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ABOVE- AND BELOW-GROUND NUTRIENT TISSUE CONCENTRATION AND LEAF PIGMENT CHANGES IN PATAGONIAN WOODY SEEDLINGS GROWN UNDER LIGHT AND SOIL MOISTURE GRADIENTS

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□ *To understand the ecophysiology of natural regeneration above- and below-ground nutrient tissue concentration and leaf pigment changes in* Nothofagus pumilio *(lenga) seedlings grown in three light intensities (4%, 26% and 64% of natural irradiance) and two soil moisture levels (40 –60% and 80 –100% soil capacity) under greenhouse controlled conditions were evaluated. Carbon (C), nitrogen (N), phosphorus (P), magnesium (Mg), potassium (K), calcium (Ca) and pigment (chlorophylls and carotenoids) were measured on seedlings. Carbon, N, Mg, K and Ca increased in low light intensity and soil moisture treatments, while P decreased. Nutrients were higher in above- than in below-ground biomass. Chlorophylls were lower in high light treatments, while carotenoids increased their content. All pigments were greater in low soil moisture treatments. These changes are closely related to their photosynthetic plasticity and biomass compartmentalization. Plants growing in high light were more efficient to produce the same amount of plant biomass.*

Keywords: silviculture, nutrient models, nitrogen, carbon, ecophysiology, regeneration

INTRODUCTION

In southern Patagonian forests, seedlings often survive for long periods of time in the shaded understory with a potential advantage in reestablishment canopy structure after natural or antropic disturbances (Rebertus and Veblen, 1993; Heinemann et al., 2000; Martínez Pastur et al., 2000). These changes modify the availability of light and soil moisture at the understory level, among others factors (Frangi and Richter, 1994; Caldentey et al.,

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2000). If new silvicultural approaches intend to manage these forests more effectively (Martínez Pastur et al., 2009), first it must understand the regeneration adaptation processes related to different environmental conditions (Aussenac, 2000; Lencinas et al., 2007; Martínez Pastur et al., 2007).

The adaptation ability of seedlings to different light and soil moisture conditions also determine recruitment and growth processes (Lieffers et al., 1999; Heinemann et al., 2000; Lencinas et al., 2007). This capability could refer to changes in photosynthetic performance, in biomass compartmentalization, in gas exchange, in leaf morphology and in water capture (Sun et al., 1995; Evans and Poorter, 2001; Damesin, 2003; Reynolds and Frochot, 2003; Sardans et al., 2006; Lencinas et al., 2007; Martínez Pastur et al., 2007). These physiological adjustments could also modify nutrient tissue concentration and their requirements (Sardans et al., 2006), as well as leaf pigment changes in the seedlings (Valladares et al., 2000; Damesin, 2003; Larcher, 2003; Zúñiga et al., 2006; Lichtenthaler et al., 2007) according to the new micro-environmental conditions.

There are some studies in functional ecology of *Nothofagus* forests (Caldentey, 1992; Heinemann et al., 2000; Hart, et al. 2003; Frangi et al., 2005; Romanya et al., 2005; Peri et al., 2006, 2008; Caldentey et al., 2009), which include changes in photosynthetic performance and biomass compartmentalization (Lencinas et al., 2007; Martínez Pastur et al., 2007). However, the influences of changes in nutrient tissue or leaf pigment contents that determine the success of seedling growth are much less known. The objective was to evaluate above- and below-ground nutrient tissue concentration (carbon and macronutrients) and leaf pigment changes (chlorophyll and carotenoids) in *Nothofagus pumilio* seedlings grown in light intensity and soil moisture gradients.

MATERIAL AND METHODS

Plant Material and Growing Conditions

Two to three year-old *Nothofagus pumilio* seedlings of 6-7 cm in height were obtained from the understory in natural old-growth forests (54°06′ SL, 68°37′ WL). The seedlings were collected in stands with high canopy cover (94% ± 5% standard deviation-SD measured with a concave spherical crown densiometer, Forestry Suppliers, Jackson, MS, USA) at the beginning of the spring season during the first week of September before budburst occurs. Seedlings were immediately 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 two days of full soil moisture.

Plants were grown in a greenhouse covered with 100 μm of plastic commercial nylon to avoid the natural rainfall at Ushuaia city (Tierra del Fuego) (54°46′ SL, 68°12′ WL) under three light intensities: 64% (L), 26% (M), and 4% (O) 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 μmol m^{-2} s^{-1} ± 534 μmol m^{-2} s^{-1} SD, with a maximum value of 2702 μmol m^{-2} s^{-1}. Temperature was controlled through forced ventilation avoiding more than 24°C at plant canopy level. Data loggers (HOBO Onset, Bourne, MA, USA) were used to measure air temperature (13.1°C ± 2.1 SD for L, 13.6°C ± 1.9 SD for M and 13.4°C ± 1.8 SD for O), air humidity (66% ± 10 SD for L, 66% ± 9 SD for M, and 68% ± 9 SD for O) and soil temperature (13.6°C ± 1.3 SD for L, 12.4°C ± 0.7 SD for M, and 12.1°C ± 0.9 SD for O). Irrigation was applied manually, maintaining half of the plants under soil moisture of 40–60% soil water capacity (S), while the other half was grown under 80–100% soil water capacity (H). The amount of irrigation was determined gravimetrically every three days.

In the greenhouse, most of seedlings survived the transplanting with a mortality rate of 0.5–2.4% during the spring. Details of the experimental design and the growing conditions were discussed in Martínez Pastur et al. (2007) and Lencinas et al. (2007).

Sampling and Measurements

For each combination of light intensity and soil moisture levels, six blocks of 20 plastic pots were individualized in the greenhouse. Plant sampling was conducted in February, and the above- and below-ground components were separated for nutrient tissue concentration (NTC) analysis and pigment determination. Plant material were dried in a forced draft oven at 65°C to constant weight and ground in a mill containing 1 mm stainless steel screen. Nitrogen (N) was determined using the Kjeldahl technique using a Büchi K350 (Büchi, Flawil, Switzerland), while carbon (C), phosphorus (P), magnesium (Mg), potassium (K) and calcium (Ca) tissue concentration were determined with a plasma emission spectrometry (ICPS 1000 III, Shimadzu, Kyoto, Japan). Chlorophyll (A and B) and carotenoids were determined using a spectrophotometric method following Sims and Gamon (2002). For photosynthetic pigment determinations, one plant per block and treatments was chosen at random, and the first fully expanded leaf was collected and analyzed immediately. Foliar disks of 0.50 cm^2 were ground in 2 mL aqueous acetone-tris buffer solution (80% v/v, pH 7.8), and centrifuged at 1610 g for 10 min at 20°C. The supernatant was used for determining absorbance at 470, 537, 647 and 663 nm to obtain carotenoid, chlorophyll A and B contents. Leaf pigment contents in mmol m^{-2} were a function of the calculated solution concentrations, the total volume of extraction solution and the extracted total leaf area.

Data Analysis

For comparison of different light intensities and soil moisture levels, the following analyses were done: (i) three-way ANOVAs for NTC including above- and below-ground compartments as the third main factor; (ii) two-way ANOVA for pigment content; (iii) a principal components analysis (PCA) using a matrix of NTC for each treatment; and (iv) a cluster analysis using a complete linkage amalgamation rule and Euclidean distance measurement based on a matrix of NTC for each treatment. A post-hoc Tukey's test was used for all mean comparisons ($p < 0.05$).

RESULTS

NTC in Above- and Below-Ground Biomass

Significant differences were found in NTC, except for C and N when soil moisture content was considered as main factor (Table 1). Thus, Mg, K and Ca were higher in the low soil moisture (S), while P was greater in the higher soil moisture contents (H). On the other hand, NTC were higher under low light intensity conditions (O), except for N and P which were higher in M and L treatments. Finally, NTC were higher in the above-ground compared with below-ground biomass. Significant interactions were found for all the nutrients, except for N (Table 1). When the interactions between factors were analyzed, it was observed that light intensity and soil moisture treatments presented some fluctuations in the variation range for each nutrient (Figure 1). For example, C concentration in below-ground biomass was higher than in above-ground biomass in the low light intensity treatments, which generates the interactions.

Carbon concentration varied from 44.4–45.3% for above-ground biomass being higher in OH treatment, and 43.2–45.4% for below-ground biomass being higher in OS treatment. Nitrogen concentration varied from 1.4–2.9% for above-ground biomass, and 0.7–1.7% for below-ground biomass being higher in MH treatment for both compartments. Magnesium concentration varied from 0.10–0.18% for above-ground biomass being higher in OS treatment, and 0.09–0.14% for below-ground biomass being higher in OH treatment. Phosphorus concentration varied from 0.13–0.19% for above-ground biomass being higher in LS treatment, and 0.10–0.13% for below-ground biomass being higher in LH treatment. Potassium concentration varied from 0.39–0.62% for above-ground biomass, and 0.24–0.45% for below-ground biomass being higher in OS treatment for both compartments. Calcium concentration varied from 0.45–0.90% for above-ground biomass being higher in OS treatment, and 0.27–0.42% for below-ground biomass being higher in MS treatment (Figure 1).

When above-ground NTC was analyzed through a PCA the axis one (eigenvalue = 3.200) give priority to the P from the other nutrients to

TABLE 1 Mean values of ANOVA analyzing nutrient tissue concentrations considering soil moisture, light intensity and biomass compartment as main factors, and percentage of carbon (C), nitrogen (N), phosphorous (P), magnesium (Mg), potassium (K) and calcium (Ca) as dependent variables

Factor	C	N	P	Mg	K	Ca
A = Light intensity						
L	44.00 a	1.14 a	0.16 c	0.10 a	0.33 a	0.39 a
M	44.35 b	2.05 c	0.14 b	0.12 b	0.34 a	0.50 b
O	44.99 c	1.72 b	0.12 a	0.15 c	0.48 b	0.62 c
F(p)	26.6 (<0.01)	40.2 (<0.01)	33.8 (<0.01)	101.3 (<0.01)	297.4 (<0.01)	68.6 (<0.01)
B = Soil moisture						
H	44.45	1.68	0.15 b	0.12 a	0.36 a	0.46 a
S	44.53	1.79	0.13 a	0.14 b	0.40 b	0.54 b
F(p)	0.01 (0.90)	3.8 (0.05)	12.4 (<0.01)	34.0 (<0.01)	55.6 (<0.01)	28.2 (<0.01)
C = Biomass						
B	44.04 a	1.22 a	0.12 a	0.11 a	0.31 a	0.35 a
A	44.85 b	2.24 b	0.17 b	0.14 b	0.44 b	0.67 b
F(p)	51.0 (<0.01)	332.7 (<0.01)	156.7 (<0.01)	68.6 (<0.01)	599.5 (<0.01)	391.3 (<0.01)
Interaction AxBxC	4.01 (0.02)	0.19 (0.82)	3.26 (0.04)	6.14 (<0.01)	23.60 (<0.01)	4.48 (0.01)

Light intensity: L = 64% incident light; M = 26% incident light; O = 4% incident light. Soil moisture: H = 80–100% water field capacity; S = 40–60% water field capacity. Biomass: B = below-ground; A = above-ground. F(p) = F statistic and probability at $p = 0.05$. Values followed by different letters in each column and for each factor are significantly different with Tukey's Multiple Range test at $p < 0.05$.

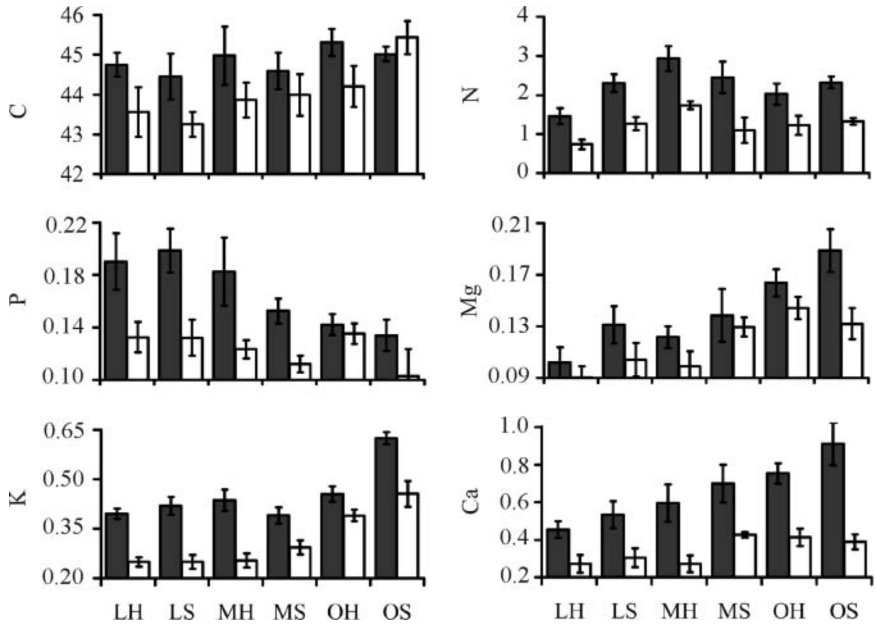

FIGURE 1 Tissue concentration (%) of carbon (C), nitrogen (N), magnesium (Mg), phosphorous (P), potassium (K) and calcium (Ca) for above ground (■) and below ground (□) biomass. Light intensity: L = 64% incident light; M = 26% incident light; O = 4% incident light. Soil moisture: H = 80–100% water field capacity; S = 40–60% water field capacity. Bars indicate standard deviation.

separate the samples, while the axis three (eigenvalue = 0.819) separated the samples using C and N among the other nutrients (Figure 2A). A gradient from left to right was observed among treatments due to light intensity, while soil moisture content treatments not present a clear disjunction except for the low light intensity treatments (O). Cluster analysis separated OS treatment from the others (Euclidean distance of 0.6), while LH treatment was separated at Euclidean distance of 0.5 (Figure 2A). When below-ground NTC was analyzed through a PCA using the axis one (eigenvalue = 3.118) and two (eigenvalue = 1.108), all nutrients participate in the treatment ordination (Figure 2B). A gradient of light intensity treatments was observed from left to right, and soil moisture content treatments present a clear disjunction except for the high light intensity treatment (L). Cluster analysis separated the treatments in two groups: (i) the first one with the low light intensity treatments (OH and OS) linkage with MS treatment; and (ii) the second one LH-MH linkage with LS treatment (Figure 2B).

Leaf Pigment Contents

Significant differences were found in pigment leaf contents, except for Chlorophyll A when light intensity was considered as main factor (Table 2). Chlorophyll B was significantly lower in high light intensity treatments (L),

TABLE 2 Means values of ANOVA analyzing pigment leaf content considering soil moisture and light intensity as main factors, and chlorophyll A (mmol m^{-2}) (A), chlorophyll B (mmol m^{-2}) (B) and carotenoids (mmol m^{-2}) (C) as dependent variables

Factors	A	B	C
A = Light intensity			
L	0.275	0.076 a	0.172 ab
M	0.337	0.112 b	0.177 b
O	0.307	0.110 b	0.148 a
F(p)	3.26 (0.05)	12.89 (<0.01)	4.04 (0.02)
B = Soil moisture			
H	0.282 a	0.091 a	0.153 a
S	0.332 b	0.107 b	0.178 b
F(p)	6.00 (0.02)	6.04 (0.02)	8.23 (<0.01)
Interaction AxB	0.50 (0.61)	1.43 (0.25)	5.06 (0.01)

Light intensity: L = 64% incident light; O = 4% incident light. Soil moisture: H = 80–100% water field capacity; S = 40–60% water field capacity. F(p) = F statistic and probability at p = 0.05. Values followed by different letters in each column and for each factor are significantly different with Tukey's Multiple Range test at $p < 0.05$.

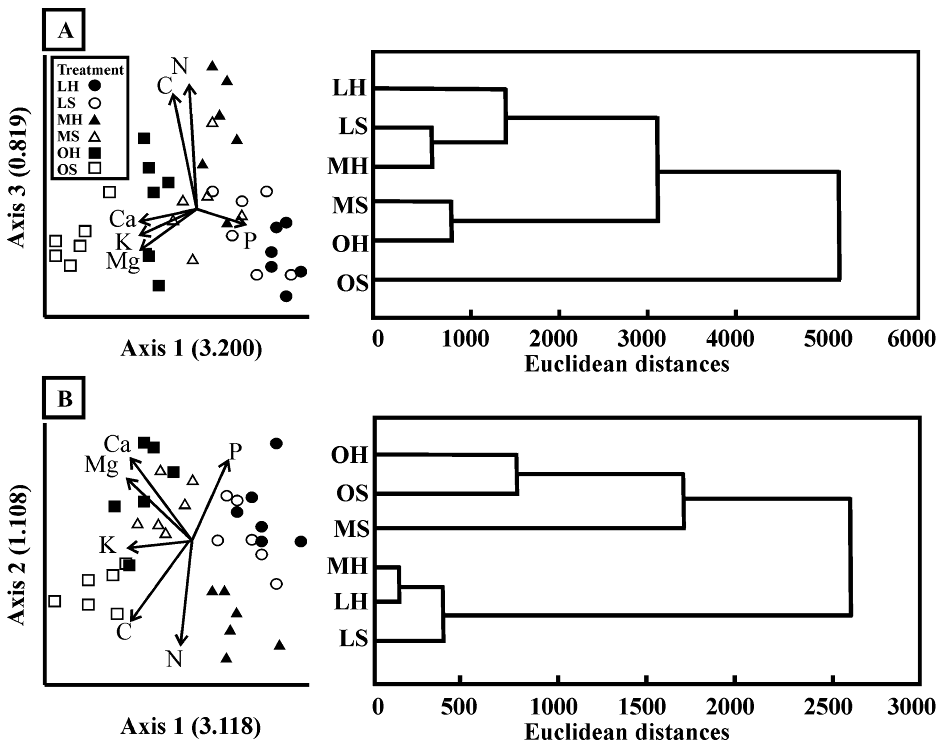

FIGURE 2 PCA ordination and cluster analysis for seedlings nutrient tissue content of carbon (C) (%), nitrogen (N) (%), magnesium (Mg) (ppm), phosphorous (P) (ppm), potassium (K) (ppm) and calcium (Ca) (ppm) for above- (A) and below-ground (B) biomass. Light intensity: L = 64% incident light; M = 26% incident light; O = 4% incident light. Soil moisture: H = 80–100% water field capacity; S = 40–60% water field capacity.

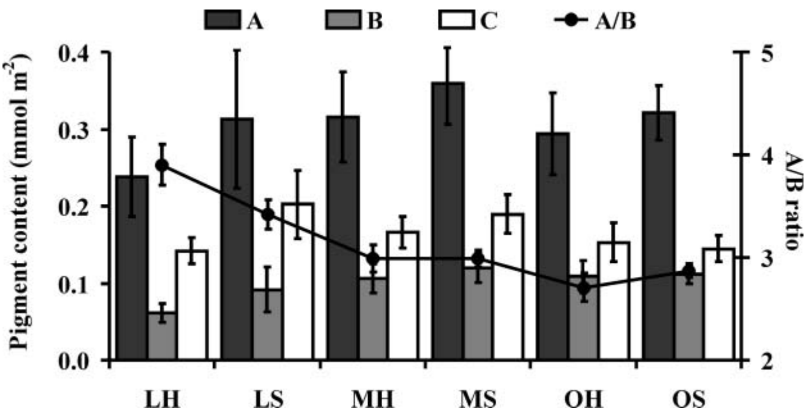

FIGURE 3 Pigment leaf content of chlorophyll A (mmol m^{-2}) (A), chlorophyll B (mmol m^{-2}) (B), carotenoids (mmol m^{-2}) (C) and ratio between chlorophyll A and chlorophyll B (A/B). Light intensity: L = 64% incident light; M = 26% incident light; O = 4% incident light. Soil moisture: H = 80–100% water field capacity; S = 40–60% water field capacity. Bars indicate standard deviation.

while carotenoids were lower in low light intensity treatments (O). Finally, all pigment leaf contents were higher in low soil moisture content treatments (S). Significant interaction was only found for carotenoids (Table 2), where soil moisture in the high light intensity treatments presented a variation in the expected range of carotenoid leaf tissue content (Figure 3). Regarding photosynthetic processes that are controlled by incident light, it is more appropriate to use the leaf area as a reference system to analyze chlorophyll and carotenoid levels (Lichtenthaler et al., 2007). Chlorophyll A and chlorophyll B varied from 0.23–0.35 mmol m^{-2} and 0.06–0.11 mmol m^{-2} respectively, being both higher in MS treatment. Carotenoids varied from 0.14–0.20 mmol m^{-2} being higher in LS treatment (Figure 3). Leaves grown in high light intensity treatments (L) and high soil moisture content treatments (H) presented significantly higher values for the chlorophyll A/B ratio (Table 2). This ratio varied from 2.71 in OH treatment to 3.90 in LH treatment.

DISCUSSION

NTC in Above- and Below-Ground Biomass

Seedling acclimation to environmental changes during the forest dynamics may influence in the success of the natural regeneration and nutritional balance at ecosystem level (Grime, 1979; Hart et al., 2003; Frangi et al., 2005; Lencinas et al., 2007). Also, NTC studies on seedlings and saplings are important because it varies according to tree component and age, e.g., in above- and below ground biomass of seedlings and saplings (5–20 years) of *Nothofagus antarctica* (Peri et al., 2006, 2008).

Most of the described NTC of *N. pumilio* seedlings are included in the range of other reported forest species. Phosphorus (0.13–0.20%), Mg (0.10–0.19%) and K (0.39–0.62%) were similar to those reported for other *Nothofagus* species (0.15–0.23%, 0.02–0.17% and 0.12–0.70%, respectively), at leaf (Caldentey, 1992; Diehl et al., 2003, 2008) or whole plant level (Hart et al., 2003; Peri et al., 2006, 2008). Beside this, Rapp et al. (1999) and Santa Regina (2000) report similar NTC for above-ground for several *Quercus* species (0.02–0.17%, 0.02–0.21% and 0.12–0.83% for P, Mg and K), and Wang et al. (2000) for *Betula papyrifera* (0.26%, 0.27% and 1.07%, respectively) and *Abies lasiocarpa* (0.17%, 0.09% and 0.49%, respectively). Peri et al. (2008), Diehl et al. (2008) and Hart et al. (2003) presented lower N values for other *Nothofagus* species (0.18–1.69%, 0.51–0.52% and 0.05–1.25%, respectively) than those described in the present work for *N. pumilio* (0.15–2.93%). Higher concentrations in seedlings in this work could be due differences in the age of the sampled trees, due to NTC decrease with age (Das and Chaturvedi, 2005; Peri et al., 2006). On the other hand, Rapp et al. (1999), Santa Regina (2000), Hart et al. (2003) and Peri et al. (2008) presented higher Ca concentrations for *Quercus* species (1.7%), *N. truncata* (1.4%) and *N. antarctica* (1.12%) than values described here (0.90%), relationated with higher Ca concentrations in mature trees, which is the main structural component in it (Peri et al., 2006, 2008).

Some works described above- and below-ground NTC in *Nothofagus* forests (Caldentey, 1992; Richter and Frangi, 1992; Veblen et al., 1996; Weber, 1999; Diehl et al., 2003, 2008; Frangi et al., 2005), but few analyze it in seedlings and saplings (Peri et al., 2006, 2008). These authors found similar N, Mg and K concentrations, but higher Ca concentrations (above-0.06–1.23% and below-ground 0.06–0.7%) and higher P concentration for below-ground biomass (0.12–0.23%). These differences can be related with the site quality of the stands (Peri et al., 2008), and the strategies of assignment of the nutrients according to the readiness in the forest soils (Newman and Hart, 2006). In example, *N. antarctica* usually grows in marginal sites than *N. pumilio*, and the higher NTC concentrations can be a strategy to survive in these extreme environments (e.g., strongest root system development against winds) (Peri et al., 2008).

Light Intensity and Soil Moisture Content Influence on NTC

NTC of different compartments (e.g., leaves, roots or stems) are related to the physiological processes associated to respiration, photosynthesis, water and nutrient uptake processes (Marschner, 1995). These differences could be originated due to the combination of environmental factors, as soil water (Austin and Sala, 2002; Peri et al., 2008) and light availabilities in the stands (Larcher, 2003).

Nitrogen concentration changed according to light intensity levels, being related to the photosynthetic capacity of seedlings, while soil moisture content not showed any evident effect. Through this study we have shown that higher N occurred in M treatments, where higher photosynthetic efficiency was attained for *N. pumilio* seedlings (Martínez Pastur et al., 2007). Similar optimum light levels for N were described for *Abies balsamea* grown under different light treatments (10, 40, 70 and 100% ambient light), where N concentration was higher (2.7%) in middle (40%) light intensities (Evans et al., 2001). The fact that foliar N concentration decreased with increasing light availability while content increased may suggest that N concentrations in seedlings grown in the lower light environments were in excess of physiological requirements and therefore represent a luxury consumption (Evans et al., 2001) if we considered the entire biomass and not only the foliar area of each seedling. This N increment in *N. pumilio* seedlings growing in middle light intensity treatments allow to the increase of above-ground biomass (Lencinas et al., 2007), but diminish the root growth (Marschner, 1995) which can affect nutrient and water uptake in the following development stages.

The higher P concentration in high light intensity and soil moisture treatments can be related to an increment in the metabolic activity of the tissues, as a consequence of the increase in the photosynthetic capability of the seedlings (Martínez Pastur et al., 2007). Beside this, water facilitates the mobilization of organic phosphorus in the soil (Schachtman et al., 1998), increasing total phosphorus and phosphate in *Fagus sylvatica* plant tissues (Peuke and Rennenberg, 2004). Forest soils in Tierra del Fuego have high P percentage (Novóa Muñoz et al., 2007) and their immobilization not represent a limiting growing factor for *N. pumilio* seedlings. Evans et al. (2001) reported that higher light availability increased P content in both, *Betula cordifolia* and *Abies balsamea* seedlings, and can be explained through the facilitation of mycorrhizal fungi.

Other nutrients, as K, Mg and Ca, were related to cell turgence, water uptake, pH regulation and cell structure (Marschner, 1995), diminishing their concentrations when the availability of light and soil water content increases, e.g. in OS treatment we found the higher concentrations of these nutrients. In the below-ground biomass, the increase in the concentrations of these nutrients in the low soil moisture level treatments (S) allows to increase the ability of the water uptake (Marschner, 1995). In the above-ground compartments, especially in leaves, these nutrients also have the same regulation purpose, which can facilitate the transport to a vascular level and the assignment of the available resources (Marschner, 1995; Larcher, 2003). Beside this, K also stimulates CO_2 fixation, improving the photosynthesis for those plants growing in low light intensity treatments (Marschner, 1995). Higher C concentration (45% for above- and below-ground) was registered in tissues of low light intensity treatments, which can be explained through to the ratio between the compartments of the above- and below-ground biomass (e.g., ratio between leaves and shoots, or fine and coarse roots) (Lencinas et al., 2007). On the other hand, stress in plants can affect the C uptake through the stomata aperture, altering the ratio among the fixed nutrients (Kendrick and Kronenberg, 1994; Larcher, 2003; Baquedano and Castillo, 2006). In Chilean forests, the evergreen *N. nitida* closed its stomata at midday for water conservation at high irradiance conditions, but maintained its photosynthetic rate (Zúñiga et al., 2006).

Seedlings growing in shaded environments (e.g., O treatments) presented lower growth biomass rates (Lencinas et al., 2007). Beside this, these treatments presented higher concentrations of some nutrients (C, Mg, K, Ca), which can indicate that those plants have a low efficiency in these nutrient uses, due to they need more nutrients per unit of biomass (e.g., seedlings growing in O treatment needs 453 mg of C, 1.89 mg of Mg, 6.25 mg of K and 9.10 mg of Ca, to produce 1 g of above-ground biomass compared to seedlings growing in L treatment, which needs 444 mg of C, 1.02 mg of Mg, 3.95 mg of K and 4.54 mg of Ca, to produce 1 g of the same biomass). Multivariate analyses on NTC showed similarities with the photosynthetic rate and biomass compartmentalization of the seedlings. Above-ground NTC separate firstly the OS treatment, which presented the higher foliar area/total biomass ratio (Lencinas et al., 2007), and secondly the LH treatment, which diminished their photosynthetic rate due to negative interactions between light and soil moisture content (Martínez Pastur et al., 2007). Below-ground NTC analysis separated the treatments into two groups, one with the higher (MH, MS and LS) and one with the lower photosynthetic rates and total biomass productions (LH, OH and OS) (Martínez Pastur et al., 2007; Lencinas et al., 2007).

Leaf Pigment Contents

Chlorophyll and carotenoid formation in plants is regulated by endogenous (e.g., enzymatic activity) and exogenous factors (e.g., nutrient, water or light availability) during growth and differentiation processes (Gehring et al., 1977; Timko, 1998; Valladares et al., 2000). Described pigment contents in leaves of *N. pumilio* are in agree with those reported for other forest species (Valladares et al., 2000; Lee et al., 2003; Zúñiga et al., 2006; Lichtenthaler et al., 2007; Aranda et al., 2008). Ratio of chlorophylls A/B in L (3.4–3.9) and O (2.7–2.8) treatments are characteristics of sun- and shade-type leaves (Damesin, 2003), which was similar or slightly lower than reported for other *Nothofagus* species (Hogan et al., 1997; Zúñiga et al., 2006), and similar than those reported for other forest species (Lee et al., 2003; Torres Netto et al., 2005).

Light intensity and soil moisture content influenced over the chlorophyll in *N. pumilio* leaves. Chlorophylls were significantly lower in the high light treatments, and could be explained through the destruction of pigments for photo-inhibition (Larcher, 2003; Zúñiga et al., 2006). Also, chlorophylls were significantly lower in the high soil moisture content treatments, and could be a consequence of the water-logging and inadequate root development (Martínez Pastur et al., 2007; Lencinas et al., 2007) affecting the nutrient uptake and subsequent pigment formation. This negative effect of water-logging was also founded for *N. nitida* (Rojas, 2007). Valladares et al. (2000) not found a clear relation among soil nutrient availability and pigments in leaves of *Quercus ilex* and *Q. coccifera*, which depends of NTC and light interactions.

Carotenoids are usually considered to perform two major functions in photosynthesis. They serve as accessory light harvesting pigments, and they act to protect the chlorophyll pigments from the harmful photo-destructive reaction (Valladares et al., 2000; Ort, 2001; Baquedano and Castillo, 2006; Lichtenthaler et al., 2007). In this work, greater carotenoid contents were also found in high light intensity and low soil moisture treatments, in response to the interaction of light intensity and soil moisture described for in *N. pumilio* seedlings (Martínez Pastur et al., 2007).

Pigment content in leaves are closely related to NTC (Larcher, 2003), due to the chemical composition of the pigments (Timko, 1998) as well as related physiological processes (Damesin, 2003). Lower N and Mg concentration in *N. pumilio* seedlings were associated to the lower chlorophyll contents in the LH treatment, affecting the chloroplast formation (Field and Mooney, 1986) and their photosynthetic efficiency (Martínez Pastur et al., 2007). Nutrient uptake efficiency was affected in plants growing in extreme environmental conditions, e.g. Mg (Barker and Pilbeam, 2006) which limiting the chlorophyll formation (Marschner, 1995; Larcher, 2003).

CONCLUSIONS

Natural *Nothofagus pumilio* seedlings varied their NTC, both in above- and below-ground biomass, as well as pigment leaf concentration, according to the availability of light and soil moisture contents. Acclimation of seedlings to the new environmental conditions includes changes in NTC and leaf pigments, which are closely related to their photosynthetic plasticity and biomass compartmentalization. Plants growing in high light environments are more efficient in the use of some nutrients per biomass unit (C, Mg, K, Ca), while seedlings growing in shaded environments need more nutrients to produce the same quantity of vegetal tissue biomass. This plasticity allows to the seedlings to a quickly adaptation to several environmental changes, e.g., new silvicultural proposals. However, these practices can increases the soil moisture content affecting the NTC, and subsequently their physiological performance. It is necessary to compare our results of light and soil moisture interactions, with natural regeneration patterns and forest dynamics according to the nutrient soil availability. In example, changes in soil properties due to forest harvesting can influence over plant nutrition and their growth or subsequent survival.

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REFERENCES

Aranda, I., T. M. Robson, J. Rodríguez-Calcerrada, and F. Valladares. 2008. Limited capacity to cope with excessive light in the open and with seasonal drought in the shade in Aranda, I., T. M. Robson, J. Rodríguez-Calcerrada, and F. Valladares. 2008. Limited capacity to cope with excessive light in the open and with seasonal drought in the shade in Mediterranean *Ilex aquifolium* populations. *Trees* 22: 375–384.

Aussenac, G. 2000. Interactions between forest stands and microclimate: Ecophysiological aspects and consequences for silviculture. *Annals of Forest Science* 57: 287–301.

Austin, A. T., and O. E. Sala. 2002. Carbon and nitrogen dynamics across a natural precipitation gradient in Patagonia, Argentina. *Journal of Vegetation Science* 13: 351–360.

Baquedano, F. J., and F. J. Castillo. 2006. Comparative ecophysiological effects of drought on seedlings of the Mediterranean water–saver *Pinus halepensis* and water-spenders *Quercus coccifera* and *Quercus ilex*. *Trees* 20: 689–700.

Barker, A. V., and D. J. Pilbeam. 2006. *Handbook of Plant Nutrition*. New York: CRC Press.

Caldentey, J. 1992. Nutrients accumulation in natural stands of lenga (*Nothofagus pumilio*) in Magallanes region, Chile. *Ciencias Forestales* 8: 19–25.

Caldentey, J., A. Promis, H. Schmidt, and M. Ibarra. 2000. Microclimatic variation caused by shelter wood cut in a *N. pumilio* forest. *Ciencias Forestales* 14: 51–59.

Caldentey, J., H. Mayer, M. Ibarra, and A. Promis. 2009. The effects of a regeneration felling on photosynthetic photon flux density and regeneration growth in a *Nothofagus pumilio* forest. *European Journal of Forest Research* 128: 75–84.

Das, D. K., and O. P. Chaturvedi. 2005. Structure and function of *Populus deltoides* agroforestry systems in eastern India. 2: Nutrient dynamics. *Agroforestry Systems* 65: 223–230.

Damesin, C. 2003. Respiration and photosynthesis characteristics of current-year stems of *Fagus sylvatica*: From the seasonal pattern to an annual balance. *New Phytologist* 158: 465–475.

Diehl, P., M. J. Mazzarino, F. Funes, S. Fontenla, M. Gobbi, and J. Ferrari. 2003. Nutrient conservation strategies in native Andean-Patagonian forest. *Journal of Vegetation Science* 14: 63–70.

Diehl, P., M. J. Mazzarino, and S. Fontenla. 2008. Plant limiting nutrients in Andean-Patagonian woody species: Effects of interannual rainfall variation, soil fertility and mycorrhizal infection. *Forest Ecology and Management* 255: 2973–2980.

Evans, J. R., and H. Poorter. 2001. Photosynthetic acclimation of plants to growth irradiance: The relative importance of specific leaf area and nitrogen partitioning in maximizing carbon gain. *Plant, Cell & Environment* 24: 755–767.

Evans, C. A., E. K. Miller, and A. J. Friedland. 2001. Effect of nitrogen and light on nutrient concentrations and associated physiological responses in birch and fir seedlings. *Plant and Soil* 236: 197–207.

Field, C., and H. A. Mooney. 1986. The photosynthesis - nitrogen relationship in wild plants. In: *On the Economy of Plant Form and Function*, ed. T. J. Givnish, pp. 25–55. Cambridge: Cambridge University Press.

Frangi, J. L., and L. Richter. 1994. Water balances within *Nothofagus* forests of Tierra del Fuego, Argentina. *Revista de la Facultad de Agronomía de La Plata* 70: 65–79.

Frangi, J., M. Barrera, L. Richter, and A. Lugo. 2005. Nutrient cycling in *Nothofagus pumilio* forests along an altitudinal gradient of Tierra del Fuego, Argentina. *Forest Ecology and Management* 217: 80–94.

Gehring, H., H. Kasemir, and H. Mohr. 1977. The capacity of chlorophyll-a biosynthesis in the mustard seedling cotyledons as modulated by phytochrome and circadian rhythmicity. *Planta* 133: 295–302.

Grime, J. P. 1979. *Plant Strategies and Vegetation Processes.* New York: John Wiley and Sons.

Hart, P., P. Clinton, R. Allen, A. Nordmeyer, and G. Evans. 2003. Biomass and macro-nutrients (above and below- ground) in a New Zealand beech (*Nothofagus*) forest ecosystem: Implications for storage and sustainable forest management. *Forest Ecology and Management* 174: 281–294.

Heinemann, K., T. Kitzberger, and T. Veblen. 2000. Influences of gap microheterogeneity on the regeneration of *Nothofagus pumilio* in a xeric old–growth forest of Northwestern Patagonia, Argentina. *Canadian Journal of Forest Research* 30: 25–31.

Hogan, K. P., I. Fleck, R. Bungard, J. M. Cheeseman, and D. Whitehead. 1997. Effect of elevated $C0_2$ on the utilization of light energy in *Nothofagus fusca* and *Pinus radiata*. *Journal of Experimental Botany* 311: 1289–1297.

Kendrick, R. E., and G. H. M. Kronenberg. 1994. *Photomorphogenesis in Plants.* Berlin: Springer.

Larcher, W. 2003. *Physiological Plant Ecology: Ecophysiology and Stress Physiology of Functional Groups.* Berlin: Springer.

Lee, D. W., J. O'Keefe, N. M. Holbrook, and T. S. Feild. 2003. Pigment dynamics and autumn leaf senescence in a New England deciduous forest, eastern USA. *Ecological Research* 18: 677–694.

Lencinas, M. V., G. Martínez Pastur, A. Moretto, E. Gallo, and C. Busso. 2007. Differential biomass productivity of *Nothofagus pumilio* seedlings under light and soil moisture gradients. *Bosque* 28: 241–248.

Lichtenthaler, H. K., A. Ac, M. V. Marek, J. Kalina, and O. Urban. 2007. Differences in pigment composition, photosynthetic rates and chlorophyll fluorescence images of sun and shade leaves of four tree species. *Plant Physiology and Biochemistry* 45: 577–588.

Lieffers, V., C. Messier, F. Gendron, K. Stadt, and P. Comeau. 1999. Predicting and managing light in understory of boreal forests. *Canadian Journal of Forest Research* 29: 796–811.

Marschner, H. 1995. *Mineral Nutrition of Higher Plants.* London: Academic Press.

Martínez Pastur, G., J. M. Cellini, P. Peri, R. Vukasovic, and C. Fernández. 2000. Timber production of *Nothofagus pumilio* forests by a shelterwood system in Tierra del Fuego (Argentina). *Forest Ecology and Management* 134: 153–162.

Martínez Pastur, G., M. V. Lencinas, P. Peri, and M. Arena. 2007. Photosynthetic plasticity of *Nothofagus pumilio* seedlings to light intensity and soil moisture. *Forest Ecology and Management* 243: 274–282.

Martínez Pastur, G., M. V. Lencinas, J. M. Cellini, P. Peri, and R. Soler Esteban. 2009. Timber management with variable retention in *Nothofagus pumilio* forests of Southern Patagonia. *Forest Ecology and Management* 258: 436–443.

Newman, G. S., and S. C. Hart. 2006. Nutrient covariance between forest foliage and fine roots. *Forest Ecology and Management* 236: 136–141.

Novóa Muñoz, J., X. Pontevedra Pombal, A. Moretto, A. Martínez Cortizas, and E. García Rodeja Ganoso. 2007. Geochemical characterization of *Nothofagus pumilio* forest soils in Tierra del Fuego, Argentina. In: *Tendencias Actuales de la Ciencia del Suelo* [Current Trends in Soil Science], eds. N. Bellinfante and A. Jordan, pp. 689–696. Seville, Spain: University of Seville.

Ort, D. 2001. When there is too much light. *Plant Physiology* 125: 29–32.

Peri, P., V. Gargaglione, and G. Martínez Pastur. 2006. Dynamics of above- and below-ground biomass and nutrient accumulation in an age sequence of *Nothofagus antarctica* forest of Southern Patagonia. *Forest Ecology and Management* 233: 85–99.

Peri, P., V. Gargaglione, and G. Martínez Pastur. 2008. Above and belowground nutrients storage and biomass accumulation in marginal *Nothofagus antarctica* forests in Southern Patagonia. *Forest Ecology and Management* 255: 2502–2511.

Peuke, A. D., and H. Rennenberg. 2004. Carbon, nitrogen, phosphorus, and sulfur concentration and partitioning in beech ecotypes (*Fagus sylvatica* L.): Phosphorus most affected by drought. *Trees* 18: 639–648.

Rapp, M., I. Santa Regina, M. Rico, and H. Gallego. 1999. Biomass, nutrient content, litterfall and nutrient return to the soil in Mediterranean oak forest. *Forest Ecology and Management* 119: 39–49.

Rebertus, A., and T. Veblen. 1993. Forest structure and tree-fall gap dynamics of old-growth *Nothofagus* forests in Tierra del Fuego, Argentina. *Journal of Vegetation Science* 4: 641–654.

Reynolds, P. E., and H. Frochot. 2003. Photosynthetic acclimation of beech seedlings to full sunlight following a major windstorm event in France. *Annals of Forest Science* 60: 701–709.

Richter, L., and J. Frangi. 1992. Ecological basis for *Nothofagus pumilio* forest management of Tierra del Fuego. *Revista de la Facultad de Agronomía de La Plata* 68: 35–52.

Rojas, P. A. 2007. Study of the effect of prolonged waterlogging on *Nothofagus dombeyi* (Mirb.) Oerst, *Nothofagus nitida* (Phil.) Krasser and *Nothofagus betuloides* (Mirb) Blume. Dr. Phil. Thesis, Universidad Austral de Chile, Valdivia, Chile.

Romanya, J., J. Fons, T. Sauras-Yera, E. Gutiérrez, and V. R. Vallejo. 2005. Soil plant relationships and tree distribution in old growth *Nothofagus betuloides* and *Nothofagus pumilio* forests of Tierra del Fuego. *Geoderma* 124: 169–180.

Santa Regina, I. 2000. Biomass estimation and nutrient pools in four *Quercus pyrenaica* in Sierra de Gata Mountains, Salamanca, Spain. *Forest Ecology and Management* 132: 127–141.

Sardans, J., J. Peñuelas, and F. Rodà. 2006. Plasticity of leaf morphological traits, leaf nutrient content, and water capture in the Mediterranean evergreen oak *Quercus ilex* subsp. *ballota* in response to fertilization and changes in competitive conditions. *Ecoscience* 13: 258–270.

Schachtman, D. P., R. J. Reid, and S. M. Ayling. 1998. Phosphorus uptake by plants, from soil to cell. *Plant Physiology* 116: 447–453.

Sims, D. A., and J. A. Gamon. 2002. Relationship between pigment content and spectral reflectance across a wide range of species, leaf structures and developmental stages. *Remote Sensing of Environment* 81: 337–354.

Sun, O. J., G. B. Sweet, D. Whitehead, and G. D. Buchan. 1995. Physiological responses to water stress and waterlogging in *Nothofagus* species. *Tree Physiology* 15: 629–638.

Timko, M. P. 1998. Pigment biosynthesis: Chlorophylls, heme, and carotenoids. In *The Molecular Biology of Chloroplasts and Mitochondria in Chlamydomonas*, eds. J. D. Rochaix, M. Goldschmidt-Clermont, and S. Merchant, pp. 377–414. Dordrecht, the Netherlands: Kluwer Academic Publishers.

Torres Netto, A., E. Campostrini, J. Gonçalves de Oliveira, and R. E. Bressan-Smith. 2005. Photosynthetic pigments, nitrogen, chlorophyll a fluorescence and SPAD–502 readings in coffee leaves. *Scientia Horticulturae* 104: 199–209.

Valladares, F., E. Martínez-Ferri, L. Balaguer, E. Perez-Corona, and E. Manrique. 2000. Low leaf–level response to light and nutrients in Mediterranean evergreen oaks: A conservative resource-use strategy? *New Phytologist* 148: 79–91.

Veblen, T., C. Donoso, T. Kitzberger, and A. J. Rebertus. 1996. Ecology of southern Chilean and Argentinean *Nothofagus* forests. In: *The Ecology and Biogeography of Nothofagus Forests*, eds. T. Veblen, R. Hill, and J. Read, pp. 293–353. New Haven, CT: Yale University Press.

Wang, J. R., T. Letchforda, P. Comeaub, and J. P. Kimmins. 2000. Above- and below-ground biomass and nutrient distribution of a paper birch and subalpine fir mixed-species stand in the sub–boreal Spruce zone of British Columbia. *Forest Ecology and Management* 130: 17–26.

Weber, V. M. 1999. Carbon budget of a virgin *Nothofagus* forest in Tierra del Fuego. *Forstwissenschaftliches Centralblatt* 118: 156–166.

Zúñiga, R., M. Alberdi, M. J. Reyes-Díaz, E. Olivares, S. Hess, L. A. Bravo, and L. J. Corcuera. 2006. Seasonal changes in the photosynthetic performance of two evergreen *Nothofagus* species in south central Chile. *Revista Chilena de Historia Natural* 79: 489–504.