



# Nitrogen loading of *Eucalyptus globulus* seedlings: nutritional dynamics and influence on morphology and root growth potential

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## Abstract

Achieving successful outplanting of trees on increasingly harsher sites is a global concern. In Chile, for example, new *Eucalyptus globulus* plantations are being targeted to poorer, low fertility sites where additional stresses often negatively affect seedling performance during the first field season. Nitrogen-loading during the later stages of nursery production can, however, significantly improve seedling physiological performance on harsher sites by increasing plant growth and nutrient storage reserves for subsequent root growth after outplanting. Maximizing these benefits, and applying the concept of loading throughout the entire crop cycle, requires a better understanding of nitrogen (N) dynamics. Thus, for container *E. globulus*, considered one of the most important plantation species worldwide, we evaluated the effects of increasing N supply (50 to 600 mg N L<sup>-1</sup>) on seedling morphological traits, biomass production, root growth potential, and foliar N. After 15 weeks of N application, seedlings showed an increase in all growth variables evaluated up to 300 mg N L<sup>-1</sup>, whereas higher rates allowed luxury consumption. Modeling growth responses revealed, however, an apparent ammonium antagonism with other macronutrient cations suggests that high rates of this N-form may negate the potential benefits of N-loading, or that other modifications to the fertilizer solution may promote additional growth at higher N rates. Foliar N concentration, which was easily and accurately measured with a leaf chlorophyll meter, showed a gradient from basal to apical leaves suggesting a strong translocation of N in *E. globulus* seedlings. Our results suggest nursery management that supplies 300 mg L<sup>-1</sup> of N through the entire growing season could improve seedling nutritional status, morphological attributes, and growth of new roots, attributes that may improve establishment on low fertility sites.

**Keywords** Seedling fertilization · Nutrient reserves · Nursery · Root growth potential · Relative chlorophyll

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## Introduction

One strategy to meet burgeoning global, societal demands for ecosystem services, while ensuring preservation of primary forests, is an increased use of forest plantations (Paquette and Messier 2010). An important plantation species throughout the world is *Eucalyptus globulus*, now growing on all continents except Antarctica (CABI 2018). With changing climate and attentive focus on reforesting degraded lands, seedlings necessary for outplanting success must have robust morphological and physiological traits (Jacobs et al. 2015). Tree physiological traits are known to exert a strong influence on survival and growth of outplanted seedlings (Oliet et al. 2013; Villar-Salvador et al. 2015). Obtaining optimum physiological conditions is challenging considering that visual morpho-functional attributes and the appropriate growing practices used to obtain them in nurseries, are still under discussion (Jacobs et al. 2015; Dumroese et al. 2016; Grossnickle and MacDonald 2017). Studies have shown that plant survival and growth after establishment are positively affected by nursery nutritional management (Villar-Salvador et al. 2004; Fernández et al. 2007; Oliet et al. 2013). Nutrient supply can modify tissue nutrient content and thereby accelerate shoot and root growth, and increase plant resistance to water stress, low temperatures, and diseases (Oliet et al. 2013). Nutrient reserves are of key importance for improving physiological conditions that secure successful establishment under unfavorable conditions (Villar-Salvador et al. 2015; Buendía Velázquez et al. 2017). The ability of seedlings to accumulate nutrient reserves for later translocation to newly formed tissues can be provided by nutrient loading during the nursery phase (Timmer 1996; Oliet et al. 2013; Schott et al. 2016). Research showing positive effects of seedling nutrient loading on outplanting performance are numerous, and include work with conifers (e.g. Timmer and Miller 1991; Salifu and Timmer 2003; Villar-Salvador et al. 2013) and hardwoods (e.g. Trubat et al. 2010; Oliet et al. 2013; Villar-Salvador et al. 2012), but less so for *E. globulus* (e.g., Grove et al. 1996; Monsalve et al. 2009) despite its worldwide use in plantations.

An improved understanding of N-loading in *E. globulus* could be beneficial to plantation establishment. This is especially true for establishment of hardwood species, particularly those in the genus *Eucalyptus*, which is planted worldwide. About 31% of the Chilean forest plantations of exotic tree species are established with *Eucalyptus* species (CONAF 2014; Salas et al. 2016) because large numbers of improved *E. globulus* genotypes, which show high productivity and fast growth, can be easily produced with vegetative propagation (INFOR 2012). Expansion of *Eucalyptus* plantations in Chile during the last decades has pushed plantations onto sites with poor conditions for establishment (extended dry periods and high temperatures), negatively affecting seedling survival and growth after outplanting (INFOR 2012). Thus, employing an optimum nutrient application protocol for *Eucalyptus* would improve plantation efficiency in Chile and have important implications for use of this species on other harsh sites worldwide.

It has been difficult to make nutritional prescriptions to reach an optimum nutrient concentration for *E. globulus* in the nursery, mainly due to unknown nitrogen (N) demands during seedling developmental stages. Moreover, the high cost per sample and required time to determine N concentration are main limitations to continuous monitoring of seedling nutritional status. Non-destructive methods have been used satisfactorily to estimate foliar N indirectly via chlorophyll measurements on many species (Schaper and Chacko 1991; Loh et al. 2002). Furthermore, chlorophyll measurements have the additional advantage that the same leaf may be resampled for additional nutritional analyses (Schaper and Chacko 1991).

A key gap in understanding *E. globulus* seedling nursery nutrition for a production cycle is knowing the optimal N concentration required to promote a specific growth rate and nutritional status in container *E. globulus* seedlings. Our study objectives were to (1) evaluate the effect of increasing N supply on morphological attributes, biomass, and root growth potential, (2) and determine the expected N sufficiency levels of *E. globulus* seedlings.

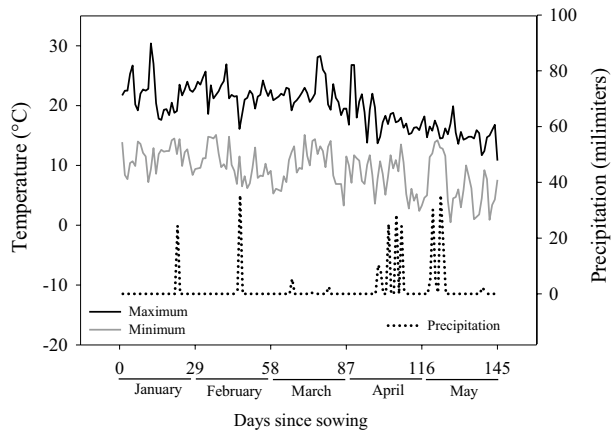
## Methods

### Study design

Seedlings were grown on benches in the open growing compound (outdoor nursery; no climate control is available) of the Forest Sciences Faculty of the Universidad de Concepción, Concepción, Chile. The ambient climate is Mesomediterranean (Amigo and Ramírez 1998). During the last 20 years, the average annual temperature and precipitation are 12.5 °C and 988 milliliters. January is typically the harshest month with mean maximum and absolute maximum temperatures of 24.4 and 28.1 °C and mean precipitation of 11.3 milliliters ([www.explorador.cr2.cl](http://www.explorador.cr2.cl)). We established a completely randomized design having 5 N treatments with 4 replications. A replicate was a single tray (typically used in Chile) containing 98 individual cavities (15 cm depth, 115 cm<sup>3</sup> volume, 397 cavities m<sup>-2</sup>). Twenty trays were washed with a mixture of copper carbonate, water, and latex for chemical root pruning following the standard practice in Chile. We filled containers with composted pine bark (pH=5.5; organic matter=56.5%; total nitrogen=0.6%; carbon-to-nitrogen ratio=27; N-NO<sub>3</sub>=140 mg kg<sup>-1</sup>; N-NH<sub>4</sub>=67.8 mg kg<sup>-1</sup>; 27% water retention and 25% aeration porosity). Seeds were from the “Chumulco” seed orchard (lat -37.76°, long -72.31°), belonging to Mininco S.A. and appropriate for our location. Seeds were sown in January. Irrigation and fertigation (irrigation amended with water-soluble fertilizer) were achieved by subirrigation of each tray until individual containers reached container capacity by capillarity movement. We monitored mass loss of each tray daily in order to irrigate when the amount of water at container capacity had been reduced by 50% (Dumroese et al. 2015); this target threshold is the standard practice at the nearby (~10 km), open growing compound operated by the Chilean Institute of Forestry (Instituto Forestal). Using the same criteria and beginning 15 days after sowing (Day 15) we fertigated once every other irrigation application until Day 145. Daily minimum and maximum temperatures, as well as precipitation, during the experiment are shown in Fig. 1.

Subirrigation water was adjusted to pH 6.5 by acid titration with phosphoric acid and was accounted for in the P addition to each treatment. We used an assortment of salts in various combinations in order to apply N in a proportion of 7 NO<sub>3</sub><sup>-</sup> to 3 NH<sub>4</sub><sup>+</sup> at concentrations of 50, 150, 300, 450, and 600 mg N L<sup>-1</sup> (hereafter 50 N, 150 N, 300 N, 450 N, and 600 N) and other nutrients at constant rates: P, K, Ca, Mg, S at 60, 100, 80, 80, and 58 mg L<sup>-1</sup>, respectively. The experiment was established as a completely randomized design with four replicates (trays) per treatment. Each experimental unit consisted of one tray (98 cavities), whereas the sampling unit consisted of 12 seedlings per treatment selected at random.

**Fig. 1** Daily minimum and maximum temperatures, as well as precipitation, during the 145 days of the experiment



### Morphological attributes and root growth potential

On Days 55, 70, 85, 100, 115, 130, and 145 (end of growing cycle), we randomly selected 12 seedlings per treatment (3 seedlings from each replicate; 60 seedlings total each sample date) to determine stem length (cm), stem diameter (mm; measured at the root collar), and biomass (g). Leaf area (cm<sup>2</sup>) was measured using an LI-3100 (LI-COR Biosciences, Lincoln, Nebraska, USA). Seedlings were separated into components (leaves, stem, and roots) and dried 48 h to equilibrium at 65 °C in a forced-ventilation oven to determine component biomass. We initiated a root growth potential (RGP) test at the end of the growing cycle (Day 145) by randomly selecting 20 seedlings per treatment (5 seedlings from each replicate; 100 seedlings total) and removing all white root tips (new and non-suberized roots) so that all seedlings start the test in an equal condition (Villar-Salvador et al. 2004; Fernández et al. 2007; Monsalve et al. 2009). Each seedling was placed in an aeroponic chamber and tap water was applied every 10 min for 6 s. We maintained a chamber water temperature of 22 °C and 16 h of light with an average photon flux of 100 μmol m<sup>-2</sup> s<sup>-1</sup>, a value consistent with other RGP evaluations of *E. globulus* (Coopman et al. 2008; Monsalve et al. 2009). After 28 days, we measured the length of the three longest roots to the nearest millimeter and recorded the number of all non-suberized (white) roots ≥ 1 cm (Burdett 1979).

### Foliar chlorophyll and N concentrations

On every other sample date (i.e., Days 55, 85, 115, and 145), the randomly selected seedlings described above were also measured for relative chlorophyll (RC) using a leaf chlorophyll meter (SPAD-502; Konica Minolta Inc., Osaka, Japan). On each seedling, leaves were numbered sequentially from the base to the apex. Avoiding the central vein, we made three measurements on every leaf. These values were used to obtain an average value for each seedling.

On Day 145, in order to calibrate our RC values with leaf N concentrations, leaves from each seedling, numbered as described above, were harvested but retained as individual seedling samples and dried for biomass. We plotted the average RC values for each leaf

of every seedling (~1100 leaves) and then divided that plot into 12 subsets based on RC values. Knowing the RC value for individual leaves, we then retrieved the leaves from our entire seedling sample (60 seedlings) within each subset range. Leaves within each subset (a minimum of 10 leaves) were homogenized and total N concentration was determined colorimetrically using Kjeldahl digestion.

## Data analysis

Final morphological attributes, biomass, and RGP were assessed using one-way analyses of variance (ANOVA) for a completely randomized design, using 3 seedlings per tray (replicate) for each of four replicates per treatment. Our ANOVA evaluated the effect of the N concentration treatments at the end of the growing cycle using a PROC GLM procedure (SAS Institute, Inc., Cary, NC, USA). We verified assumptions of normality and homoscedasticity using Shapiro–Wilk and Levene tests respectively. Differences among means were determined using a Tukey (HSD) test for multiple comparisons. A Weibull model was adjusted for growth and biomass development during the entire crop cycle (180 days) based on the average measurements of each treatment  $\times$  replicate combination obtained every 15 days during the 130-days of fertilization. Using the models, we subsequently estimated growth increments. Also, the RC readings along the stem were modeled with a cubic polynomial model. All analyses were made using SAS 9.1 statistical software and the PROC NLIN procedure (SAS Institute Inc., Cary, NC, USA) using the Gauss–Newton method through a derivative-free algorithm. We used linear regression to explore the relationship between RC values and leaf N concentrations at the final harvest, using PROC REG procedure (SAS Institute Inc., Cary, NC, USA). The RC readings taken during the course of the experiment were used to perform a nutrient vector analysis (Timmer 1991; Haase and Rose 1995). The RC values were analyzed as repeated measures (Kuehl 2001), modeling the variance and covariance structure with a 95% level of confidence. Statistical differences between means were performed with a Tukey (HSD) test for multiple comparison, using PROC MIXED procedure (SAS Institute Inc., Cary, NC, USA). Visualizations were made using SigmaPlot 10 (Systat Software Inc., San Jose, CA, USA).

## Results

### Morphological attributes and root growth potential

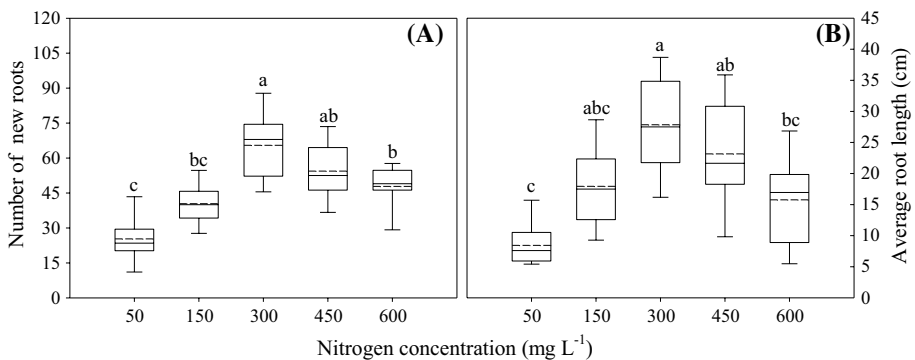
The concentration of applied N was significant for final values of all morphological attributes except stem diameter and root biomass (Table 1). We observed no differences in response when N concentration increased from 50 to 150 N. Increasing from 50 to 300 N yielded, however, significant increases in stem length (54%), leaf area (107%), and leaf (65%) and stem (129%) biomass (Table 1). No additional increases in growth were noted for applied concentrations > 300 N. The 600 N rate had significantly less stem biomass than the 300 N rate (Table 1).

The rate of N was significant for the number of new roots and average root length in the RGP test. As was the case with morphology, we observed no differences in response when N concentration increased from 50 to 150 N; these treatments averaged 33 roots per seedling with the average length of the three longest roots being 17 cm (Fig. 2). Similarly,

**Table 1** Final morphological attributes and biomass component mean values ( $\pm$  standard deviation;  $n=12$  for each concentration) for container *Eucalyptus globulus* seedlings grown under a gradient of nitrogen concentrations during a 145-day growth period

Nitrogen ( $\text{mg L}^{-1}$ )	Stem length (cm)	Stem diameter (mm)	Leaf area ( $\text{cm}^2$ )	Biomass		
				Leaf (g)	Stem (g)	Root (g)
50	$20.50 \pm 3.00\text{b}$	$2.18 \pm 0.35\text{a}$	$83.30 \pm 15.40\text{c}$	$0.66 \pm 0.11\text{c}$	$0.31 \pm 0.08\text{c}$	$0.34 \pm 0.10\text{a}$
150	$24.40 \pm 2.10\text{ab}$	$2.22 \pm 0.25\text{a}$	$98.20 \pm 20.00\text{bc}$	$0.77 \pm 0.17\text{bc}$	$0.37 \pm 0.07\text{bc}$	$0.35 \pm 0.13\text{a}$
300	$31.30 \pm 4.00\text{a}$	$2.71 \pm 0.56\text{a}$	$172.60 \pm 37.70\text{a}$	$1.14 \pm 0.40\text{a}$	$0.71 \pm 0.31\text{a}$	$0.44 \pm 0.17\text{a}$
450	$28.20 \pm 3.30\text{ab}$	$2.47 \pm 0.47\text{a}$	$150.40 \pm 32.50\text{ab}$	$0.93 \pm 0.26\text{ab}$	$0.60 \pm 0.22\text{ab}$	$0.35 \pm 0.14\text{a}$
600	$25.90 \pm 4.00\text{ab}$	$2.32 \pm 0.50\text{a}$	$142.90 \pm 35.50\text{ab}$	$0.92 \pm 0.23\text{ab}$	$0.51 \pm 0.33\text{b}$	$0.36 \pm 0.12\text{a}$

Different letters indicate statistical differences among means ( $p \leq 0.05$ )

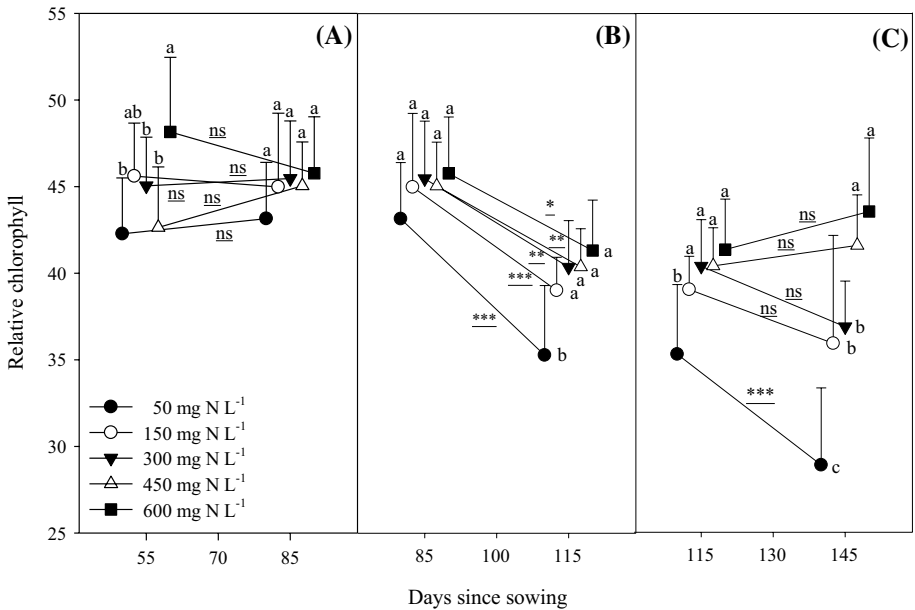


**Fig. 2** Effect of applied nitrogen concentration on the number of new roots (a) and the average length of the three longest roots (b) produced by container *Eucalyptus globulus* seedlings during a 28-day root growth potential test following 145 days of growth in the nursery. Letters indicate significant differences ( $p \leq 0.05$ ). Vertical boxes represent approximately 50% of the observations and lines extending from each box are the upper and lower 25% of the distribution. The solid horizontal line in the center of each box is the median value and the dotted line is the mean

increasing from 50 to 300 N was significant for the number of new roots and the average length of the three longest roots; the averages were 65 new roots and 27 cm of length per seedling in the 300 N treatment. The 600 N treatment yielded significantly fewer new roots with less average root length than the 300 N treatment (Fig. 2). Based on Burdett's (1979) semi-quantitative RGP scale, our observations ranged between category 4 (11 to 30 new roots > 1 cm) and 5 (31 to 100 new roots > 1 cm) for the 50 N and 300 N treatments, respectively.

### Foliar chlorophyll, foliar N concentration, and plant growth curves

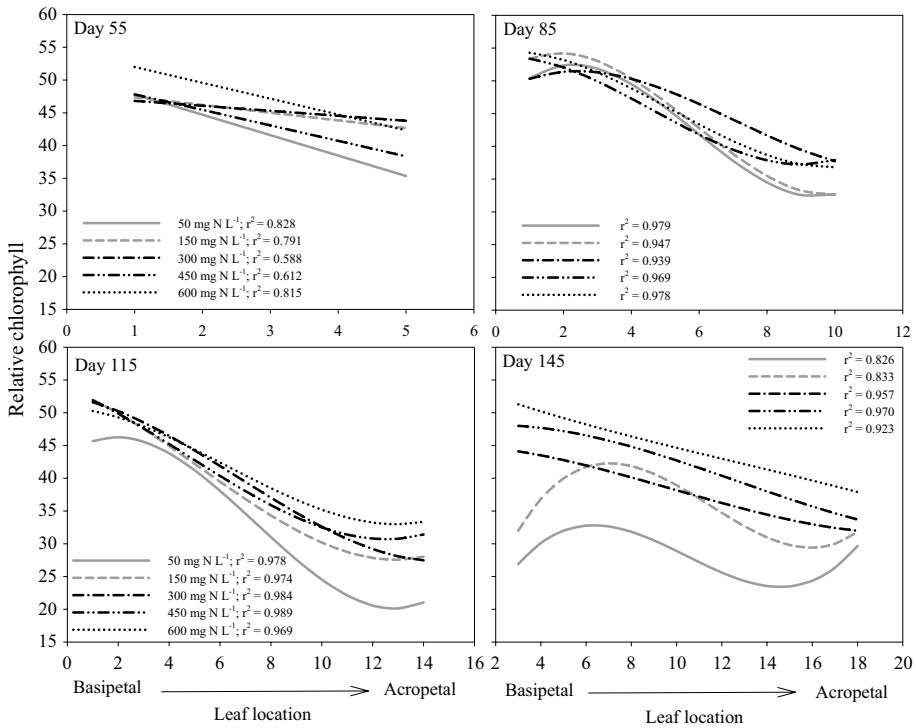
Relative chlorophyll changed during the course of the experiment in response to each N concentration (Fig. 3). From the initial measurement (Day 55) until Day 85, RC remained stable; at Day 85 we observed no significant difference among N concentrations (Fig. 3a). From Day 85 through Day 115, we observed significant decreases in RC regardless of N supply, and at Day 115 the 50 N treatment had a significantly lower RC than the other N concentrations (Fig. 3b). From Day 115 until the end of the experiment



**Fig. 3** Mean and standard deviation of relative chlorophyll (RC) for Days 55 to 85 since sowing (a) Days 85 to 115 since sowing (b) and Days 115 to 145 since sowing (c) in response to applied nitrogen (N) concentration to container *Eucalyptus globulus* seedlings (n=12 for each data point). Different letters, according to Tukey (HSD), differ significantly ( $p < 0.05$ ) between N concentration for each measurement interval. Underlined letters indicate significant differences between consecutive time intervals within N concentrations (ns=non-significant; \* significant at  $p < 0.05$ ; \*\* significant at  $p < 0.01$ ; \*\*\* significant at  $p < 0.001$ )

(Day 145), the RC in 50 N continued to decline, whereas we noted no significant differences for N rates > 300 N (Fig. 3c). This same pattern was evident in our measurement of RC; the slope of RC when moving from the basal to apex leaves appears to decline less as N concentrations increase (Fig. 4). Seedlings supplied with  $\leq 150$  N showed a decline in RC in basal leaves and an increase in RC in apex leaves during the Day 115 to 145 period. For the Day 145 sample, we found a strong linear correlation between leaf N concentration and RC values (foliar N concentration =  $0.0418 \times$  relative chlorophyll;  $p < 0.001$ ;  $r^2 = 0.8961$ ).

Vector analysis for RC, based on Figs. 3 and 4, indicates that, progressing from Day 55 to 85, only the 150 N concentration appeared to provide sufficient levels of N, with deficient levels provided by the 50 N, 300 N, and 450 N rates, and dilution occurring in seedlings in the 600 N rate. From Day 85 to 115, all seedlings exhibited dilution. Toward the end of the growing season (i.e., Day 115 to 145) seedlings in the 150 N and 300 N concentrations, continued to experience dilution, the 600 N rate appeared to provide sufficient N levels, and seedlings receiving 50 N were actively translocating N from their older, basal leaves to their newer, apical leaves. As the concentration of applied N increased, so did the resulting foliar nutrient concentrations of N, P, and S (Table 2). Conversely, the foliar nutrient concentrations of the cations (K, Ca, Mg) tended to decrease. Boron was generally unaffected. A vector nomogram for leaf characteristics (dry mass and nutrient concentration and content) using 300 N as the reference



**Fig. 4** Relative chlorophyll (RC) modeled over time (Day 55, 85, 115, and 145), and RC behavior along the stem in response to the application of five nitrogen concentrations to container *Eucalyptus globulus* seedlings during a 145-day growth period. Models derived using an average of 12 leaves per location, with locations numbered sequentially from the base to the apex

**Table 2** Final foliar nutrient levels obtained after 145 days of growth in response to five nitrogen (N) concentration treatments applied to container *Eucalyptus globulus* seedlings

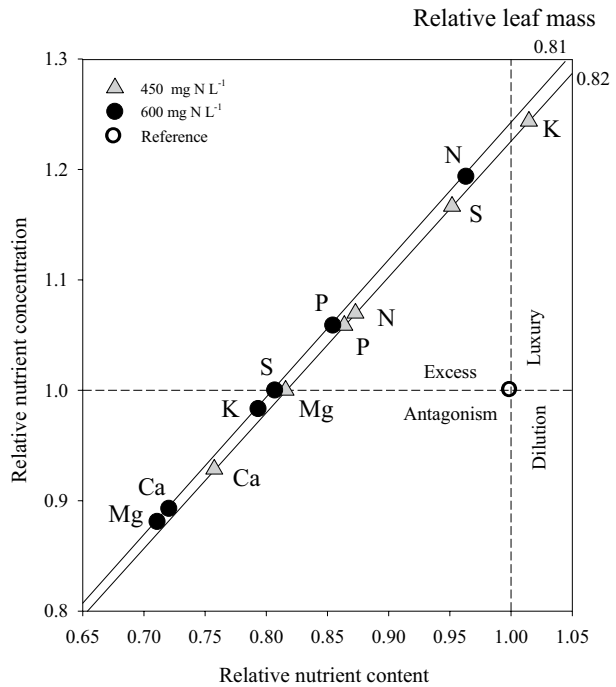
Nutrient	Applied N concentrations (mg L <sup>-1</sup> )				
	50	150	300	450	600
	Foliar nutrient levels (%)				
N	1.21	1.48	1.86	1.99	2.22
P	0.15	0.15	0.17	0.18	0.18
K	1.38	1.43	1.19	1.48	1.17
Ca	0.60	0.57	0.56	0.52	0.50
Mg	0.45	0.51	0.42	0.42	0.37
S	0.08	0.10	0.12	0.14	0.12
B*	30	30	27	32	30

\*Concentration in mg kg<sup>-1</sup>

revealed excess N, P, and S with the 450 N and 600 N, antagonism with Ca and Mg at 450 N, and luxury consumption with K, antagonism with K, Ca, and Mg at 600 N (Fig. 5).



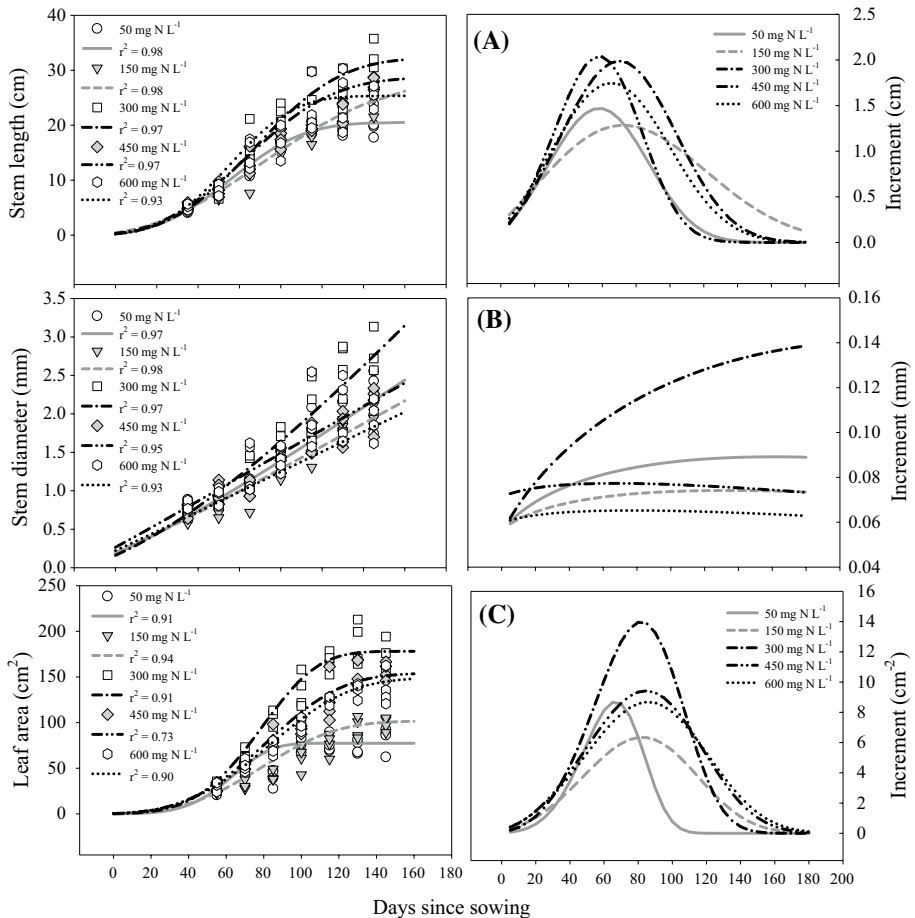
**Fig. 5** Vector nomogram of relative changes in leaf dry mass, leaf nutrient content, and leaf nutrient concentration of container *Eucalyptus globulus* seedlings supplied either 450 or 600 mg N L<sup>-1</sup> during a 145-day growth period. Characteristics of seedlings supplied with 300 mg N L<sup>-1</sup> were used as the reference values



Our Weibull models for development of seedling morphological traits yielded high  $r^2$  values. For stem length, stem diameter, and leaf area,  $r^2$  were  $>0.9$ , except for a single  $r^2$  of 0.73 (Fig. 6). For biomass, all  $r^2$  were  $>0.67$  and most were  $>0.9$  (Fig. 7). Using the models, we estimated increments for the morphological traits. For stem length, stem diameter, and leaf area, the largest increments were observed with 300 N, usually followed by the 450 N and 600 N rates (Fig. 6). Interestingly, increments achieved with the 50 N were higher than those observed with 150 N. Similarly, the 300 N treatment yielded the largest increments in biomass, only surpassed by the leaf biomass increment that occurred for a shorter period (Fig. 7). Seedlings generally followed a sigmoidal growth curve, thus the increments were best estimated as bell-shaped curves; the exception was stem diameter (Figs. 6 and 7). Except for the 50 N and 600 N treatments, seedlings had larger increments of stem growth than root growth. For every rate, leaf biomass increment exceeded that of root and stem and leaf biomass was always the main component of the seedlings (Table 1).

## Discussion

A linear relationship between mean RC values and foliar N concentrations indicated a robust indirect estimation of leaf N concentration. A similar relationship has been found between leaf chlorophyll concentration and foliar N level for several tree species (Schaper and Chacko 1991) and this relationship has been used to indirectly estimate foliar N concentration (Follet et al. 1992). Pinkard et al. (2006) found a significant and strong non-linear relationship between SPAD and chlorophyll for 2.5-year-old *E. globulus* planted trees. In addition, the same authors found a weak ( $R^2=0.47$ ) relationship between SPAD and



**Fig. 6** Growth dynamics for stem length (a), stem diameter (b), and leaf area (c) in response to the nitrogen fertilization treatments applied to *E. globulus* plants produced in containers. Right-hand panels show the periodic increments for this variable

foliar N content. Using SPAD to monitor N in forest nurseries may be an effective, practical way to monitor plant development and establish seedling quality criteria; these critical aspects of seedling production are often overlooked (Landis et al. 2005).

We found substantial evidence that N concentration affected the growth rate of container *E. globulus* seedlings. Growth response to N supply followed a curvilinear pattern with discrete phases that ranged from N deficiency to N sufficiency, similar to the results of other studies that have evaluated the effectiveness of nutrient loading (Oliet et al. 2013; Uscola et al. 2015).

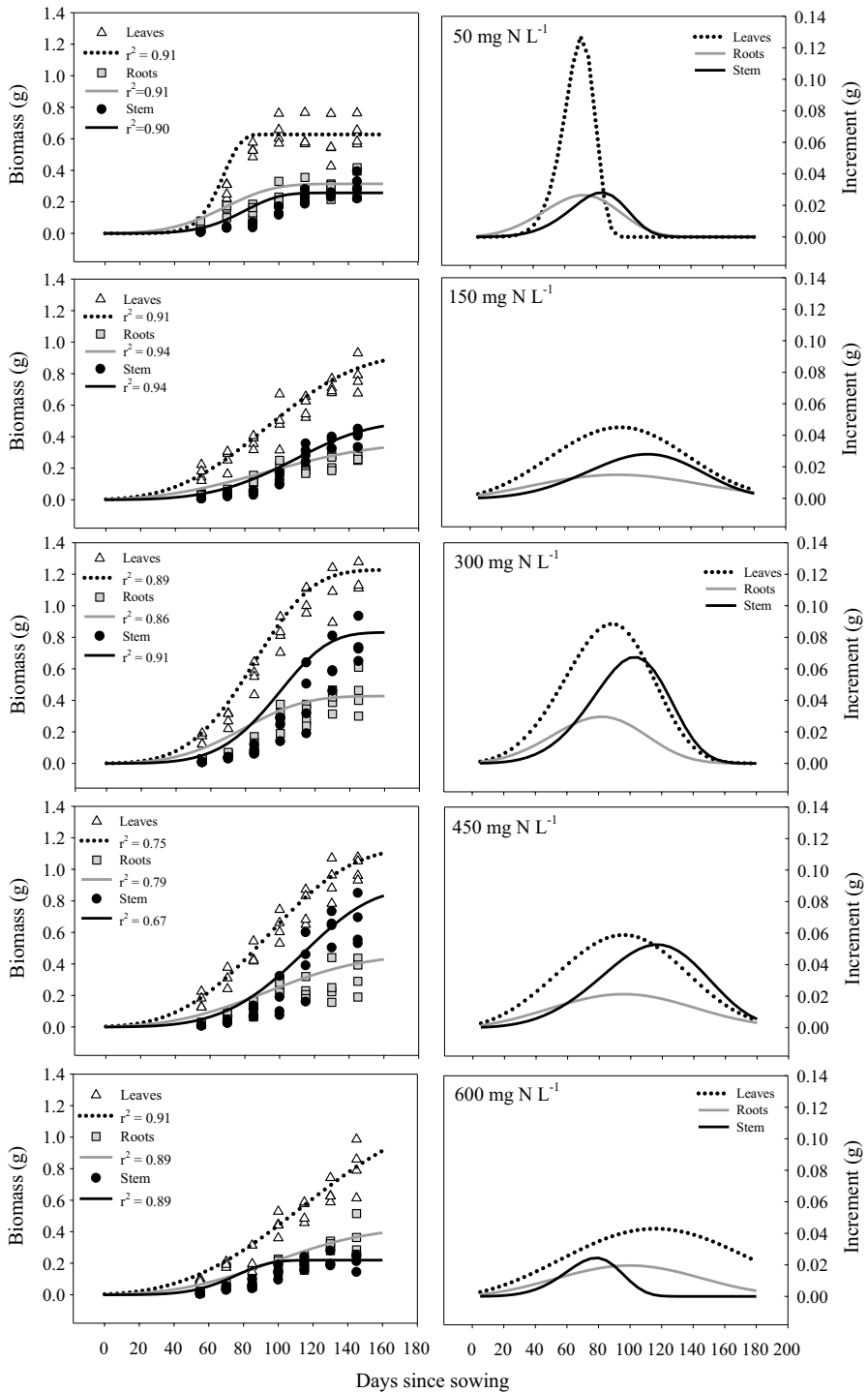
In this study, we observed a trend of increasing values for morphological characteristics and new root development during the RGP test as N increased from 50 to 300 N. The 300 N rate was sufficient to maximize seedling growth, to enhance RGP, and to accumulate

nutritional reserves (not N because 450 and 600 continue to accumulate N), confirming the ability of seedlings to use it later for new formed tissues (Timmer 1996; Olier et al. 2013; Schott et al. 2016). Our RGP results at 300 mg L<sup>-1</sup> of N supply were similar to the results of Fernández et al. (2007) who found that increasing foliar N from 0.89 to 1.58% at out-planting increased first-year survival for *E. globulus* from 40 to 80%.

At the lower levels of N, a decrease in RC of the most basipetal leaves, concurrent with an increase in the most acropetal leaves, indicates N translocation due to insufficient N supply (Fig. 4). For seedlings under the 150 N treatment at Day 145, average RC dropped considerably in the first two pairs of leaves (basal leaves), and leaves close to the apex showed an increase in RC. However, the results showed that RC readings tended to be homogeneous along the stem over time and with increasing N concentrations. This behavior is similar to a “steady-state nutrition” (Ingestad and Lund 1986), in which fertilization tends to maintain a relatively stable level of internal concentration during plant development (Buendía Velázquez et al. 2017). This steady state favors plant nutrient reserves period (van den Driessche 1985), and such reserves may remain available for future retranslocation and use on the outplanting site (Timmer and Munson 1991). After Day 55, the RC gradient tended to increase from basal to apical leaves, indicating that in this period the nutrient supply failed to satisfy plant N demand and therefore, the phenomenon of dilution occurred (Andivia et al. 2014).

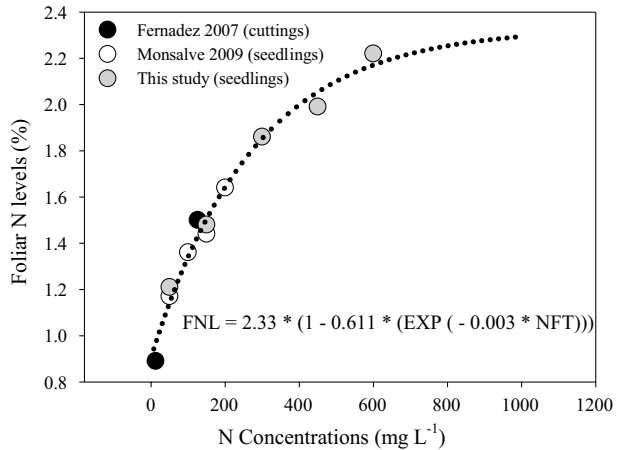
Our results concur with, and extend those reported by Close et al. (2005) and Monsalve et al. (2009); these authors obtained maximum growth responses of *E. globulus* seedlings with 200 mg N L<sup>-1</sup> but they indicate that concentrations closer to 300 mg N L<sup>-1</sup> may be optimum, assuming that concentrations of other elements (P, K, Ca, Mg, S, and micronutrients) were sufficient. Using the relationship between N supply and foliar N levels reported by Fernández et al. (2007) and Monsalve et al. (2009), we developed a N saturation curve for N (Fig. 8), which shows saturation would occur at about 2.3% (coefficient of the model) and this would be achieved with application concentrations greater than 600 mg L<sup>-1</sup>. Although our study shows that the highest N concentration was achieved with the 600 N rate (2.22% N, Table 2), we did not, however, see an increase in growth with N rates > 300 N despite an apparent N sufficiency during most of the growing cycle (Fig. 3).

A vector nomogram for leaf dry mass, using the 300 N rate as reference, shows a decrease in cation (i.e., K, Ca, Mg) concentration and content concurrent with the 450 N and 600 N rates, interpreted as antagonism (Fig. 5; Timmer 1991; Haase and Rose 1995). Nutrient uptake by plants is a complex interaction of cell membranes and the physiochemical properties of ions, and to maintain a balanced pH inside their cells, plant roots taking up positively charged ammonium (NH<sub>4</sub><sup>+</sup>) release an identically charged molecule that leads an increase in rhizosphere pH (Marschner 2012). High levels of NH<sub>4</sub><sup>+</sup> have been shown to inhibit the uptake of Ca<sup>2+</sup> and Mg<sup>2+</sup> from the substrate leading to plant tissue deficiencies (Adams 1966; Siddiqi et al. 2002). In addition, lower rhizosphere pH associated with applications of NH<sub>4</sub><sup>+</sup> was associated with Ca<sup>2+</sup> and K<sup>+</sup> deficiencies in *Rosa* (rose) leaves (Bar-Yosef et al. 2009). In our study, rates of macro- and micro-nutrients were held constant as the amount of NH<sub>4</sub><sup>+</sup> was increased. Given that the pH of our applied solutions was constant at 6.5, we speculate that the abundance of NH<sub>4</sub><sup>+</sup> and subsequent competition for exchange sites, rather than changes to rhizosphere pH, are the source of the antagonism.



**Fig. 7** For each pair of panels (a set for each of the five applied N concentrations), the left panel displays the dynamics of *Eucalyptus globulus* biomass production whereas the right panel provides the increments of roots, stems, and leaves during the 145-day cultivation period

**Fig. 8** Relationship between nitrogen (N) concentrations ( $\text{mg L}^{-1}$ ) supplied to *Eucalyptus globulus* plants and foliar N levels (%) observed by Fernández et al. (2007), Monsalve et al. (2009), and this study. In the mode. FNL: Foliar N levels; NFT: N fertilizer treatments



## Conclusions

It is difficult to obtain fertilization protocols that efficiently deliver nutrients at an appropriate time (Oliet et al. 2011). A better nutritional management strategy for container seedlings of *E. globulus* and other tree species could improve plant production in the nursery and result in optimal nutrient states for improving plant initial resources at outplanting and early establishment. Modeling growth responses to N inputs improves our understanding of physiological processes, and when combined with timely feedback systems, such as the SPAD method described here, could be employed to allow optimal nutrient management in forest nurseries, yielding substantial improvements in the establishment and growth of outplanted seedlings. Finally, the use of SPAD can be a rapid and effective diagnostic tool to monitor N status in container nurseries.

Our results indicate that *E. globulus* seedlings respond differently to various levels of N supply. We found that the  $300 \text{ mg N L}^{-1}$  rate provided optimum seedling growth in this study. This specific N fertilization rate could positively affect the early establishment of seedlings on more stressful planting sites because of the greater plant growth, enhanced new root growth, and higher tissue nutrient concentrations it yielded. This constant fertilization regime produced N dilution; however, we observed that increasing N rates reduce the effects of dilution at the end of the cultivation period (fall–winter). This finding suggests that N fertilization at the end of cultivation could be a useful tool to increase plant N loading. However, our observation of potential ammonium antagonism with other macronutrient cations is of concern as it may negate the potential benefits of N-loading unless other modifications are made to the nutrient regime. Additional work is needed to determine appropriate levels of the other macronutrients to leverage any potential growth benefits that higher rates of N might provide.

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