 \pm 534 \mu\text{mol m}^{-2} \text{s}^{-1}$  S.D., with a maximum value of  $2702 \mu\text{mol m}^{-2} \text{s}^{-1}$ . Temperature was controlled through forced ventilation avoiding more than 24 °C at plant canopy level. Air humidity, air and soil temperatures were measured in each light intensity treatment using data loggers along the studied period. Irrigation was done manually, maintaining half of the plants under soil moisture of 40–60%

soil capacity, while the other half was grown under 80–100% soil water capacity. The amount of irrigation was determined gravimetrically every 3 days.

In order to compare light intensities and microclimatic conditions in the greenhouse and in the natural forests, two stands growing at a site quality III ( $SI_{60} = 16.5 \text{ m}$ ) (Martínez Pastur et al., 1997) were sampled ( $n = 20$  plots) near where seedlings were collected. In each forest plot, trees were sampled by the “angle count sampling” method (BAF 6) (Bitterlich, 1984). The first stand corresponded to an old-growth pure forest with a basal area of  $69 \pm 10 \text{ m}^2 \text{ ha}^{-1}$  S.D., a quadratic mean diameter of  $40.6 \pm 5.8 \text{ cm}$  S.D., a density of  $471 \pm 66 \text{ trees ha}^{-1}$  S.D., a total over bark volume of  $825 \pm 118 \text{ m}^3 \text{ ha}^{-1}$  S.D. and a crown cover of  $91 \pm 4\%$  S.D. The second selected stand was previously harvested using a shelterwood cut method (Martínez Pastur et al., 2000), which left a remnant overstory with a basal area of  $30 \text{ m}^2 \text{ ha}^{-1}$ , and a crown cover of  $49 \pm 21\%$  S.D. Two data loggers were installed in each stand to measure air humidity, air temperature and 30 cm depth soil temperature. Light intensities in the two stands were recorded with a lux-meter using lux units (visible flux density) (Model Extech, Waltham, MA, USA) in eight plots of 25 points conformed a grid of  $5 \text{ m} \times 5 \text{ m}$ , during January in mid-days without clouds.

### 2.2. Sampling and measurements

A three factor ANOVA was performed, where light level (4, 26 and 64% of the natural incident irradiance), soil moisture (40–60 and 80–100% soil capacity) and month along the growing season (October to March) were the main factors. For each combination of light level and soil moisture, 6 blocks of 20 plastic pots were individualized in the greenhouse. During the first week of each month one plastic pot per block per treatment was randomly chosen for measurements ( $n = 6$  per treatment).

The net respiratory  $\text{CO}_2$  efflux were measured using an infra red gas analyzer (Model S151—Qubit Systems, Kingston, Ontario, Canada) with a  $9 \text{ cm}^2$  leaf chamber and electronic thermal mass flow-meter. The instrument use an open flow system that determines  $\text{CO}_2$  flux based on differential measurements. Data were taken in controlled laboratory conditions (12–14 °C in complete darkness) after an 8 h accustoming period of the plants to the new environmental conditions. Leaf chamber temperature was  $19.6 \pm 2.9 \text{ }^\circ\text{C}$  S.D., and the supplied air humidity was  $44.8 \pm 7.2\%$  S.D., while air  $\text{CO}_2$  concentration was  $315 \pm 43 \text{ ppm}$  S.D. Air supply was taken from outside and varied according to climatic conditions during the growing season.

Leaf phenology was determined in the greenhouse along the studied period. Phenological stages were defined as budburst (B), leaf unfolding (E1), leaf expansion (E2), leaf reddening (R) and leaf fall (F). The first open leaf (October) and the newly full expanded leaf (November to March) per plant were selected for measurements. To develop the photosynthetic light response curves, ten irradiance levels were assayed (0, 25, 50, 75, 100, 200, 300, 500, 750 and  $1000 \mu\text{mol m}^{-2} \text{s}^{-1}$ ). The leaves were allowed 3–5 min to accustoming to light intensity changes before

measurements. CO<sub>2</sub> measurements were taken 500 times min<sup>-1</sup> and data were collected when the concentrations were stabilized at least 150 times with a variation of ±1 ppm. In each curve, the rate of dark respiration ( $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ) (RDR), photosynthetic efficiency ( $\mu\text{mol CO}_2 \mu\text{mol PPDF}^{-1}$ ) (PE), leaf level light-saturated net photosynthesis rate ( $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ) (NPR), light compensation point ( $\mu\text{mol PPDF}^{-1} \text{ m}^{-2} \text{ s}^{-1}$ ) (LCP) and light saturation point ( $\mu\text{mol PPDF}^{-1} \text{ m}^{-2} \text{ s}^{-1}$ ) (LSP) were obtained. Data were subjected to an analysis of variance, where means were separated through a Tukey multiple range test at  $p \leq 0.05$ . The measured plants were discarded to avoid the influence of photo-inhibition damages in the following samplings.

### 3. Results

#### 3.1. Leaf phenology and microclimate growing conditions

In the greenhouse, average air temperature increased in all treatments from the beginning of the experiment at the end of the winter (5.2–6.2 °C), reaching a maximum in summer (13.9–14.4 °C) and then decreased in autumn (7.3–7.9 °C) (Fig. 1). In the field, the old-growth forests and the harvested stands increased their average air temperatures from winter (1.7 and

1.9 °C, respectively) to a maximum in summer (11.2 and 12.5 °C, respectively), and then decreased at the beginning of autumn (6.1 and 6.3 °C, respectively).

All treatments in the greenhouse and in the field presented a mean soil temperature following the pattern of air temperature (from 4.2 to 14.2 °C) (Fig. 1), but with a smoothing delay in the increase and decrease over time. In the primary forests, soil did not freeze during winter (1.1 °C) and reached its maximum value in summer (9.2 °C). In contrast, the harvested stand presented soil temperatures below zero at the beginning of spring (−0.3 °C) and reached the highest values in summer (11.9 °C during the first February weeks).

In the greenhouse, average air humidity varied according to air temperature from 62 to 82% (Fig. 1), while in the primary forest varied between 63 and 94%, and in the harvested stand between 61 and 88%.

The frequency and amount of irrigation in the greenhouse varied according to the treatments. Low and medium light intensity treatments required less frequent watering (12–23 times along the assay with 5.7–6.2 mm each), while high light intensity treatments needed between 36 and 46 times with 5.3–6.8 mm each. Total irrigation increased with the light intensity and soil moisture treatments (68.2 and 142.9 mm in low light intensity, 103.9 and 120.2 mm in medium light intensity, 191.6 and 315.1 mm in high light intensity, for low and high soil moisture treatments, respectively). It could be seen that the soil moisture was quickly reduced between irrigations at high levels of light intensity, and especially in the advanced growing season. In contrast, low levels of light did not result in low levels of soil moisture before late in the growing season.

In the greenhouse, most of seedlings survived the transplanting with a mortality rate of 0.5–2.4% during the spring. Budburst began in September in all treatments, followed by a massive leaf unfolding, which was more concentrated in low light intensity treatments and high soil moisture treatments. Leaf expansion began in December and was related to the formation of new leaves. In medium and high light intensity treatments leaf reddening occurred in March before the massive leaf fall in April. In the low light intensity treatment leaf reddening was not present (Figs. 2 and 3).

The measured natural incident light in the field was  $2384 \pm 90 \mu\text{mol m}^{-2} \text{ s}^{-1}$  S.D., which was higher than those measured near the greenhouse ( $2085 \mu\text{mol m}^{-2} \text{ s}^{-1}$ ). In the primary forest, the incident light diminished to 12%, with  $272 \pm 366 \mu\text{mol m}^{-2} \text{ s}^{-1}$  S.D., and the average extreme values of the plots were 31–1484  $\mu\text{mol m}^{-2} \text{ s}^{-1}$ . The low and medium light intensity treatments of the greenhouse represent the lower and higher average values that could be possible to find in the primary forests. In the harvested stand of the field, the incident light decreased to 49% with  $1150 \pm 913 \mu\text{mol m}^{-2} \text{ s}^{-1}$  S.D., and the average extreme values of the plots were 186–2553  $\mu\text{mol m}^{-2} \text{ s}^{-1}$ . The medium and high light intensity treatments of the greenhouse represent the lower and higher average values that could be possible find in the harvested stands.

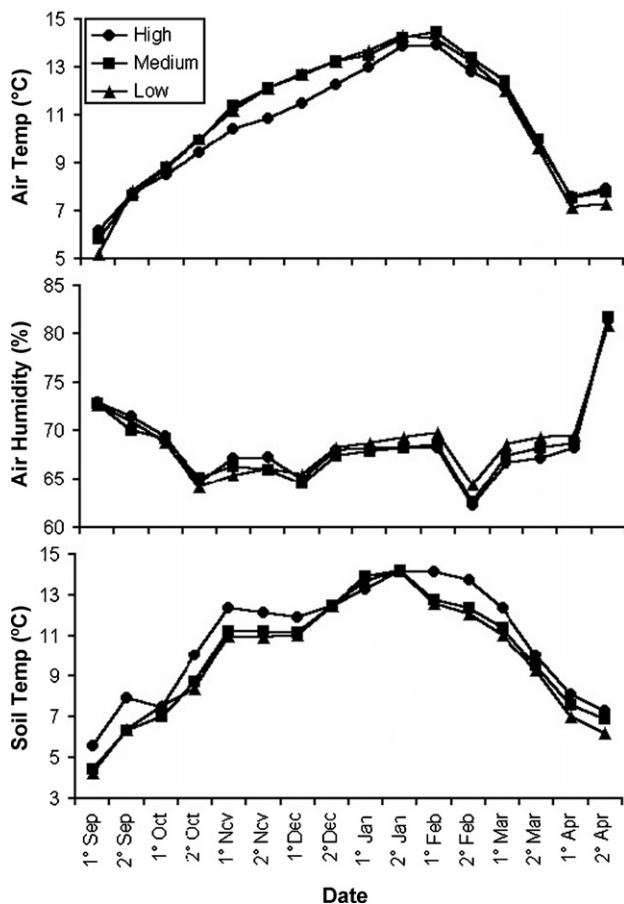


Fig. 1. Means for the first and second two weeks of each month for the greenhouse microclimatic variables through the studied period for the three light intensity treatments.

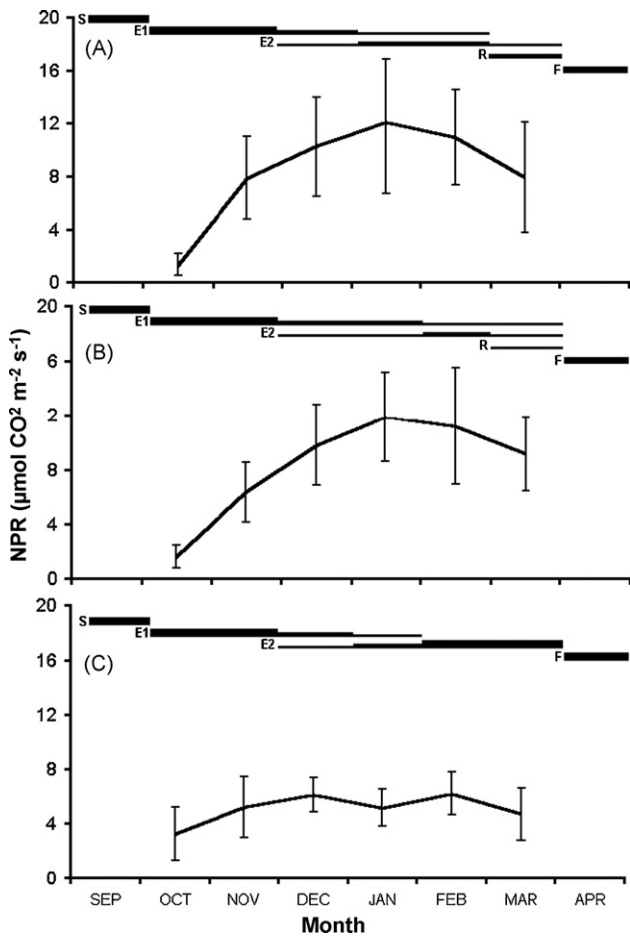


Fig. 2. Leaf light-saturated net photosynthesis rate (NPR) and leaf phenology (S, budburst; E1, leaf unfolding; E2, leaf expansion; R, leaf reddening; F, leaf fall) over the studied period for the three light intensity treatments: (A) high; (B) medium; (C) low. Error bars represent the standard deviation.

### 3.2. Net respiratory $\text{CO}_2$ efflux

The rate of dark respiration significantly varied during the growing season and with light intensity treatments (Table 1). It was lower at the beginning and the end of the experiment ( $-0.7$  to  $-0.8 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ), compared to the other months ( $-1.6$  to  $-1.8 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ). The rate of dark respiration increased with the light intensity treatment from  $-0.9$  to  $-1.7 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ . Also, photosynthetic efficiency significantly varied over the growing season and with light intensity treatments. It increased from the early spring ( $0.02 \mu\text{mol CO}_2 \mu\text{mol PPDF}^{-1}$ ) to the mid-summer ( $0.08 \mu\text{mol CO}_2 \mu\text{mol PPDF}^{-1}$ ) and decreased before the end of the growing season ( $0.06 \mu\text{mol CO}_2 \mu\text{mol PPDF}^{-1}$ ). Photosynthetic efficiency was significantly different between low light intensity treatment ( $0.05 \mu\text{mol CO}_2 \mu\text{mol PPDF}^{-1}$ ) and the other treatments ( $0.06$ – $0.07 \mu\text{mol CO}_2 \mu\text{mol PPDF}^{-1}$ ). The leaf level light-saturated net photosynthesis rate significantly varied among the three studied factors (Table 1). As for photosynthetic efficiency, values increased from the beginning of the growing season ( $2.1 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ) to mid-summer ( $9.7 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ) and then decreased in March ( $7.3 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ). In

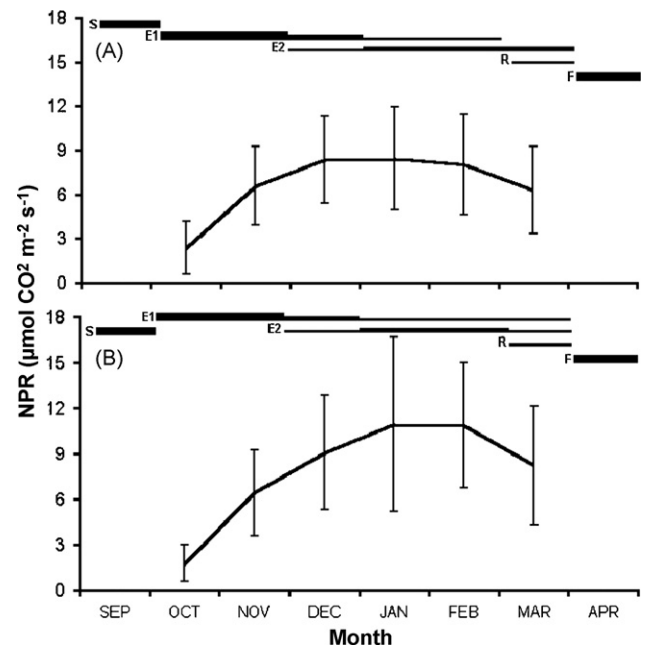


Fig. 3. Leaf level light-saturated net photosynthesis rate (NPR) and leaf phenology (S, budburst; E1, leaf unfolding; E2, leaf expansion; R, leaf reddening; F, leaf fall) during the studied period for the two soil moisture treatments: (A) high; (B) low. Error bars represent the standard deviation.

the low soil moisture treatment, the leaf level light-saturated net photosynthesis rate was significantly higher ( $7.9 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ) than in the high soil moisture treatment ( $6.7 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ) (Fig. 3). As in photosynthetic efficiency, the leaf level light-saturated net photosynthesis rate was significantly different among the low light intensity treatment ( $5.1 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ) and the others ( $8.3$ – $8.4 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ) (Fig. 3). The light compensation point significantly varied over the growing season and light intensity treatments (Table 1), being highest in the early spring ( $28.5 \mu\text{mol PPDF m}^{-2} \text{ s}^{-1}$ ) and then decreasing up to a minimum at the end of the growing season ( $12.3 \mu\text{mol PPDF m}^{-2} \text{ s}^{-1}$ ). This variable was significantly different between the high light intensity treatment ( $30.3 \mu\text{mol PPDF m}^{-2} \text{ s}^{-1}$ ) and the lower light intensity treatments ( $12.5$ – $16.5 \mu\text{mol PPDF m}^{-2} \text{ s}^{-1}$ ). Also, the light saturation point varied significantly over the growing season and light intensity treatments, with minimum values in the early spring ( $276 \mu\text{mol PPDF m}^{-2} \text{ s}^{-1}$ ) and then increased during the growing season reaching  $530 \mu\text{mol PPDF m}^{-2} \text{ s}^{-1}$ . This variable increased with the light intensity treatment from  $328$  to  $549 \mu\text{mol PPDF m}^{-2} \text{ s}^{-1}$ .

Significant interactions were found for photosynthetic parameters between main factors and the growing season (Table 1 and Figs. 2 and 3) where leaf light-saturated net photosynthesis rate presented most of the interactions (Table 1), e.g. the low light intensity treatment had higher leaf light-saturated net photosynthesis rate values at low irradiances, while high light intensity treatment had higher leaf light-saturated net photosynthesis rate values at high irradiances (Fig. 4). When the interactions between light intensity and month main factors were analyzed, it was observed that the

Table 1  
Means values of ANOVA analyzing net respiratory CO<sub>2</sub> efflux in *Nothofagus pumilio* seedlings considering soil moisture, light intensity and month as main factors and rate of dark respiration ( $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ) (RDR), photosynthetic efficiency ( $\mu\text{mol CO}_2 \mu\text{mol PPDF}^{-1}$ ) (PE), leaf light-saturated net photosynthesis rate ( $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ) (NPR), light compensation point ( $\mu\text{mol PPDF m}^{-2} \text{ s}^{-1}$ ) (LCP) and light saturation point ( $\mu\text{mol PPDF m}^{-2} \text{ s}^{-1}$ ) (LSP) as dependent variables

Main effects	RDR	PE	NPR	LCP	LSP
A = soil moisture					
Low	-1.30a	0.064a	7.89b	20a	450a
High	-1.37a	0.059a	6.68a	20a	432a
<i>F</i> ( <i>p</i> )	0.37 (0.545)	2.72 (0.101)	11.50 (0.001)	0.05 (0.828)	0.44 (0.509)
B = light intensity					
Low	-0.89c	0.051a	5.11a	12a	328a
Medium	-1.41b	0.065b	8.34b	17a	446b
High	-1.72a	0.069b	8.41b	30b	549c
<i>F</i> ( <i>p</i> )	21.03 (0.000)	10.93 (0.000)	37.96 (0.000)	39.55 (0.000)	21.90 (0.000)
C = month					
October	-0.68b	0.024a	2.08a	28c	276a
November	-1.84a	0.054b	6.48b	24bc	488bc
December	-1.60a	0.074cd	8.73cd	15a	388ab
January	-1.57a	0.083d	9.71d	19ab	474bc
February	-1.55a	0.072cd	9.45d	20ab	489bc
March	-0.79b	0.063bc	7.26bc	12a	530c
<i>F</i> ( <i>p</i> )	13.86 (0.000)	27.02 (0.000)	42.85 (0.000)	7.73 (0.000)	7.82 (0.000)
Interactions <i>F</i> ( <i>p</i> )					
A × B	0.08 (0.922)	3.81 (0.023)	9.71 (0.001)	0.51 (0.603)	1.20 (0.302)
A × C	0.72 (0.609)	1.40 (0.226)	2.73 (0.021)	0.59 (0.704)	1.73 (0.130)
B × C	3.52 (0.001)	4.18 (0.000)	5.46 (0.001)	0.50 (0.889)	2.87 (0.002)
A × B × C	0.67 (0.748)	0.78 (0.644)	2.28 (0.015)	0.92 (0.519)	0.81 (0.617)

*F* (*p*), *F*-statistic and probability at *p* = 0.05. Values followed by different letters (a–d) in each column and for each factor are significantly different with Tukey multiple range test at *p* < 0.05.

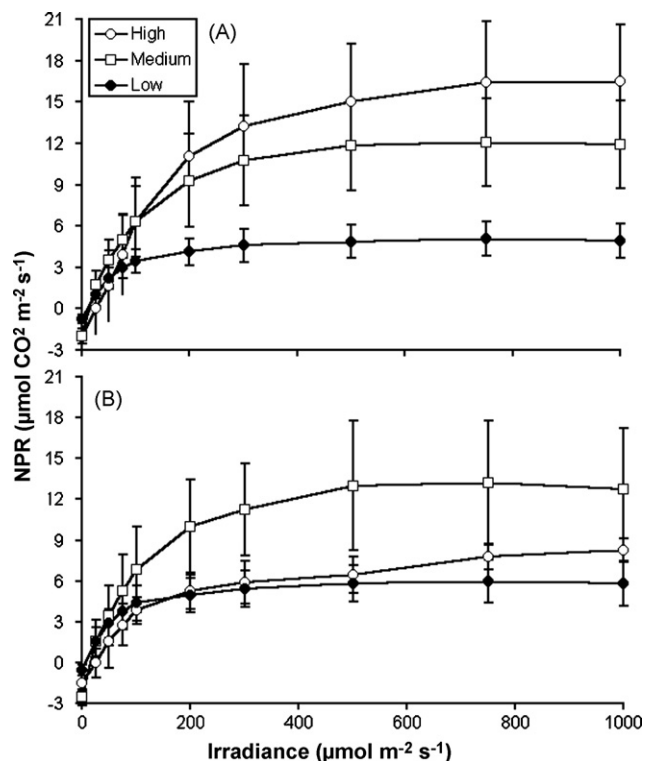


Fig. 4. January photosynthetic light response curves for the two soil moisture treatments: (A) low; (B) high in the three light intensity growing conditions.

slopes varied along the growing season. The values of the low light intensity treatment were lower in all months except in October. Similarly, there were interactions between soil moisture and month main factors. The values of the high soil moisture treatment were lower in all months except in October. Finally, it was observed interactions between light intensity and soil moisture. In high soil moisture levels the leaf light-saturated net photosynthesis rate value was higher in dark light treatments, while in low soil moisture levels it was higher in high light treatments.

Another interaction between light intensity and soil moisture treatments was observed when the photosynthetic light response curves were compared, e.g. during the month with the maximum leaf light-saturated net photosynthesis rate (Fig. 4). The low light intensity treatment had a higher photosynthetic rate in low irradiance levels ( $75\text{--}100 \mu\text{mol m}^{-2} \text{ s}^{-1}$ ) in both soil moisture treatments. In the low soil moisture treatments, as well as the medium and high light intensity treatments, the leaf level light-saturated net photosynthesis rate at high irradiance levels (up to  $100 \mu\text{mol m}^{-2} \text{ s}^{-1}$ ) was significantly higher than the low light intensity treatment. The leaf light-saturated net photosynthesis rate of the high light intensity treatment was affected by the high soil moisture treatment, decreasing its values and response. The obtained values were intermediate between the medium and low light intensity treatments.

## 4. Discussion

### 4.1. The experimental growing conditions

The registered temperature conditions in the greenhouse were slightly higher than in the natural forest stands. The higher temperatures at the beginning of the spring advanced the budburst, while during the summer the phenological period of new leaf formation was extended. In contrast, air humidity was similar between greenhouse and field conditions, and the experimental light intensities in the greenhouse reflected the natural limits of the two studied forest stands. The low light intensity treatments corresponded to a primary forest with a full closed canopy, while the medium light intensity treatment was comparable to the most open areas inside the primary forest or the closed sectors in the harvesting stands (up to 30 m<sup>2</sup> ha<sup>-1</sup> basal area), e.g. in mature southern beech stands, light levels in the understory are typically below 5% incident radiation (Reynolds and Frochot, 2003). The high light intensity treatment is comparable to many of the post-harvesting conditions in managed forests with large open areas (10–15 m<sup>2</sup> ha<sup>-1</sup> basal area) (Gea et al., 2004). A shelterwood-managed forest stand with 50% original basal area have a photosynthetically active radiation of 2.4–2.9 lx than of unmanaged stands (Caldentey et al., 2005a).

Most studies have collected the data in the field (e.g. Reynolds and Frochot, 2003), which makes it necessary to standardize the data before comparing them (Damesin, 2003). As a result, in this study the data were taken in the same light intensity, temperature, humidity and CO<sub>2</sub> levels in laboratory conditions through the growing season, by accustoming plants obtained from the greenhouse. The data were obtained at a leaf chamber temperature near 20 °C, which was reported as an optimum range of net photosynthesis (17–24 °C) for Australian *Nothofagus* species (Read, 1990).

The differences observed in the phenology between treatments were due to light intensities and soil moistures. The influence was greater in treatments with a better leaf level light-saturated net photosynthesis rate, affecting the extent of reddening and leaf unfolding. Light intensity affected the phenology, where leaf unfolding was more extended in time in the medium light level. In the higher light level treatments leaf reddening occurs due to the higher carotenoid production or chlorophyll degradation (Larcher, 2003; Hormaetxe et al., 2004). The leaf phenology in the experimental conditions was comparable to the natural growing conditions (Rusch, 1993; Barrera et al., 2000), except for leaf unfolding, which was more concentrated at the beginning of the summer in the natural forests.

### 4.2. Net respiratory CO<sub>2</sub> efflux changes along the growing season

The rate of dark respiration was correlated with the observed phenology stages and temperatures. At the beginning, the values were lower due to the leaves are not fully expanded. However, in *Litchi chinensis*, an evergreen tree, the rate of dark

respiration decreased as the leaves expanded (–6 and –2 μmol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup>) (Hieke et al., 2002). In our experiment, the rate of dark respiration reached its maximum value at the end of the spring, and decreased along the summer. At the end of growing season, the rate of dark respiration abruptly decrease due to the senescence of tissues. This is consistent with Kramer and Kozłowski (1979) who reported that respiration in young leaves was greater than in older leaves due to mature tissue contain less physiologically active material.

The photosynthetic efficiency and leaf light-saturated net photosynthesis rate were related to the developmental stage of the leaves, the photoperiod and temperatures. The leaves that are in the processes of unfolding and have not yet attain their full size did not have fully active chloroplasts, while well develop foliage is at the peak of its photosynthetic performance (Hieke et al., 2002; Larcher, 2003). At the end of growing season, leaf light-saturated net photosynthesis rate rapidly decrease due to chlorophyll breakdown and degeneration of the chloroplast (Larcher, 2003). The light compensation point was higher at the beginning of the growing season due to the incomplete development of the photosynthetic structures, and decreased in old tissues. In contrast, the light saturation point increased through the growing season. Leaves do not begin to contribute to the carbon budget of the plant until they are about half expand, which occur earlier in temperate deciduous than in tropical evergreen trees (Hieke et al., 2002).

### 4.3. Net respiratory CO<sub>2</sub> efflux changes with the light intensities

The rate of dark respiration was correlated with light intensities due to the demand of metabolic energy increases in the plant tissues growing in lighted environments. Lichtenthaler et al. (1981) report an increase of three-folds in sun exposed compared to shaded leaves of *Fagus*, while the increase in this study was on the order of two-fold. Heliophytic tree species have characteristics that favor high-light growth, which include higher relative growth rates and higher respiration under all environmental conditions (Larcher, 2003; Kneeshaw et al., 2006; Niinemets, 2006).

The photosynthetic efficiency and leaf light-saturated net photosynthesis rate were related to light availability, as was found in other studies (Tognetti et al., 1998; Larcher, 2003). *N. pumilio* seedlings can grow and survive with low light intensities (Rebertus and Veblen, 1993; Cuevas and Arroyo, 1999), which is the main limiting factor to achieve greater growth rates (Heinemann et al., 2000). However, through this study we have shown that 26% of the natural incident irradiance is enough to attain higher photosynthetic efficiency. Sun-tolerant plants typically have a higher light saturated photosynthetic capacity when exposed to high light intensities than sun-intolerant plants (Griffin et al., 2004). Lichtenthaler et al. (1981) found for *Fagus* an increase of 2.7 lx in sun exposed compared to shaded leaves, while our ratio was 1.6 lx.

Shaded leaves have lower light compensation points than sun-exposed leaves (Kramer and Kozłowski, 1979; Larcher,

2003), which was related to the rate of dark respiration. In our study, it was found an increase of 2.4 and 1.7 lx in sun-exposed leaves compared with shaded leaves for light compensation point and light saturation point, respectively. Similar values were found in *Fagus* by Lichtenthaler et al. (1981). Light saturation point was related to light intensities due to the photosynthetic structures, which are more efficient in light use. In our experiments it was necessary 14, 3 and 1.5 lx more light in the low, medium and high light level treatments, respectively, to saturate photosynthesis, compared to the average available to each treatment type.

#### 4.4. Net respiratory CO<sub>2</sub> efflux changes with the soil moisture

For *N. pumilio*, soils with high humidity were unfavorable compared to drier ones (40–60% soil capacity), decreasing the photosynthetic performance in higher light levels. For several oak species, the effects on seedling performance of a dry or wet summer vary under different light and competition conditions. Although leaf light-saturated net photosynthesis rate with competitors increase in the shade under dry conditions, it tend to decline in the shade under wet conditions (Davis et al., 1999). In *N. solandri* and *N. menziesii*, the rate of net photosynthesis diminishes about 60–65% in severe water-logged conditions (Sun et al., 1995). Regeneration of *N. pumilio* is produced mainly through gap dynamics (Veblen, 1989) due to the light availability under the overstory. Another limiting factor in this response is the water stress, which could switch the processes from a light- to a water-limited system (Heinemann et al., 2000; Heinemann and Kitzberger, 2006).

#### 4.5. Photosynthesis plasticity

Phenotypic plasticity is a modification adaptation that adjusts plants to the average conditions of radiation during morphogenesis and includes short- and long-term responses (Larcher, 2003). One growing season was enough to generate a significant plasticity in seedlings of *N. pumilio*, which germinated and grew during the two preceding years in primary forest. This plasticity allowed the plants to improve the efficiency of their photosynthetic systems to the new growing conditions. Light compensation and saturation points changed among light intensity treatments from the beginning of the measurements. The rate of dark respiration showed differences among light intensity treatments starting at the second month after sprouting, and photosynthetic efficiency expressed differences after the third month. As was described here, high-light adapted-plants presented greater respiration and light compensation point (Griffin et al., 2004; Kneeshaw et al., 2006). Finally, leaf light-saturated net photosynthesis rate changed during the second month for the light intensity treatments, while the same was not observed in the soil moisture treatments until the third month. In the high-light treatment, the leaf light-saturated net photosynthesis rate advanced the leaf senescence in comparison with the other treatments during the last two months (February and March).

The greater leaf level light-saturated net photosynthesis rate in the high soil moisture treatment could be due to the need for water during the new fine root formation at the beginning of the growing season (Larcher, 2003). Another explanation could be through the carry over effect of the preformed tissues (Tognetti et al., 1998). Some of the responses delay could possibly be related to the environment under which seedlings have developed, as was described in *Fagus sylvatica* seedlings after a windstorm (Reynolds and Frochet, 2003). In this study, the experiment was conducted for 1 year, but the plasticity of *N. pumilio* could also be a gradual process, occurring over successive growing seasons (Larcher, 2003; Reynolds and Frochet, 2003).

#### 4.6. Silviculture implications

In forest ecosystems, both natural and managed stands, plasticity to changing conditions plays a major role in tree recruitment and competition processes. The increase in resource availability can lead to differences in photosynthetic characteristics, leaf anatomy and whole plant growth. The overall response of tree seedlings to canopy opening depends on their ability of adaptation to the new conditions in managed forests (Tognetti et al., 1998; Heinemann et al., 2000).

*N. pumilio* primary forests have high crown closure, which retains a large percentage of the rainfall (13–25% for interception and 15–50% for evapo-transpiration) and diminishes the light availability at the understory level (Frangi and Richter, 1994; Caldentey et al., 2005a). Soil moisture can reach up to 50–60% of capacity during the late spring and summer (Mormeneo et al., 2004) in better quality sites in southern Patagonian primary forests (Martínez Pastur et al., 1997), which was quite similar to our low soil moisture treatment. In addition these primary forests have no long-term seed bank and seedling often survives and grows slowly for a long time in the shaded understory (Rebertus and Veblen, 1993). These seedlings are adapted to this limited light resource environment, developing photosynthetic machinery that allows them to grow and survive less than 20 years with no more than 0.5 m height (Martínez Pastur et al., 1999; Gea et al., 2004).

Natural dynamics (e.g. gaps) (Veblen, 1989), catastrophic events (Rebertus et al., 1997) and harvesting (Martínez Pastur et al., 2000; Gea et al., 2004) all open the overstory canopy in *Nothofagus* forests, thus increasing light availability (e.g. three-fold in shelterwood cuts) and the effective rainfall at understory level (Caldentey et al., 2005a). Higher global radiation, temperature and wind speed in the managed forest causes greater evapo-transpiration than in the unmanaged stands (Caldentey et al., 2005b). Several regeneration harvesting systems were applied in *N. pumilio* forests to assist regeneration already present or to make the natural regeneration possible, from light selective cuts to clear-cuts (Gea et al., 2004). The most applied method was the shelterwood cuts (Martínez Pastur et al., 2000; Rosenfeld et al., 2006), which open the canopy and generate light conditions similar to our experiment. According to our experiment, seedlings can be adapted to the new environmental conditions by optimizing their photosynthetic

systems. In contrast, high levels of soil moisture are not favorable to seedling growth, with a negative interaction that can influence the fine root dynamics thus affecting nutrient and water uptake. *N. pumilio* forests diminish drastically their site quality when the soils increase their soil moisture (Martínez Pastur et al., 1997), which often allows to the establishment of *N. antarctica* (Lencinas et al., 2005) or *N. betuloides* (Schlatter, 1994).

Plasticity to a high light environment is likely related to the plants successional stage for some species (Reynolds and Frochot, 2003). In a pure *N. pumilio* forest of southern Patagonia, the seedlings are the main woody component of the understory, constituting the pioneer species after large disturbances, and the climax species of the final succession. For this reason, many authors classified it as a shade intolerant species (Richter and Frangi, 1992; Veblen et al., 1997). However, when we analyzed its photosynthesis performance, we determined that the species can be considered as mid-tolerant, due to the shade tolerance in early development stages and for the fact that it reaches the maximum photosynthesis efficiency at relatively low light levels (26% of the natural incident irradiance). *N. pumilio* is not a shade tolerant species because is not able to reproduce and survive for long periods of time under closed canopy. Nor it is shade intolerant, due to the fact that its photosynthesis performance is not significantly improved in high light compared to medium light treatments. Gutiérrez (1994) suggest that Tierra del Fuego *Nothofagus* species are mid-tolerant due to their colonization abilities.

The photosynthesis performance of *N. pumilio* seedlings along light intensity and soil moisture gradients has important implications for forest management. In mesic *N. pumilio* forests, small- to moderate-sized gaps typically result in abundant regeneration, while in drier conditions soil moisture is a dominant factor and regeneration only occurs under special conditions (Heinemann et al., 2000). According to these results, the established seedlings of *N. pumilio* forests have the capacity to a quickly adaptation to the new environmental conditions generated by forest management practices. Gradual openings in the canopy of the overstory appears to be the most convenient (e.g. shelterwood cuts in Chile), due to the fact that seedlings in the understory reach their maximum photosynthetic potential. Water availability positively controls seedling survival and growth in the xeric forest, while in the mesic forest, survival and growth are differentially controlled by soil moisture and light availability, respectively (Heinemann and Kitzberger, 2006).

When ambient conditions are favourable, such as light (up to  $150\text{--}200\ \mu\text{mol m}^{-2}\ \text{s}^{-1}$ ) and soil moisture (40–60% soil capacity) for *N. pumilio*, seedling plants could exhibit their maximum photosynthetic capacity. If one of these factors is a limited resource, the plants reduce their photosynthesis, e.g. seedlings with enough light and high levels of soil moisture, probably decrease their growth and fine roots activity. For this, application of silviculture systems must take in account the changes in both factors (light and soil moisture) for maximize the growth potential in the natural regeneration.

## 5. Conclusions

Natural *N. pumilio* seedlings grow below their optimum photosynthetic potential in the understory, and quickly accustom to dramatic changes in the forest structure acting as pioneer plants in the succession process that follows. This photosynthesis plasticity allows the adaptation to several silviculture proposals. However, small gaps in the canopy are enough to attain the maximum photosynthetic performance. These findings must be combined with morphological variables obtained at whole-plant, shoot, crown and leaf levels, e.g. medium and high light intensity treatments presented the same photosynthesis efficiency, but if we combine it with the foliar area, the achieved growth could be different. Finally, it is necessary to compare our results of light and soil moisture performance with natural regeneration patterns and forest dynamics.

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