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Sprouts and saplings regeneration niche, crown carbon balance, and crown photosynthetic limitations of *Eucryphia cordifolia* Cav. plants at field and nursery conditions

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ANTONIO BARTOLOMÉ ESCANDÓN MONARDES

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Profesor Guía: Luis Julián Corcuera Pérez

Profesor Co-guía: Rafael Eduardo Coopman Ruiz-Tagle

Dpto. de Botánica, Facultad de Ciencias Naturales y Oceanográficas

Universidad de Concepción

A todo aquello que permite alzar el vuelo…

A todo aquel que en mi ha labrado y pulido los sentimientos…

- Espíritu… Música -

Comisión evaluadora

Sprouts and saplings regeneration niche, crown carbon balance, and crown photosynthetic limitations of *Eucryphia cordifolia* Cav. plants at field and nursery conditions.

Luis Corcuera Pérez (Profesor tutor) ____________________________
Profesor de Biología, Ph.D.
Facultad de Ciencias Naturales y Oceanográficas
Universidad de Concepción

Rafael Coopman Ruíz-Tagle ____________________________
Ingeniero Forestal
Dr. en Ciencias Forestales
Instituto de Conservación, Biodiversidad y Territorio
Universidad Austral de Chile

Alfredo Saldaña Mendoza ____________________________
Biólogo
Dr. en Ciencias Biológicas
Facultad de Ciencias Naturales y Oceanográficas
Universidad de Concepción

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General Introduction

The increase of CO_2 in the atmosphere contributes to a greater increment of the greenhouse effect and thus global warming. Carbon sequestration by plants has become a tool (e.g. carbon credits) that seeks, in part, maintaining and / or reducing these levels of atmospheric CO_2. Although plants respire between 30-80% of the assimilated CO_2 (Loveys et al. 2002), the rate of climate change could diminish over time if they respond by increasing the photosynthetic activity in this new environment. Concomitantly with the rise in environmental CO_2, it has been forecasted increased temperature and decreased rainfall for many regions of the world (Solomon et al. 2007). The predictive models of climate change predict a decline between 25-40% of rainfall for the growing season in the next 70 years for South Central Chile (Fuenzalida et al. 2006, Solomon et al. 2007). Within this geographic area, there are several forest types, being the evergreen temperate rain forest the one that covers the largest area (INFOR s / f). However, this type of forest is one of the rarest in the world (WWF). Furthermore, a number of metabolic processes in plants are dependent on environmental conditions (Bernacchi et al. 2001, Flexas & Medrano 2002, Campbell et al. 2007), which largely determine its responsiveness. Having predictive models is critical to address the effects of climate change on ecosystems and their component species (Diaz-Espejo et al. 2012). Therefore, it is necessary to understand how CO_2 capture and fixation in plants is modulated by the interaction of environmental factors and to what extent these environmental factors determine the success of establishment and survival of plants in their natural environment.

The availability of resources, specifically light and water, is crucial to plant establishment, species distribution, and community composition and diversity (Nicotra et al. 1999 Rozenbergar et al. 2007, Engelbrecht et al. 2007). Light in the forest is the most variable and heterogeneous abiotic factor in time and space. Much of it is intercepted by the forest canopy (Larcher 1980, Valladares 2006, Pugnaire & Valladares 2007). Therefore, light is likely the major environmental limiting factor of growth and survival of many forest species that can influence stand-level regeneration patterns (Nicotra et al. 1999). The vertical and horizontal canopy structure in temperate and tropical forests determines light availability in the understory, modulating the amount of direct, diffuse, and total incident light (Küppers et al. 1996, Valladares 2005, Valladares et al. 2012). Diffuse light has been shown to make a high contribution in temperate rain forest (Valladares et al. 2012), even to photosynthesis (Pearcy & Yang 1998). In turn, direct light (sunfleck) is scarce but highly important for understory plants in tropical forests, because its contribution is about 10 to 80% of the total photosynthetic photon flux density (PPFD) (Chazdon & Pearcy 1986, Pearcy et al. 1994). Nevertheless, this high photon contribution may decrease the photochemical efficiency of photosystem II under shade (Tobita et al. 2010). Hence, shaded plants are exposed to low potential carbon gain and a high relative cost of PSII repairing when photoinhibition occurs, reducing potentially the carbon gain (Tezara et al. 1998, Valladares & Pearcy 2002).

Regeneration of forest tree species is regulated by canopy height, seeds, soil type, microsites availabilities, light intensity, and the size of gap-light formation (Welden et al. 1991, Eriksson & Ehrlén 1992, Canham et al. 1994, Hubbell et al. 1999, Kobe 1999, Modrý et al. 2004). Light availability increases with gap size (Canham et al. 1990, Denslow et al. 1990, Gálhidy et al. 2006). The smaller gaps seem to be important especially for the occurrence of mid and shade-tolerant species (Hubbell et al. 1999). In consequence, many studies are concentrated on shade-tolerance understanding. In turn, open sites are stressful environments, due to high irradiance (i.e. high photoinhibition risk) and evaporative demand, being the latter especially relevant for shallow rooted seedlings (Bullock 2000). Regeneration dynamics of plant communities could be driven by seed and sprout origin (e.g. Clarke et al. 2013). Most of the studies on the ecophysiology of regrowth in ecosystems have been conducted in subjects with recurrent severe disturbances, where much of the aboveground biomass is removed and regenerated from the substance of reserves (i.e. carbohydrates) (e.g. Goorman et al. 2011). In ecosystems under a low-severity disturbance regime, sprouting of woody plants occurs in absence of major disturbances, suggesting other functional meanings of sprouting (Jeník 1994, Peterson & Jones 1997), different to that well known functions (i.e. exploitation, colonization, reproduction, and persistence; van Groenendael et al. 1996, del Tredici 2001). Clonal integration (i.e. the production of spreading non-splitted sprouts, sensu van Groenendael et al. 1996) is very important for plants invading stressful microhabitats (e.g. see Pennings & Callaway 2000). The flux of water, nutrients, and photosynthates are effectively transported between parent-genet plants (Mao et al. 2009, and references therein), increasing the chance of survival of non-splitting sprouts under abiotic stressful conditions (Wiehle et al. 2009). In the high productive rain forests, competition induces biotic stress and strongly determines species assemblage (Kraft et al. 2008). In these ecosystems, woody plants show almost all forms of sprouting and most of them increase colonization rates and thus competitive ability (Grubb 1987, Jeník 1994). Nevertheless, few studies tried to show some physiological light-related difference responses between seedlings and sprouts. For instance, photosynthesis capacity of *Betula pubescens* Ehrh and *B. pendula* Roth stump sprouts showed a better response during the first growing season than seedlings, which could be related with leaf structure (Kauppi et al. 2001). In turn, *Fagus grandifolia* does not show photosynthetic differences between its sprouts and saplings, even when leaf traits (leaf mass area, LMA) differ between them (Farahat & Lechwicz 2013). In addition, a higher relative growth rate has been shown in sprouts compared with saplings (Kauppi et al. 2001, Muñoz & González 2009, Farahat & Lechwicz 2013). Despite the profuse regrowth phenomenon in the temperate rain forest (González et al. 2002, Gutiérrez et al. 2008), there are no studies focused to determine the functional ecological role of this putative reproductive strategy in regenerative dynamics of these systems. Specifically, architectural strategies for light capture and photosynthetic activity have not been studied in depth for temperate rain forest species. Thus, regeneration strategies and physiology of mid-tolerant species in a second-growth temperate rainforest have not been fully studied and remain unclear.

Tolerance of plant species to more or less light availability is determined by both plasticity level, and by the arrangement of crown traits (Valladares & Niinemets 2007). This adjustment is crucial for light capture, carbon uptake, and survival of individual plants especially in low light regeneration sites (Givnish 1988, Pearcy & Yang 1996). Concomitantly with the seasonal changes there may be a decline in soil water, because both soil drainage capacity and yearly oscillation of rainfall (Davidson et al. 1998, Wilson et al. 2000, Chaves & Oliveira 2004, Fisher et al. 2007). In spite of this, foliar deployment and plant growth occur throughout the season. Stress combination responses to light and water availability occur frequently in natural environments. However, this combined stress effects have been only recently addressed (Guidi et al. 2008, Niinemets 2010, Cavatte et al. 2012, Tosens et al. 2012). Physiological, biochemical, and molecular plant responses to low light are commonly opposite to those described for drought (Smith & Huston 1989, Valladares & Pearcy 2002). In this sense, leaf orientation could influence light interception (Falster & Westoby 2003), which varies with direct and diffuse light. For instance, leaves of pioneer tropical tree species were oriented to increase diffuse light interception. This makes sense when low light acclimated leaves are saturated at low PPFDs (Ackerly & Bazzaz 1995, Muraoka et al. 2003), which clearly represents a photo-protection mechanism (Ort 2001). Under limiting light conditions, plant responses are focused to maximize light interception and capture by increasing the concentration of chlorophyll and proteins in the antennas (Demmig-Adams & Adams 1992, Marsuki et al. 2003), leaf area ratio (LAR) (Kitajima 1994, Broncano et al. 1998, Gardiner & Hodges 1998, Pattison et al. 1998), but decreasing leaf thickness (LMA) (Abrams & Kubiske 1990), root:shoot ratio, and growth rate (Markesteijn & Poorter 2009). In fact, shade-tolerant species under shade regularly have higher structural investment (Givnish 1988), greater leaf area per plant mass (LAR), higher specific leaf area (SLA), and a poor initial root investment than shade intolerant species (Kitajima 1994, Lusk 2002). Meanwhile, these traits are reversed in sun leaves. Light interception efficiency (or STAR: ratio of displayed to total leaf area, averaged over the entire sky hemisphere; Farque et al. 2001, Delagrange et al. 2006) has been shown to increase among contrasting crown architectures of understory plants (Valladares et al. 2002). STAR is affected by crown shape and leaf arrangement in the crown (Valladares & Niinemets 2007) and decreases with high irradiance and self-shading increment (Falster & Westoby 2003, Delagrange et al. 2006). However, the architectural traits have been mainly studied in order to compare the effect of light on shade tolerant or intolerant species under different light availabilities (see Pearcy et al. 2005), which is currently well understood. However, water availability should be integrated to the analysis, because the architectural traits are depending on growth and leaf area displayed, which could relate with carbon assimilation (Falster & Westoby 2003).

Commonly, biomass allocation, physiological, biochemical and molecular responses lead to improve carbon balance as adaptive responses to light and water stress (Valladares et al. 2000, Gratani et al. 2006, Coste et al. 2010, Wyka et al. 2011, Egea et al. 2011). Several studies have analyzed water stress effects on photosynthetic parameters (Limousin et al. 2010, Misson et al. 2010). In other studies, the authors have scaled up from leaf assimilation and water relations to higher levels (i.e. whole-plant, canopy, ecosystem; Rambal et al. 2003, Bucci et al. 2004, Santiago & Mulkey 2005). For instance, photosynthetic activity at leaf level decreases due to the increment of limitations provoked by drought and ontogeny (Flexas et al. 2002, Grassi &

Magnani 2005). In this sense, the individual leaf performance should depend on its acclimation level to the prevailing environmental conditions. The impact of acclimation to stress on photosynthetic limitations of different leaf cohorts deployed under different environmental conditions along growth season has been partially studied. Limousin et al. (2010) found comparing two leaf cohorts of different growing seasons that leaf and canopy acclimation to progressive, long-term drought occurred through changes in leaf area index, leaf mass per area, and leaf chemical composition, but not through modifications of physiological parameters. In addition, Mulkey et al. (1995) found that fully expanded leaves produced during the early rainy season showed lower photosynthetic capacities and longer mean longevity compared with leaves of the end of the rainy season. The limitation in plant growth imposed by low water availability has been commonly attributed to reductions in plant carbon balance (Flexas el al. 2005, 2006). Since there is structural and biochemical acclimation to varying environmental conditions during the season, it is logic to expect that under different conditions plants will generate leaf cohorts with different morpho-physiological traits. It may also be expected that the successive leaf cohorts will show differential responses to light and water stress. Thus, differences in photosynthetic limitations are expected along the crown. There are no records on limitations analysis that integrate metabolic differences in foliar photosynthesis at whole plant level in relation to the seasonal availability of light and water during the deployment of foliage during the growing season. Therefore, it is unknown how photosynthetic limitations contribute differentially to leaf cohorts deployed at different times, or its relation to carbon gain when plants are facing such environmental variables during growth and development.

Approaching to the problem

Eucryphia cordifolia Cav. is an abundant species in the Chilean evergreen temperate rain forest along its geographical distribution, specifically on the south-central Chile. It has the ability of regrowth from root suckers in undisturbed areas. *E. cordifolia* has shown a high frequency in both primary and secondary forests (Lusk & Piper 2007, Lusk et al. 2011). Its preference for recruitment occurs in rather shady sites. However, it can be found at sites of increased light availability (Escobar et al. 2006, Figueroa et al. 2010). It is an economically and socially promising and highly valued species due to its high quality for use in construction, furniture, and as lumber by its caloric value. Moreover, the nectar of the flowers allows the production of high quality honeybee, for the national and international market. This species responds well to cultivation showing a high relative growth rate (Escobar et al. 2006). Nevertheless, plantations of *E. cordifolia* are difficult to establish in the field because of the high mortality of seedlings and saplings after transplanting to open sites (González et al. 1997, Donoso 2008), possibly due to drought events during the growth season combined with high solar radiation. This weakness may be exacerbated by the predicted drier growing seasons than at present. This prediction fits very well with recorded data at 30 km of the study site (Tepual Airport weather station: 41° 25' S, 73° 05' 85" W; Meteorological Office of Chile, http://www.meteochile.gob.cl/), where rainfall has decreased at a rate of 5.8 mm year^{-1} since 1861 until today (more than 30% decrease over the last 150 years). Low water availability could affect photosynthesis, growth, and survival of seedlings. Moreover, shade and semi-shade plants might be especially sensitive to water stress, generating unknown consequences on regeneration dynamics of this type of forest. Therefore, in addition to study the recruiting behavior of *E. cordifolia* in the field, it is required to disclose the effects of light and water availability on its responsiveness on the architectural and physiological acclimation during the continuous leaf displaying along the growth season. The results of this thesis work will provide the basic ecophysiological understanding to develop more effective procedures for restoration and field regeneration of this species.

The following hypotheses are proposed:

1. Niche differentiation between sprouts and saplings extends the regeneration niche towards non optimal conditions for sexual recruitment. This is proposed because of the role of resources gaps colonization of the sprouts and by the susceptibility to mortality of *Eucryphia cordifolia* seedlings.
2. Sprouts and saplings show similar crown architectural traits growing under similar light environments, independently of the biological age and subsidy of the parent plant. In addition, *E. cordifolia* sprouts show higher photosynthetic capacities than saplings, which allows the higher relative growth rates.
3. Leaf cohorts of *Eucryphia cordifolia* displayed during gradual increase in water deficit in both high and low light preserve their biochemical metabolism compared against well

watered. Thus, they show a lower non-stomatal and a higher stomatal limitation contributions at crown level, explaining the decrease of daily crown assimilation.

4. Well watered and water stressed *E. cordifolia* plants under low light conditions have a similar effect on plant architecture and daily crown assimilation. However, daily crown assimilation balance of water stressed plants is lower or even negative compared with well watered.
5. *E. cordifolia* leaves displayed towards the end of the growth season show higher rates of gas exchanges, contributing more to daily crown assimilation than the older leaf cohorts. This may be due to the acclimation response to environmental conditions to which they were subjected during the leaf-plant growth and development.

The general objective of this thesis was determinate the effect of light and water availability on regeneration, architecture and photosynthetic performance at crown level of *Eucryphia cordifolia* Cav.

Chapter I

Sprouting extends the regeneration niche in temperate rain forests: The case of the long-lived tree *Eucryphia cordifolia*.

Antonio B. Escandón[a,†], Susana Paula[b,†], Roke Rojas[c], Luis J. Corcuera[a], Rafael E. Coopman[c,*]

[a]Laboratorio de Fisiología Vegetal, Facultad de Ciencias Naturales y Oceanográficas, Universidad de Concepción, Casilla 160-C, Concepción, Chile.

[b]Instituto de Ciencias Ambientales y Evolutivas, Facultad de Ciencias, Universidad Austral de Chile, Casilla 567, Valdivia, Chile.

[c]Forest Ecophysiology Laboratory, Conservation, Biodiversity and Territory Institute, Universidad Austral de Chile, Casilla 567, Valdivia, Chile.

[†]These two authors contributed equally to this work.

*Corresponding author: tel. +56-63-293037; fax: +56-63-221230, e-mail: rafael.coopman@uach.cl

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ABSTRACT

Sprouting is a widespread trait of geographical, ecological and taxonomical scales that allows resource exploitation, space colonization, vegetative reproduction and post-disturbance persistence. It is considered that persistence is the most frequent functional role of sprouting in woody plants. Here we propose that niche differentiation between sprouts and saplings extends the regeneration niche to suboptimal conditions for sexual recruitment. To test this hypothesis, we sampled root suckers (sprouts) and saplings of the long-lived tree species *Eucryphia cordifolia* Cav. in an even-aged coastal temperate rain forest. Canopy openness was measured over each recruit as a proxy of regeneration niche. Sapling survival was checked after two years. The entire forest canopy openness range was used as an estimation of niche availability. Light and microclimatic conditions were recorded for different canopy openness. Sprouts and saplings occupied a narrow range of the canopy openness near to the closest extreme of the gradient (4.8-14.1%). The distribution of saplings was explained by the lower survival of small saplings under more open canopies, as a consequence of the interactive effect of higher evaporative demand and radiation load in more opened canopies. Although the niches of sexual and vegetative recruits slightly overlap (31% of the interquartile range on average), sprouts inhabit the more opened sites. Therefore, sprouts extend the regeneration niche to suboptimal conditions for sapling establishment. Our results contribute towards explaining the high colonization ability of *E. cordifolia* in this kind of forest, whose composition is strongly driven by gap dynamics.

Highlights:

- Saplings and sprouts of *Eucryphia cordifolia* show niche selection
- Sapling survival is driven by the interactive stress of drought and high irradiance
- Niche differentiation between sprouts and saplings extends the regeneration niche

Keywords: Niche breadth, root suckers, gap colonization, sapling survival, interactive abiotic stress

1. Introduction

Sprouting is a widespread, ancestral trait, appearing in a wide range of ecosystems and lineages (Wells, 1969; del Tredici, 2001; Bond and Migdley, 2003; Vesk and Westoby, 2004). The very high frequency of sprouting at both ecological and taxonomical scales reflects its functional diversity (van Groenendael et al., 1996; del Tredici, 2001); it allows populations: (1) to exploit resources when they are scarce or heterogeneously distributed in the space (exploitation function);(2) to increase the competitive ability by fast colonization of gaps in high productive ecosystems (colonization function); (3) to increase reproduction in stressful environments or in species with low sexual reproductive success (reproduction function); and (4) to regenerate vegetatively after disturbances of different severities (i.e. resprouting *sensu* Clarke et al., 2013; persistence function). It is considered that clonal growth in herbaceous species mostly allows gathering resources, space colonization and reproduction, whereas the primary benefit of sprouting for woody species is recovering after disturbances (Peterson and Jones, 1997). In fact, resprouting is a cornerstone trait determining resilience to disturbances in woody plant communities (Keeley, 1986; Bond and Migdley, 2001).

In ecosystems under a low-severity disturbance regime, sprouting of woody plants occurs in the absence of major disturbances, suggesting other functional meanings of sprouting (Jeník, 1994; Peterson and Jones, 1997). For instance, clonal growth by spreading non-splitting sprouts is frequent in stressful environments such as marsh wetlands or tree-lines (Pennings and Callaway, 2000; Peltzer, 2002). The subsidy of water, sugars and nutrients from parent plants increases the chance of survival of non-splitting sprouts under abiotic stressful conditions (Wiehle et al., 2009). In the high productive rain forests, competition induces biotic stress and strongly determines species assemblage (Kraft et al., 2008). In these ecosystems, woody plants show almost all forms of sprouting and most of them increase colonization rates and thus competitive ability (Grubb, 1987; Jeník, 1994).

Opportunities of colonization in rain forests mainly occur after canopy openness, such as tree-fall gap formations (Denslow, 1987). Commonly, sexual regeneration is in competitive disadvantage against sprouts due to their lower growth rates during early developmental stages (Farahat and Lechowicz, 2013). This is exacerbated in gaps where microsite conditions are likely to become unsuitable for seedling establishment. The interaction between the sudden increment of high irradiance and subsequent evaporative demand in gaps might lead to some level of drought (Tognetti et al., 1994; Flexas et al., 1999). This is especially true for shade saplings, which present several traits towards the maximization of light capture, which are antagonistic to those described for water stress resistance, resulting in an exacerbated water stress sensibility (Valladares and Pearcy, 2002). In these cases, sprouts would extend the regeneration niche to suboptimal conditions where the chance of sexual recruitment is low.

The regenerative dynamics of the coastal temperate rain forest of south-central Chile is strongly driven by the occurrence of individual tree-fall gaps, which increases environmental heterogeneity, thus allowing the coexistence of different plant functional types (e.g. Armesto and Fuentes, 1988; Gutiérrez et al., 2008). Specifically, gaps are suitable for the seedling establishment of shade-intolerant species, whose survival is negligible in deep shaded microsites because the low carbon gain induces carbon starvation (Givnish, 1988). For intermediate shade-intolerant species, such as the long-lived tree species *Eucryphia cordifolia* Cav., small gaps seem to be adequate for sexual recruitment (Lusk et al., 2006; Gutiérrez et al., 2008). This species produces high amounts of viable seeds from an early age onwards, which can be dispersed over long distances (Escobar et al., 2006). Although germination is usually greater in understory conditions, it is not negligible in gaps (Figueroa and Lusk, 2001). Therefore, the spatial pattern of sexual recruitment of *E. cordifolia* (i.e. at intermediate canopy openness) is not explained by seed availability and germination but by seedling establishment and survival. In fact, although seedlings of this species have been recorded immediately after gap formation, most of them fail to survive after two years (González et al., 2002).In addition to sexual recruitment, this species profusely sprouts from roots, even in the absence of disturbances; contrary to sexual recruits, root suckers show very low mortality (Donoso et al., 1985; Veblen, 1985; González et al., 2002). The coexistence of the two regeneration mechanisms in *E. cordifolia* and gap dynamics of the coastal temperate rain forest provides an excellent model to evaluate the role of sprouting in extending the niche regeneration to limiting conditions for sexual recruitment. To test this hypothesis, we addressed the following questions: (1) does niche selection exist in sprouts and saplings of *E. cordifolia*?; (2) which environmental condition constrains the survival of *E. cordifolia* saplings?; (3) does niche differentiation between sprouts and saplings extend the niche regeneration? In order to fulfill these aims, we considered the canopy openness as a proxy of ecological niche, since it involves the availability of key resources like light and water (Brown, 1993; Maherali et al., 1997).

2. Material and methods

2.1. Study site

This study was conducted during the growing seasons of 2011 to 2013 in a 30 ha secondary coastal temperate rain forest located in south-central Chile (Katalapi Park: 41°31′8″ S, 72°45′2″ W, elevation ca. 90 m a.s.l.). The forest structure corresponds to ca. 35 years old even-age forest where the taller individuals reach up to 10 m, but with some old-growth remnant trees. The study site presents several open areas previously used for cattle activities. For the last 27 years, the forest has been protected from anthropogenic alterations (logging, cattle), allowing for regeneration of both sun and shade species. The most frequent tree species are *Nothofagus nitida* (Phill.) Krasser, *Nothofagus dombeyi* (Mirb.) Oerst, *Laureliopsis philippiana* (Looser) Schodde, *Aextoxicum punctatum* R. et P., *Eucryphia cordifolia* Cav., *Drimys wintery* J.R. et G. Forster, and several Mirtaceae (Saldaña et al., 2005; Lusk and Corcuera, 2011; Coopman et al., 2011).

This area presents a maritime temperate climate, with annual rainfall of ca.1900 mm concentrated between April to November (ca. 77% annual rainfall; data from 1861 to 2001; Tepual Airport weather station: 41° 25' S, 73° 05' 85" W; Meteorological Office of Chile, http://www.meteochile.gob.cl/) and a mild dry season during December to March. In this period, the mean air temperature reaches 15º C, the lowest air relative humidity range between 45-55% and a 15-day-long dry period frequently occurs within each summer. Climatic details for the study area are shown in Coopman et al. (2010).

2.2. Forest canopy openness

The forest canopy openness gradient was determined on 8 transects 100 m long on average randomly distributed along the forest. To ensure that we included the entire light gradient, transects were started in open sites located at the edge of the forest and continued through the closed forest. Hemispherical photography was used to measure canopy openness (Chazdon and Field, 1987) by using a Coolpix 4500 digital camera equipped with a FC-E8 fisheye lens (Nikon, Tokyo, JP). A photograph was captured at 5 m intervals along each transect at 40-60 cm height, resulting in a total of 161 photographs. The camera was hand leveled and oriented so that the top of the image faced north. Photographs were taken on homogeneous overcast days. The resulting photographs were analyzed for the percentage of canopy openness (hereafter CO) with the Gap Light Analyzer 2.0 software (GLA; Frazer et al., 1999).

2.3. Canopy openness and microsite environment

To relate CO with microsite conditions we recorded photosynthetic photon flux density (PPFD), air temperature (Ta) and relative humidity (RH) at different CO ranging from 2 to 100% (see Fig. 1). In the open site (100% CO), we used a Li-1400 data logger connected to LI250, LI 1400-104 and LI 1400-106 sensors (Li-Cor Inc., NE., USA). For measurements inside the forest, we installed H21-002 HOBO meteorological stations connected to S-LIA-M003 and S-THA-M0xx sensors (Onset, MA, USA). Data were recorded at 30 minute intervals from 2007 until 2013 (except 2010 due to technical troubles). The air vapor pressure deficit (VPD) was determined according to Murray (1967):

$$VPD = Pv - ((RH/100)*Pv),$$

where Pv is calculated as follows:

$$Pv = 0.611 \exp [17.27\, Ta/(Ta + 237.3)].$$

2.4. Regeneration niche

More than 90% of the surface area of the forest was inspected looking for saplings. Regarding sprouts, previous observations indicated that they only appear close to mature trees. Therefore, sprouts were recorded in15 plots of 200 m^2established around mature trees, in such a way each plot covered a wide range of canopy openness (i.e. from big gaps to closed forest). *Eucryphia cordifolia* sprouts from the root collar and from roots (root suckers; e.g. Veblen, 1985). However, for the aim of this study, we only sampled root suckers, because basal sprouts are generally associated to severe damage (persistence function; Jeník, 1994). Sampled recruits were taller than 2 cm in height but shorter than 175 cm. We excluded older recruits (i.e. taller), because environmental conditions at the establishment time would have been different to current conditions. Each plant was carefully inspected and recognized as originating from sprout or seed.

Specifically, recruits were identified as sprouts when they showed a “T-inverse” at the underground level, meanwhile saplings did not show any subsidiary root connection or root scar indicating past connections. A hemispherical photograph over plant apex was recorded (see section 2.2 for details). If recruits were clustered and the canopy structure was homogeneous over them, one photograph was taken directly above the group of recruits. Because the sensitivity of woody plants to environmental conditions changes with the ontogeny (Coopman et al., 2008), the height of both sprouts and saplings was measured. All saplings, except 50 (that were harvested in a parallel study), were tagged to check survival two years later (March 2013). A sapling was considered dead when the above-ground biomass was totally necrosed or when no leaves or axillaries buds were observed on the stem.

2.5. Statistical analyses

For each CO, we calculated daily (from 7:00 to 19:00 h) 90% percentile of PPFD and VPD. We used percentile instead maximum and minimum values to avoid extreme (non-frequent) conditions. Then, the percentiles were averaged for each summer period (i.e. December to February) and independently correlated with CO. After visual inspection, linear regression was tested for VPD, whereas logistic regression was used in case of PPFD. Because most measurements were concentrated in the closed end of the canopy openness gradient, we log-transformed this variable.

Niche selection was evaluated by comparing the distribution of the CO for each recruit type with those of the forest by means of the Kolmogorov-Smirnov test. Because we were aware that sampling design would bias the distribution of the forest CO towards the upper extreme of the gradient, we repeated the analyses excluding those CO values higher than 31.4% which corresponded to the 75% quantile of the forest. In addition, we conducted similar analyses comparing independently the distribution of forest CO with that of recruits of different height classes defined on the quartile basis. Height classes were established separately for sprouts and saplings to ensure a large enough sample size for each recruit type and height class.

Changes of the sapling survival probability along the light availability gradient and plant height were analyzed by means of Generalized Lineal Model (GLM), assuming a binomial distribution error and logit link function, and tested by an analysis of deviance (McCullagh and Nelder, 1989). Because of the high skewness of the distribution of saplings towards the shaded extreme of the gradient (Fig. 2), differences in survival under low light availability would be difficult to detect with raw data. Therefore, CO was divided into quartiles, and differences in survival tested among CO classes. Plant height classes were defined in the same way and included as independent variable in the model. Because the analysis of survival was conducted with a subset of the initial saplings (see section 2.4.), height classes slightly differed from those established in the evaluation of niche selection (see Table 1 and Figure 3). The canopy openness and sapling height distributions for this subset did not significantly differ from those of the initial set of saplings (P = 0.828 and P = 0.398 respectively in the Kolmogorov-Smirnov test).

Comparison of the mean CO between sprouts and saplings was performed by means of permutational ANOVA (PERMANOVA; Anderson, 2001). This statistical tool was chosen because the response variable was not normal and any transformation corrected this issue. PERMANOVA analysis was conducted based on 10,000 permutations with the function *adonis* of the library *vegan* of the R program (Oksanen, 2011). This function is less sensitive to differences in the dispersion between groups than other sister functions (Oksanen, 2011).

To evaluate niche overlap between the two types of recruits, the first and third quartiles of CO for both sprouts and saplings were calculated. Then, the 95% confidence intervals of the quartiles were estimated by bootstrapping (n = 10,000). They were considered significantly different when the proportional overlap of the confidence interval was less than 0.5 (Cumming, 2009). Finally, in order to evaluate if sprouts increase niche breadth, we calculated the Levins' index for saplings and saplings+sprouts according to Colwell and Futuyma (1971). This index has been suitable for assessing niche breadth in the CO dimension in temperate rain forests (Saldaña et al., 2005). The 95% confidence intervals of the Levins' indexes were estimated by bootstrapping (n = 10,000) and the proportional overlap calculated to test for differences between saplings and saplings+sprouts.

3. Results

Canopy openness (CO) and photosynthetic photon flux density (PPFD) were related in a threshold fashion, in such a way that PPFD abruptly increases from 10% CO (Fig. 1a). Accordingly, vapor deficit pressure (VDP) was positively correlated with CO (Fig. 1b).

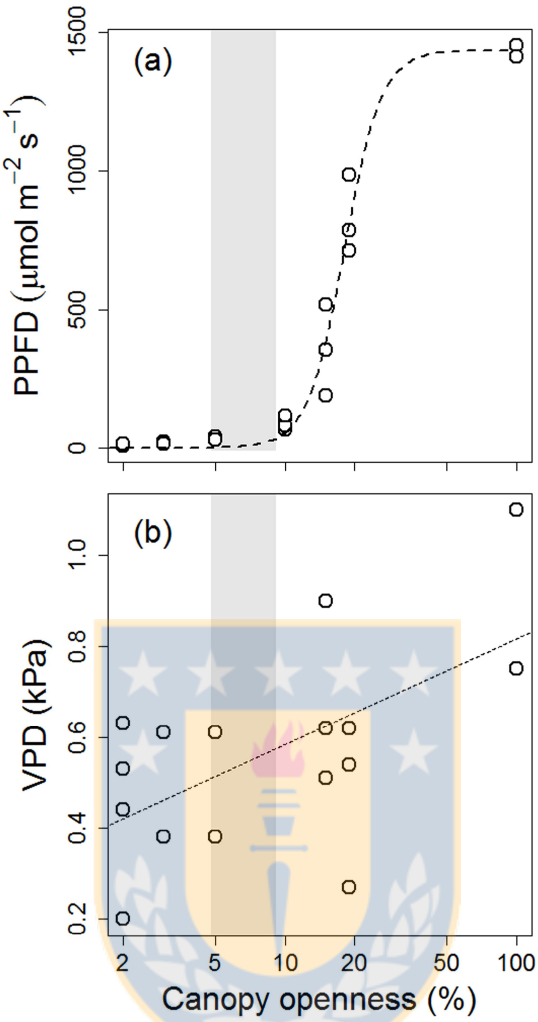

Fig. 1. Changes in the 90 percentile of daily (from 7:00 to 19:00 h) photosynthetic photon flux density (PPFD) and vapor pressure deficit (VPD) along the canopy openness gradient for the study area in summer (December to February). Discontinuous lines represent fitted models: logistic for PPFD (R^2 = 0.99) and linear for VPD (R^2 = 0.35). Grey area indicates interquartile range of canopy openness for *E. cordifolia* saplings. The estimated PPFD at 5% of canopy openness (7.3 μmol m^{-2} s^{-1}) is quite similar to the light compensation point (LCP) of *E. cordifolia* saplings (5.3±1.2 μmol m^{-2} s^{-1}). Notice the absence of VPD values at 10% of canopy openness due to erroneous humidity data logging.

The Kolmogorov-Smirnov test indicates that neither sprouts nor saplings are randomly distributed along the CO gradient of the study area (*P*< 0.001 in both cases; Table 1). Similar results were obtained when the higher CO values were excluded from the analyses (i.e. higher than the 75% quantile of the forest CO; *P* <0.001), indicating that such differences were not due to the long tail of the distribution of forest CO, and thus to a possible bias in the sampling design. However, whereas the distribution of CO for the forest was mesokurtic (kurtosis = 0.23; *P* = 0.280), the distribution of CO for sprouts and especially for saplings was leptokurtic (kurtosis = 3.82, kurtosis = 16.85 respectively; *P*< 0.001 in both cases), indicating that most recruits were concentrated around the corresponding mean values of CO (Fig. 2). The fact that sprouts were rare in very open sites supports our assumption that they were not related to disturbances. When comparisons were conducted independently for each height class, we found that the CO distribution of sprouts significantly differed with that of the forest for all height classes except for sprouts between 6 and 35 cm in height (Table 1). For saplings, we found significant differences in the CO distributions for all height sapling classes except for the tallest one (Table 1).

Table 1. *P*-values of the Kolmogorov-Smirnov tests comparing canopy openness distribution between the forest and each recruit type (sprouts and saplings) of *Eucryphia cordifolia*. Analyses were conducted pooling all height classes and independently for each height class.

	Sprouts			Saplings		
Height class	Height (cm)	n	*P*-value	Height (cm)	n	*P*-value
All	6-164	297	<0.001	2-155	219	<0.001
Very small	6-35	74	0.488	2-9	55	0.012
Small	35-53	76	0.002	9-16	55	<0.001
Intermediate	53-87	73	0.035	16-30	58	0.028
Large	87-164	74	<0.001	30-155	51	0.089

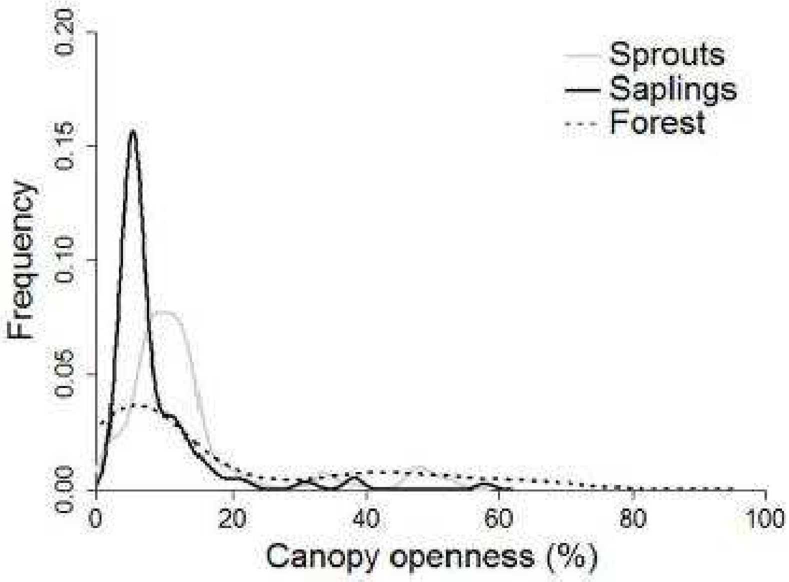

Fig. 2. Canopy openness distribution of *E. cordifolia* sprouts, saplings and the forest in the study area. Interquartile ranges of canopy openness were 7.6-14.1, 4.8-9.2 and 4.9-31.4%, respectively.

Table 2. Analysis of deviance for the probability of *E. cordifolia* sapling survival with canopy openness (CO) and heights (H) classes.

Trait	Df	Deviance	Residual Df	Residual Deviance	*P*-value
All height classes					
Null			168	232.14	
CO	3	5.85	165	226.30	0.119
H	3	10.90	162	215.39	0.012
CO x H	9	5.81	153	209.58	0.759
Overdispertion		1.38			
75% smallest heights					
Null			127	172.92	
CO	3	9.03	124	163.89	0.029
Height	2	8.10	122	155.79	0.017
CO x Height	6	2.14	116	153.66	0.907
Overdispertion		1.36			

Survival probability increases with sapling height, but no differences were detected among CO classes (Table2). However, when the tallest plants were excluded from the analysis, differences between CO classes emerged, with sapling survival higher in closed sites (Table 2, Fig. 3); differences between height classes remained significant in this last analysis (Table 2).

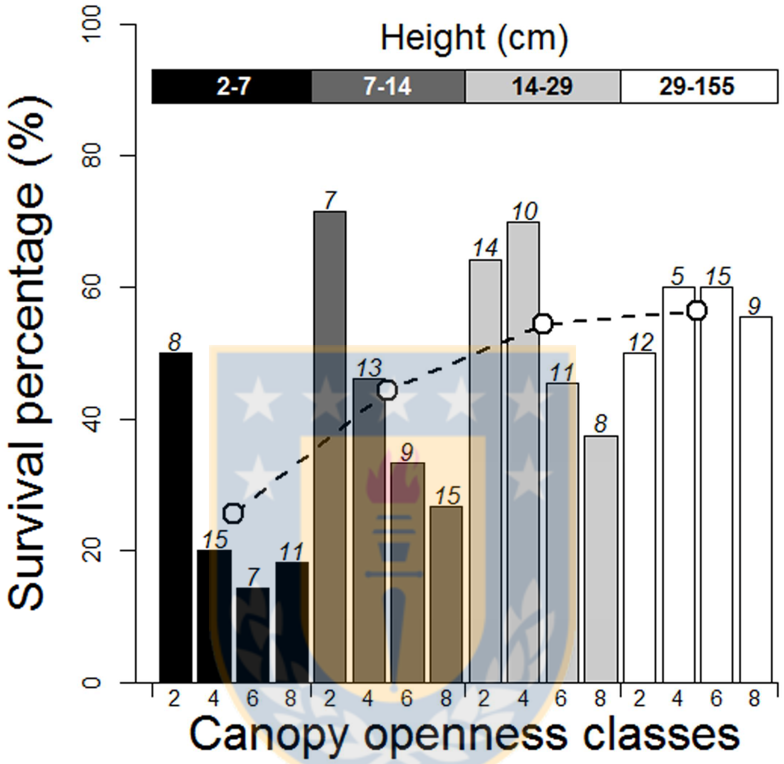

Fig. 3. Percent of survival of *E. cordifolia* saplings for each canopy openness and height classes. Height classes were defined on the quartile basis. For canopy openness classes, the value of the upper limit of each class is indicated. Symbols indicate the mean survival for each height classes regardless of the canopy openness. The sample size for each combination of canopy openness and height classes is indicated over the corresponding bar. Notice that height classes slightly differ from the height classes shown in Table 1 (see detail in the main text).

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Table 3. Results of the PERMANOVA analysis comparing the mean canopy openness between *E. cordifolia* recruit types (sprouts and saplings).

	Df	SS	MS	pseudoF	*P*-value	Explained variance (%)
Recruit type	1	3555	3554.8	34.663	<0.001	6.2
Residuals	523	53637	102.6			93.8
Total	524	57191	1			

The upper quartile of the CO of saplings (9.2%; bootstrapping confidence interval: [5.2,11.3]) significantly overpassed the lower quartile for sprouts (7.6%; bootstrapping confidence interval: [7.0,13.4]). However, the interquartile ranges of the two recruits only overlapped in a 31% on average (25% of the interquartile range of sprouts and 38% for saplings; see ranges in the Figure 1 caption). Accordingly, PERMANOVA analyses detected significant differences in CO between sprouts and saplings (Table 3). Specifically, saplings were found, on average, at more closed sites than sprouts. Consequently, the Levins' index for saplings (B = 0.58; bootstrapping confidence interval: [0.52,0.63]) was significantly lower than the index for saplings+sprouts (B = 0.70; bootstrapping confidence interval: [0.66,0.73]).

4. Discussion

Sprouting as are productive strategy commonly occurs in stressful environments where seedling establishment is negligible (van Groenendael et al., 1996). However, sprouting under suboptimal environmental conditions for sexual recruitment would be an advantage through regeneration niche extension. This is the case of the long-lived pioneer tree species *Eucryphia cordifolia*, which has yearly sexual recruitment non-randomly distributed in the forest (Escobar et al., 2006 and this study). The range of canopy openness (CO) occupied by *E. cordifolia* saplings was narrower than the entire CO gradient available in the forest, and concentrated in the shadiest portion (4.8-9.2% CO; Fig. 2), thus reflecting niche selection (Table 1). Sprouts also select for the closed end of the CO gradient (7.6-14.1% CO; Table 1, Fig. 2). Because CO ranges of sprouts and saplings do not fully overlap, the regeneration niche is wider when both recruit types are pooled together, instead of considering saplings alone. This extended regeneration niche likely explains why *E. cordifolia* is frequently considered an emergent species (Escobar et al., 2006) despite the higher frequency of saplings in closed sites.

The fact that both sprouts and saplings recruit near to the closest extreme of CO gradient could have different explanations. Shady habitats of sprouts are likely due to the spatial association with the parent plant and not to environmental constraints, since juvenile non-splitting sprouts are subsided by parent plants (Oborny et al., 2000; Wiehle et al., 2009). On the contrary, saplings would maintain a higher dependency to environmental conditions. In this sense, the scarcity of saplings recruiting at CO lower than 5% is likely determined by high mortality at light intensities below their light compensation point (LCP= 5.3±1.2 µmol m^{-2} s^{-1}; Fig. A1 supplementary data). Usually more than half of daily CO_2 uptake is lost in respiratory processes necessary for growth and maintenance (Turnbull et al., 2001; Flexas et al., 2006). Consequently, these quite similar LCP and midday PPFD availability (7.3 µmol m^{-2} s^{-1}: 90% percentile of daily PPFD at 5% CO), mean that the daily-based carbon balance is severely restricted, reaching frequently negative values. In agreement with our findings, a high mortality of *E. cordifolia* seedlings has also been reported by Lusk and Del Pozo (2002) at similar low light intensity (12 µmol $m^{-2}s^{-1}$).

At the other extreme of the CO gradient (i.e., more open sites), both irradiance and evaporative demand increases (Fig. 1). The prevailing rainfall regime induces low VPDs during the beginning of the growing season. This allows seedling survival regardless of CO, despite their small and shallow rooting systems, commonly developed into the highly porous litter. In summer, the interaction of increased VPD and the abrupt rise of midday PPFDs from 10% CO would generate water stress (Fig. 1A). During periods of insufficient water supply, the otherwise beneficial light conditions become excessive because of reduced carbon assimilation. This imbalance leads to photoinhibition and photooxidative stress (Flexas et al., 1999). Consequently, environmental conditions might become critical during the short dry periods in summer (see section 2.1) and likely explains the mortality of small saplings at more open sites (Fig. 3). Accordingly, survival of large saplings (29-155 cm in height) were affected by CO (Table 1 and 2, Fig. 3), possible because their root systems are able to supply water requirements even at more open sites.

Tree-fall gap formation is a key process driving vegetation dynamics and composition in coastal temperate rain forests (Gutiérrez et al., 2008). The role of advance (i.e. pre-disturbance) regeneration to refill gaps is well established (Denslow, 1987 and references therein). In this regard, the extended regeneration niche of *E. cordifolia* improves its competitive ability within forest gaps. In fact, the fast gap colonization of *E. cordifolia* is not only due to its capacity to reoccupy their own gaps (i.e. resprouting), which are quite frequent in temperate rain forests (Gutiérrez et al., 2008), but also to growth stimulation of pre-disturbance sprouts in response to gap formation (González et al., 2002). In this sense, pre-disturbance sprouts might function as a suppressed sapling bank (*sensu* Grime, 1979), able to grow and reach maturity when conditions become favorable (e.g. gaps), but become suboptimal for sexual regeneration.

Here, we provide new knowledge on sprouting ecology that also reveals important implications for forest management. *Eucryphia cordifolia* is an economically promising tree species, due to its fast growth, its highly appreciated wood, and the melliferous quality of its flowers. The high mortality of saplings under the prevailing light and water availabilities of opened canopies will be exacerbated in the study area under the predicted 40% decline in summer precipitation over the next 70 years (Fuenzalida et al., 2006; Solomon et al., 2007). In this scenario, current fail of sexual recruitment would be exacerbated and forest regeneration dominated by clonal growth, leading to a reduction in the genetic diversity with the aforementioned endogamous depression and susceptibility to pathogens (Honnay and Bossuyt, 2005). Our results also highlight the relevance to distinguish between vegetative and sexual recruitment in relation to shade-tolerance and longevity, which are cornerstone considerations in regeneration dynamics studies and forest management (Valladares and Niinemets, 2008).

5. Conclusions

Eucryphia cordifolia saplings show niche selection, which is modulated by the differential survival of small plants along forest environmental gradients. Sapling survival is mainly driven by the interactive effect of water stress and high irradiance. Sprouting allows regeneration of *E. cordifolia* under suboptimal conditions for sexual recruitment, expanding its niche regeneration.

Acknowledgements

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Appendix A. Supplementary data

Fig. A1. Light-response curve of *E. cordifolia* saplings.

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Appendix A. Supplementary material.

Photosynthetic light response curves: Measurements for seedlings were performed in November 2012 in young but fully expanded leaves were clamped into the cuvette of an open gas-exchange system with the Li-6400 led source. (Li-Cor Inc., Nebraska, USA). Block temperature was kept at 18ºC during all measurements (registered leaf temperatures ranging 15-19 ºC) and vapor pressure deficit (VPD) at around 0.6 kPa. Given the low photosynthetic rates of these species and the small area (2 cm^2) of the leaf cuvette, the flow rate was adjusted to ensure that CO_2 differentials between the reference and the sample IRGAs were > 4 μmol mol^{-1} air. Photosynthetic light response curves were performed at a CO_2 concentration (C_a) of 400 μmol mol^{-1}, and consisted in 10 different light intensities between 0 and 1000 μmol m^{-2} s^{-1}. Desired levels of PPFD were achieved using the Li-6400 led source with a 90% red and a 10% blue light. Mitochondrial respiration (R_n) rates were measured after darkening the plants for 30 min. Leaf absorptance (α) was determined with the Li-6400 LED light using a spectroradiometer (HR2000CG-UV-NIR; Ocean Optics Inc., Dunedin, USA), as described (Gago et al. 2013). Light responses curve parameters were calculated with the Photosynthesis tool software (Li-Cor Application Note #105).

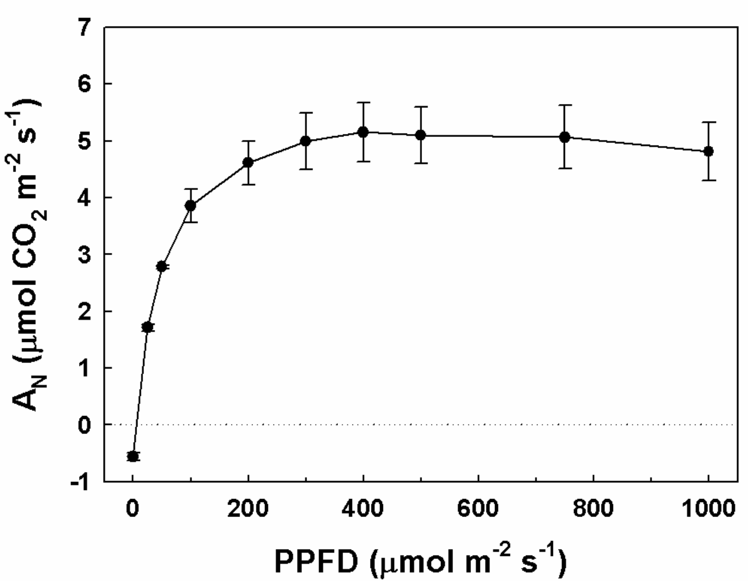

Fig. A1. Light response curve of saplings of *E. cordifolia* growing at field condition. Plant canopy openness was ca. 5% CO. Light compensation and saturation points averaged (± SD) were 5.3 (1.2) and 301.6 (71.2) µmol m^{-2} s^{-1}, respectively. *n* = 3.

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Chapter II

Deep-shade crown carbon balance of sprouts and saplings of *Eucryphia cordifolia*.

Escandón, A.B. [1], Coopman, R.E.[2], Paula S[3], Rojas R[2]., Corcuera L.J. [1].

[1]Laboratorio de Fisiología Vegetal, Facultad de Ciencias Naturales y Oceanográficas, Universidad de Concepción, Concepción, Chile.

[2]Forest Ecophysiology Laboratory, Conservation, Biodiversity and Territory Institute, Universidad Austral de Chile, Casilla 567, Valdivia, Chile.

[3]Facultad de Ciencias, Instituto de Ciencias Ambientales y Evolutivas, Universidad Austral de Chile, Valdivia, Chile.

1. Introduction

The ability of regrowth is a trait found in a wide range of ecosystems and lineages, suggesting that it is an ancestral trait (Wells 1969, Del Tredici 2001, Bond & Migdley 2003 Vesk & Westoby 2004). The outbreak is considered a key character in the dynamics of communities subjected to recurrent disturbances (e.g. Clarke et al. 2013). However, there are several mechanisms underlying the outbreak of regrowth: (1) asexual regeneration mechanism (when sexual regeneration is limiting), (2) vegetative colonization of space (when resources are heterogeneously distributed in space), (3) increased storage reserves (when resources are limiting) and (4) regeneration of the biomass after disturbance. While the first mechanism involves vascular separation from the mother plant, the remaining regrowth mechanisms require genet remain connected (e.g. Groenendael et al. 1996).

Most of the studies on the ecophysiology of regrowth in ecosystems have been conducted in subjects with recurrent severe disturbances, where much of the aboveground biomass is removed and regenerated from the substance of reserves (i.e. carbohydrates) stored in underground organs protected from disturbance. In these studies, it was determined that the absorption capacity of the sprouts is substantially higher than the undisturbed adult plants (e.g. Goorman et al. 2011). The differences in the ratio biomass / underground, appears to be the mechanism underlying these differences, by an increase in hydraulic conductivity and therefore stomatal conductance (Kruger & Reich 1993). However, to our knowledge, there are no similar studies in volunteers not associated with severe disturbances (i.e. the adult individual's biomass is not completely eliminated). In these cases, there is a change in the ratio biomass / ground, and therefore, it is expected that there are changes in the absorption. Although one study shows some differences at the level of photosynthetic capacity between root suckers vs. seedlings of *Fagus grandifolia* (Farahat & Lechowicz 2013), the results are confusing and preliminary. Despite the profuse regrowth phenomenon in the temperate rain forest (González et al. 2002, Gutiérrez et al. 2008), there are no studies to determine the ecological role of this putative reproductive strategy in regenerative dynamics of these systems. The other related architectural strategies for light capture and photosynthetic activity have not been studied in depth either. The temperate rainforest of Chile is mainly composed of evergreen species. *Eucryphia cordifolia* Cav., one species in this forest, lies between latitudes 37-41° S. It is an evergreen shade semi tolerant species (Lusk et al. 2008), which shows characteristics with respect to plasticity in leaves, self-shading, internodes which depend on environmental conditions. It usually grows near the edge of the forest, but it can also grow in the shade and full sun (Escobar et al. 2006). Seedling establishment in full sun is limited by light and humidity (Escandón et al. 2013, Chapter I). This species shows a remarkable capacity for regrowth, especially in spaces generated after small perturbations (González et al. 2002, Gutiérrez et al. 2008), although regrowth has been observed in undisturbed places. The ability to regenerate from seed occurs preferentially in areas with lower canopy opening the volunteers (Escandón et al. 2013, Chapter I). However, there are few studies which compare photosynthetic architectural attributes and between volunteers and young individuals. The aim of this work is to study the morpho-architectural features and underlying carbon assimilation associated to regrowth vegetative colonization of space. Thus, to assess whether the sprouts originated in the absence of severe disturbance show assimilation rates higher than undisturbed plants, as it has been reported for sprouts arising after the complete removal of aboveground biomass, we compare these features between root sprouts and seedlings of *E. cordifolia* of similar size and age. The restricted range of ages and sizes used in this study minimizes the effect of ontogeny.

2. Material and methods

2.1 Study site

This study was conducted towards the end of growth season in Katalapi Park (41°31′8″ S, 72°45′2″ W, elevation ca. 90 m a.s.l.), within of ca. 35 years old even-age temperate rainforest. The park has been protected from anthropogenic alterations for the last ca. 20 years and therefore, sexual regeneration of sun and shade tree species is frequent. The plant growth activity is of 8.3 month (di Castri & Hajek, 1976). This area presents a maritime temperate climate, with annual rainfall of ca. 1900 mm concentrated between April to November (Escandón et al. 2013, Chapter I) and a mild dry season during December to March. In this period, the mean air temperature reach 15º C, the lowest air relative humidity range between 45-55% and a 15-days-long dry period frequently occurs within each summer. The photosynthetic photon flux density (PPFD) can reach a maximum of 2200 at 35% of canopy openness (CO). Climatic details for the study area are shown in Coopman et al. (2010) and Escandón et al. (2013, Chapter I). A plot of 50 x 50m was delimited, in which one was able to found sprouts and saplings.

Fig 1. Left: Katalapi Park, yellow line representing the limits. Red circle indicate the site where the study was conducted. Right: Study site within the forest.

2.2 Plant material

Sprouts and saplings were found and labeled corresponding. Height, growth of the season, and relative chlorophyll content (SPAD) were measured. Specifically, SPAD measures were conducted over three leaves of each individual plant and averaged. Age estimation was conducted according to growth scars. Finally, a maximum of 43 sprouts and 26 saplings were considered for following analyses.

2.3 Light environment characterization

A hemispherical photograph was taken under diffuse light and homogenous sky conditions (cloudy days) to minimize variations due to exposure and contrast (Valladares 2005, Valladares et al. 2012). An individual photo above the apex was considered. For that, a Nikon Coolpix 4500 digital camera (Nikon Corporation, Japan) equipped with a Nikon FC-E8 (182º) fish eye was used. This camera was manually leveled and compass oriented considering that the top of the picture corresponds with the magnetic north. The processing of colored image files to determine light characteristics corresponding to each photo was done with Gap Light Analyzer (GLA) software (Frazer et al. 1999). It is well known that the hemispherical photography is adequate and accurate, especially in heterogeneous canopies and gaps abundances (Machado & Reich 1999, Bellow & Nair 2003). The outputs were obtained from canopy structure and transmitted gap light calculations (Frazer et al. 1999). The photo-data were fractions of gaps from 160 sky sectors (36 azimuth and 9 zenith regions) used to calculate the incident radiation from all directions by the standard algorithm of overcast sky (Steven & Unsworth 1980). The amounts of total, direct and diffuse incident irradiance on a tilted or horizontal surface when there is blockage of light from the overlying forest canopy or topography were calculated for each sprout and sapling.

2.4 Instantaneous measurements and daily crown estimation of gas exchange

Photosynthetic activity was measured with an IRGA Li-6400 (LiCor, Inc., Lincoln, NE, USA) during the morning (9.00 to 13.00), where the maximum gas exchange activity is reached in this species (Morales et al., manuscript in preparation). Photosynthetic light response curves (A-Q) were performed over a one year-old leaf fully expanded at 10 different light intensities between 0 and 1000 µmol m^{-2} s^{-1}, [CO_2] of 400 mol mol^{-1}, and relative humidity of 65% ± 5. To stimulate the photosynthetic activity, plants were subjected to a red (85%), blue (10%), orange (2%), and yellow (3%) light supply with a led lamp. Dark respiration (R_d) was measured after 60 minutes of leaf obscured in the same leaf where A-Q was recorded. For this latter, light condition was 0 µmol $m^{-2}s^{-1}$, and the cuvette environmental conditions were the same that for A-Q. Leaf optical properties (transmittance, reflectance and absorptance) were measured with the light source of IRGA cuvette using a spectroradiometer (HR2000CG-UV-NIR; Ocean Optics Inc., Dunedin, USA), following Gago et al. (2013). Given the low photosynthetic rates of these species and the small area (2 cm^2) of the leaf cuvette, the flow rate was adjusted to ensure that CO_2 differentials between the reference and the sample IRGAs were > 4 µmol mol^{-1} air. Light compensation (LCP) and saturation (LSP) points were calculated with the Photosynthesis tool software (Li-Cor Application Note #105).

In order to approach from leaf to daily crown assimilation (DCA) and respiration (DCRd) level, A-Q of sprouts and saplings was fitted using CurveExpert Professional V1.5 (http://www.curveexpert.net/, USA). The resulting equation was used for determinations of assimilation rates (A_N) at sunny and cloudy midday photon flux density averaged (PPFDm; 11.00 – 13.00 h). PPFDm was determined from data of 5% of canopy openness (CO, the closer for CO of sprouts and saplings, 4.4% and 6.7%, respectively) registered with HOBO micro stations at the field for both sunny and cloudy days of summer season (for climatic details, see Escandón et al. 2013, Chapter I). To achieve that, data of midday 100% CO was used to identified sunny and cloudy days corresponding to PPFDm higher than 1000 µmol m^{-2} s^{-1} and lower than 300 µmol m^{-}

^{2} s^{-1}, respectively. The resulting A_N was multiplied by leaf area (m^2) of the plants (for this latter see section 2.6), and 12 hours (in seconds). For daily crown respiration (DCRd), we considered the sum of R_d and respiration in the light (R_l), which were calculated by 12 hours following a similar protocol used for DCA. R_l was calculated as 0.5 times R_d (Gallé et al. 2011). Carbon balance for both sunny and cloudy days was estimated by simple subtraction between DCA and DCRd.

Fig. 2. *E. cordifolia* plant photosynthetically stimulated with a led lamp (see above) at the field before the gas exchange measurement. Obscured leaf with aluminum paper for R_d measurement (right).

Fig. 3. *A*-*Q* curves measured in the field with IRGA Li-6400.

2.5 Plant architecture capture

Each plant was leaf-north marked, excavated removed carefully from the field in a living soil of sufficient width and depth to include the root system. Sprouts and saplings were put immediately in containers and watered at field capacity. After this, plants were carried quickly to a field closed laboratory and digitized in order to keep the architectural trait, especially for sprouts. Plant crown architecture was obtained from 3D FASTRACK® (Polhemus, Colchester, VT, USA) and FLORADIG (CSIRO Entomology, Brisbane, Australia) software. The digitazing system involves a magnetic signal receptor and pointer, which allows to record spatial coordinates (*x*, *y*, *z*) of a whole-plant within three meters of a magnetic hemispher diameter. The digitation was conducted on 29 sprouts and 26 saplings. In addition, basal and apical stem and peciolus diameter were measured and used for plant architecture modelling. A leaf file was created in a flat surface over cartesian coordinates considering at least 25 points of the leaf border. Virtual plants (Fig. 4) were created from R software (R Development Core Team 2010), package YplantQMC (Duursma & Cieslak 2012), which one uses the same "plant" and "leaf" files that YPLANT (Pearcy & Yang 1996).

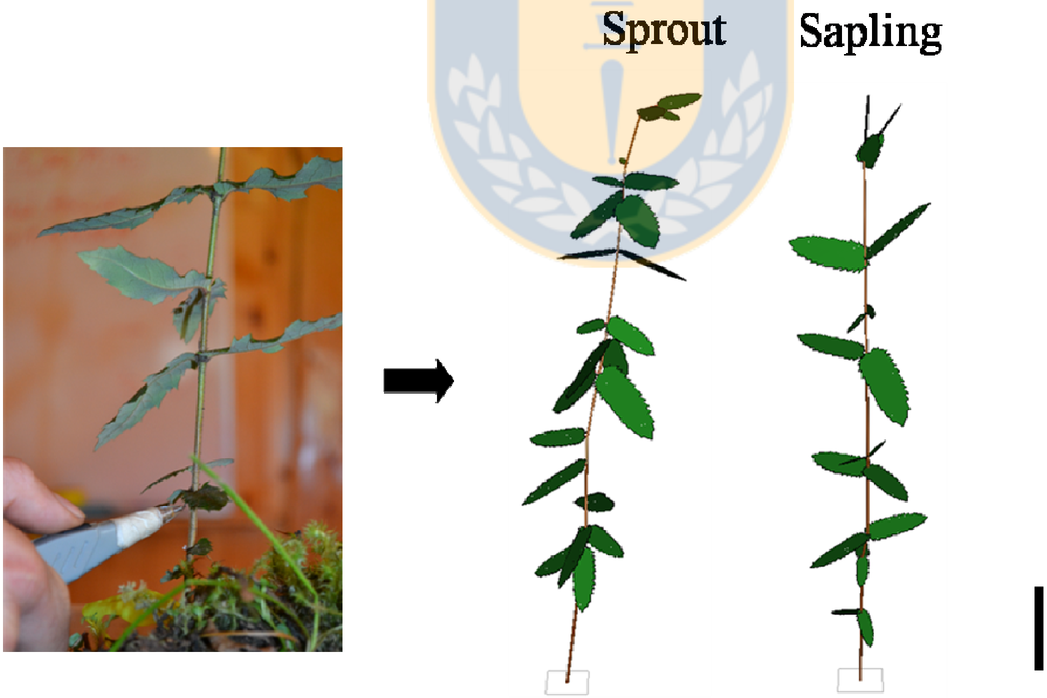


Fig. 4. Digitizing process (left). Resulting sprout (mid) and sapling (right) plant after virtual construction with Yplant-QMC. Black scale at right = 10cm.

2.6 Biomass allocation

After digitations, leaves were removed and scanned in order to obtain leaf area. Leaf area was determined from each leaf-group of images using the software ImageJ 1.47q software. Roots were cleaned and washed before drying. Leaves, stems, and roots were separated and kept in paper bags and dried in a forced air oven for 72 hours at 60º C. Specific leaf area (SLA), Leaf mass area (LMA), leaf area ratio (LAR), and leaf to stem mass (LSM) were determined. SLA was calculated dividing leaf area by leaf dry mass. LMA was calculated dividing leaf dry mass by leaf area. LAR is the relation of leaf area and total plant weight. LSM is the ratio between leaf and stem dry mass.

2.7 Statistical analyses

In order to reach the statistical precepts, light environment (total, direct and diffuse light), leaf area, leaf area ratio (LAR), specific leaf area (SLA), daily crown assimilation (DCA-sunny and cloudy), and carbon balance (for sunny and cloudy days) were Log-transformed, while the chlorophyll concentration was quadratic-transformed. One-way ANOVA (Duncan test $P < 0.05$) was conducted over all the variables measured in order to determine differences between recruit type respects. From virtual plants, STAR, SS, mean leaf angle weighted by leaf area, and crown density (AL/AC) were analyzed. From leaf optical properties transmittance, reflectance, and absorptance were calculated and analyzed subjected. Canopy openness and daily crown respiration (DCRd) were not normalized and finally were assessed for differences with Kruskal-Wallis test ($P < 0.05$). Because of the age estimated (mean ± S.D.) of sprouts (2.9 ± 0.7) and saplings (2.8 ± 0.7) were not statistically different ($P = 0.88$), they were not used as co-variables. Statistical analyses were carried out with STATISTICA7 (StatSoft).

3. Results

3.1 Light availability

Saplings received significantly more diffuse and total light than sprouts. There was no difference for direct light irradiance between them (Table 1). Diffuse light for saplings was almost two folds than for sprouts. There were no differences with respect to direct light irradiance, despite the higher canopy openness of saplings than sprouts.

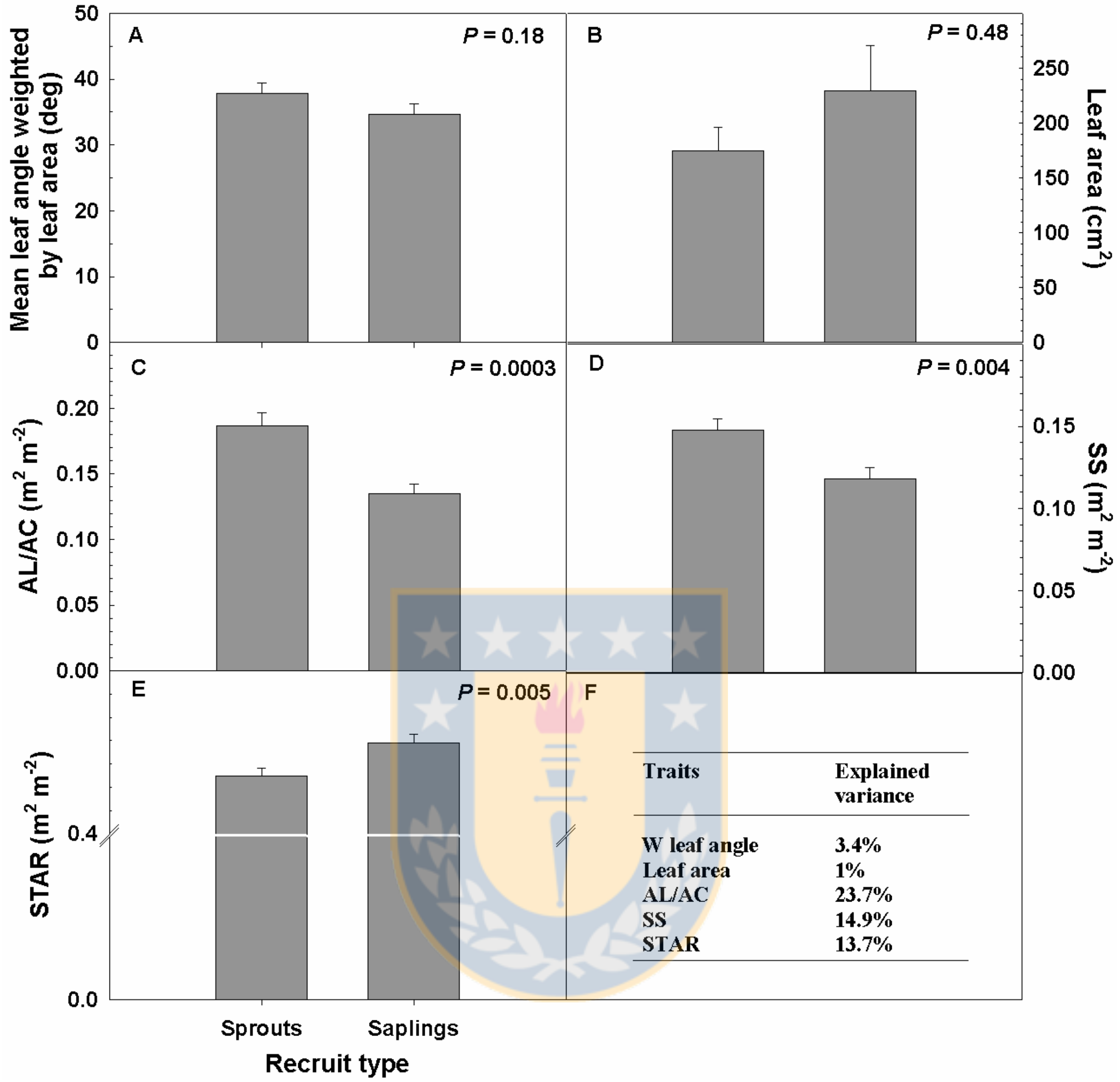
Table 1. Light availability measured on sprouts and saplings of *E. cordifolia*. Canopy openness, total, direct, and diffuse light. *P*-values of ANOVAs and Kruskal-Wallis (*) are shown comparing each variable between sprouts and saplings. Mean ± S.D. are showed. Sprouts n = 42; saplings n = 30.

	Sprouts	**Saplings**	***P*-value**
Canopy openness (%)*	4.4 ± 2.95	6.7 ± 3.26	0.001
Total light (mol photons m^{-2} day^{-1})	2.05 ± 2.04	2.87 ± 1.55	0.006
Direct light(mol photons m^{-2} day^{-1})	0.99 ± 1.23	0.93 ± 0.80	0.47
Diffuse light (mol photons m^{-2} day^{-1})	1.06 ± 0.85	1.94 ± 1.06	<0.001

3.2 Architectural traits and biomass distribution

Mean leaf angle weighted by leaf area (α) and leaf area did not show statistical differences (Fig 5A and B). However, saplings showed in average a lower α and higher leaf area than sprouts. The components of crown architecture, crown density (AL/AC), self-shading (SS) and the index of light interception efficiency (STAR) differed statistically between both sprouts and saplings (Fig. 5C, D, E, respectively). AL/AC and SS were higher in 27% and 19% for sprouts than saplings. Meanwhile, the STAR was lower in 4.6% for sprouts compared with saplings. The explained variance was a range of 13.7 to 23.7% for these three variables (see insert table, Fig. 5F).

The biomass distribution of sprouts and saplings did not show differences between total aerial biomass, but it did differ in their distribution. Leaf area ratio was not significantly different between recruits. On the other hand, sprouts allocated higher biomass to leaves (30.8%, stem related; LSM) than saplings. Specific leaf area (SLA) was lower in sprouts than saplings in 14.6%. Both LMA (total and expanded leaf cohort) were higher in 13% for sprouts than saplings (Table 2).



Traits	Explained variance
W leaf angle	3.4%
Leaf area	1%
AL/AC	23.7%
SS	14.9%
STAR	13.7%

Fig. 5. Architectural traits of *E. cordifolia* recruit types (sprouts and saplings) growing at field condition. *P*-values of each variable are shown. Explained variances are in the right inferior table. Weighted mean leaf angle (A), leaf area (B), crown density (AL/AC) (C), self shading (SS) (D), light interception efficiency (STAR) (E), and explained variance (F) are shown. Mean ± S.E. are showed. Sprouts *n* = 29; saplings *n* = 26.

Table 2. Leaf area ratio (LAR), leaf to stem mass (LSM), specific leaf area (SLA), leaf mass area (LMA), and chlorophyll density of sprouts and saplings of *E. cordifolia*. *P*-values of ANOVAs are showed comparing each variable between sprouts and saplings. Mean ± S.D. are showed. Sprouts *n* = 29; saplings *n* = 26.

	Sprouts	**Saplings**	***P*-value**
LAR (only aerial biomass; cm^2 g^{-1})	91.94 ± 24.75	93.66 ± 29.13	0.85
LSM (g g^{-1})	1.72 ± 0.80	1.19 ± 0.54	0.02
SLA (cm^2 g^{-1})	142.9 ± 3.6	167.4 ± 5.8	<0.001
LMA (all cohorts; g m^{-2})	67.04 ± 12.46	58.20 ± 10.63	0.007
LMA (expanded cohorts; g m^{-2})	71.35 ± 10.14	61.55 ± 10.97	0.001
Chlorophyll density (SPAD units)	36.17 ± 6.03	46.21 ± 4.48	<0.001

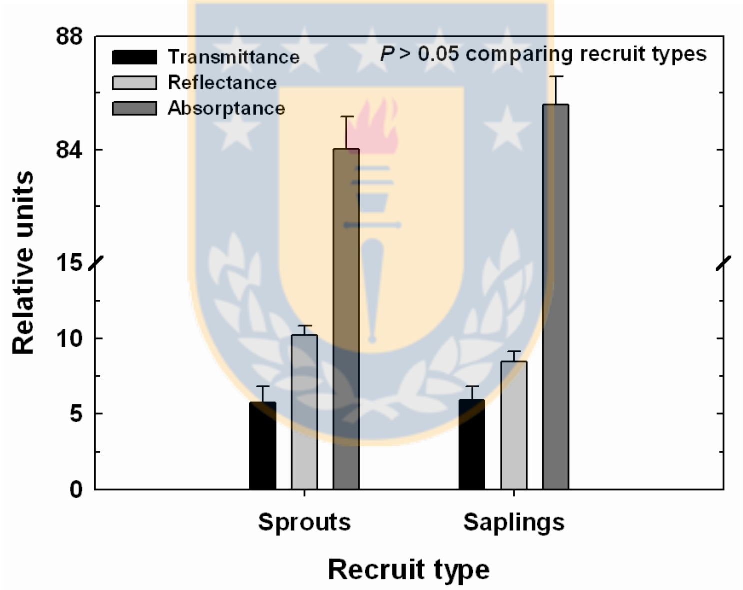


Fig. 6. Leaf transmittance, reflectance, and absorptance of *E. cordifolia* recruit types (sprouts and saplings) growing at field condition. Note that a break is showed in the *y* axis. Mean ± S.E. are showed. Sprouts *n* = 12; saplings *n* = 11. No differences between sprouts and saplings were found.

3.3 Photosynthetic apparatus functioning

Chlorophyll density was significant lower in sprouts (21.7%) than saplings (Table 2). This difference did not influence the leaf optical properties (Fig. 6), where not significant differences were found for transmittance, reflectance, and absorptance (*P* values are: 0.91; 0.068; and 0.32, respectively). In spite of that, saplings showed a higher absorptance and lower reflectance than sprouts, while the reflectance remained almost constant (Fig. 6).

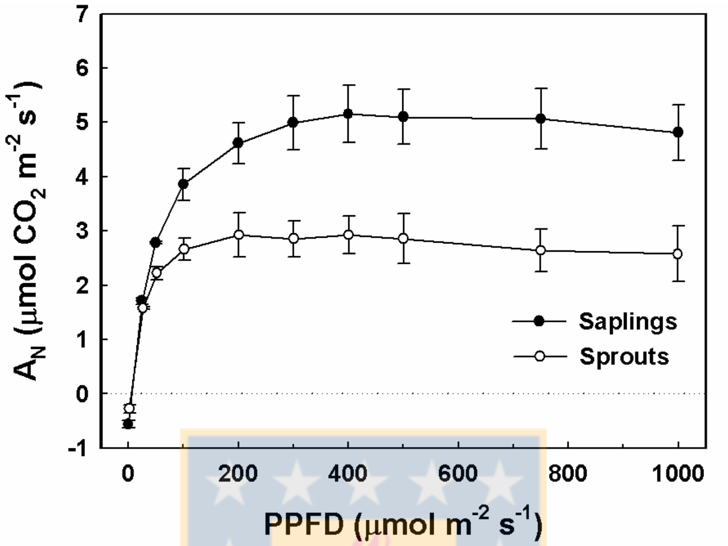


Fig. 7. Light response curve of *E. cordifolia* recruit types (sprouts and saplings) growing at field conditions. Plant canopy openness was 4.3% ± 0.45 and 6.6 ± 0.6% CO (mean ± S.E.) for sprouts and saplings, respectively. Dotted line is A_N zero. For both recruit types, *n* = 3.

Net assimilation of CO_2 in response to light intensity showed a lower maximum in sprouts than saplings in 42.5% (Fig. 7). The analysis of the light response curve determined that sprouts had a significant lower LCP (49%) and LSP (55.6%) than saplings.

The scaling up from leaf to daily crown assimilation (DCA), using the light response of both sprouts and saplings, and the PPPFm averaged for sunny and cloudy days during summer time, showed not differences between recruits for DCA-sunny neither for his carbon balance (Table 3). Regardless, sprouts had a lower DCA-sunny and carbon balance (ca. 28.6%) than saplings. Nevertheless, cloudy days showed a significant negative impact on saplings DCA and carbon balance. The DCA /DCRd ratio between sprouts and saplings showed almost the same relation of CO_2 assimilated to CO_2 respired for sunny days. Meanwhile, this ratio was ca. two folds higher for sprouts than for saplings in cloudy days.

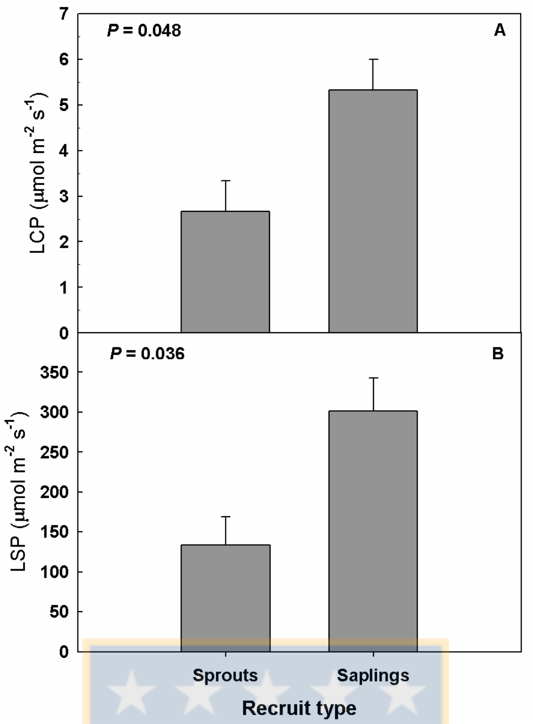


Fig. 8. Light compensation (A) and saturation (B) points of *E. cordifolia* recruit types (sprouts and saplings) growing at field condition. Mean ± S.E. are showed. *P*-values are showed. *n* = 3.

Table 3. Daily crown assimilation (DCA) and respiration (DCRd), carbon balance (Cbal), and absolute ratio DCA/DCRd estimation for sunny and cloudy days. Means ± S.E. are showed. DCRd was subjected to Kruskal-Wallis test (*P* < 0.05) for comparisons (*). Sprouts *n* = 29; saplings *n* = 26.

	Sprouts	**Saplings**	***P*-value**
DCA-sunny (mmol CO_2 plant^{-1} day^{-1})	1.69 ± 0.2	2.37 ± 0.36	0.13
DCA-cloudy (mmol CO_2 plant^{-1} day^{-1})	1.02 ± 0.12	0.72 ± 0.11	0.038
DCRd (mmol CO_2 plant^{-1} day^{-1})*	-0.1 ± 0.01	-0.13 ± 0.02	0.18
Cbal-sunny (mmol CO_2 plant^{-1} day^{-1})	1.53 ± 0.19	2.24 ± 0.34	0.13
Cbal-cloudy (mmol CO_2 plant^{-1} day^{-1})	0.92 ± 0.11	0.59 ± 0.09	0.01
DCA-sunny/DCRd ratio	18.41	18.36	
DCA-cloudy/DCRd ratio	10.63	5.54	

4. Discussion

Previous studies have shown a faster growth and use of the space by *E. cordifolia* sprouts ten years after of *Chusquea quila* dieback, concomitantly with a high mortality of saplings in the coastal temperate rain forest of southern Chile (e.g. Muñoz & González, 2009). However, in this latter forest regenerative study, light environment, architectural traits and photosynthetic apparatus functioning details of sprouts *vs.* saplings were not considered, as in many others (e.g. Donoso et al. 1985, Gonzalez et al. 2002). Besides, it has been demonstrated that sprouts inhabit preferentially in sites with higher light availability than saplings. Nevertheless, they also share some light recruitment sites within the secondary rain forest (Escandón et al. 2013, Chapter I). In this study, sprouts and saplings were found in very similar canopy openness (CO), which showed a 2.2% of difference between the both recruit types. This difference was enough to differentiate the amount of daily total and diffuse light irradiance between them. But, it was not the case with direct light irradiance (Table 1). Therefore, It is demonstrated that the increment of the CO in understory did not necessarily entail an increment in direct light irradiance. The small magnitude of change of CO in the understory showed a higher contribution to diffuse light availability, which influences directly the difference of total light irradiance for plants in the understory. Under shade, diffuse light is considered an important input of photosynthetic flux density, because it is coming from the all blue hemisphere and penetrates the canopy uniformly from all directions (Parent & Messier 1996). Thus, the low light availability could drive accordingly architectural, morphological, and photosynthetic traits in tree species (Pearcy & Valladares 2004, Pearcy et al. 2005, Valladares et al. 2012). However, these light availability conditions did not determine some of the architectural traits for sprouts (Fig. 5) neither morpho-anatomical ones (Table 2). Crown density (AL/AC) was higher in sprouts than saplings, involving a higher self-shading (SS) and, consequently, lower light interception efficiency (STAR). This latter architectural response to low light availability is opposite to that expected for plants growing under this light condition, and especially for plants of the same species, supposing a global pattern in response to light regardless the plant genesis. Even, taking into account just saplings randomly distributed in Katalapi Park under CO lower than 20%, neither STAR nor SS were affected by CO (r = -0.03, P > 0.05; r = 0.05, P > 0.05, respectively; Fig. A1). This, in part determines a low plasticity of this both architectural traits to light availability considering the change of PPFD at CO higher than 10% (Escandón et al. 2013, Chapter I). Compared to sprouts, saplings showed a better response under diminished light availability. This is why saplings had a lower AL/AC and SS, and a higher STAR than sprouts, which agrees with most literature reports for temperate forest (Flaster & Westoby 2003, Delagrange et al. 2006). Thus, our results suggest that sprouts differ in the strategy for light interception and, may be, for light capture. This could be explained by 1) the parental subsidy received by sprouts and/or 2) a different functional role of the sprouts related with his parent plant (Farahat & Lechowicz 2013, Escandón et al. 2013, Chapter I).

Light is considered the most important determinant of variation in leaf traits (i.e. SLA, LMA, LAR) (Poorter 1999). Our results show that saplings SLA was higher and LMA was lower than in sprouts. Some reports suggest that a change in SLA allows a change in STAR in the same direction, which is an important trait for light interception in *E. cordifolia* saplings showed herein (Schieving & Poorter 1999, Lusk et al. 2011, also see Fig. A1). Consistently, the lower SLA of sprouts decreases their STAR. Moreover, the higher SLA under shaded environments allows an improvement of the probability for light interception at the same weight of biomass. Then, a likely increment in light capture and carbon fixation could be possible. The rate of change of morphological traits (i.e. SLA and LMF) would affect relative growth rate (RGR) in the same way of the rate of change of morphological traits, if the other components that determine RGR are kept constant (Poorter & Nagel 2000). It has been reported that *E. cordifolia* saplings do not have enhanced light interception efficiency under shade during early stages of the ontogenetic development (Lusk et al. 2011). Leaf mass area (LMA) was higher in sprouts than saplings, which agrees with values reported by Farahat and Lechowicz (2013), who compared root sprouts and seedlings of *Fagus grandifolia*. Moreover, LMA of sprouts was lower than high-lighted and higher than shaded plants of *E. cordifolia* grown in a nursery (e.g. Morales et al., manuscript in preparation). This is also contrary to that found in the field for several shade-and mid tolerant species of the temperate rain forest (Lusk 2002; considering the inverse of specific leaf area as LMA). These morphological differences could be a consequence of the higher investment in leaf mass than for stem mass (Table 2), independently of the higher investment of saplings in stems. Leaf chlorophyll density was significant lower for sprouts than saplings under low light availability, which have a remarkable difference with that shown in other studies (Givnish 1988, Farahat & Lechowicz 2013). This suggests that sprouts leaf nitrogen investment was likely for enzymatic components than for the increment of light capture. In this sense, the previous analyses suggest that architecture and leaf morphology of sprouts had sunny plants characteristics and that could be driven by some subsidy from the parent plant.

The photosynthetic capacity in response to light was relatively lower for sprouts than saplings. This difference is widely reported for shaded compared to sunny plants (Givnish 1988) and also for temperate rain forest species (Lusk 2002). This lower photosynthetic capacity of sprouts could be attributed to the lower light saturation point (LSP) than in saplings. Concomitantly with this, sprouts showed a lower light compensation point (LCP) than saplings. Therefore, sprouts had a lower light requirement for reaching their maximum assimilation rate of CO_2 and to compensate their leaf respiration, compared to saplings. Nevertheless, the lower chlorophyll density and photosynthetic capacity observed in sprouts likely means that the nitrogen investment is not allocated to enzymatic machinery neither to chlorophyll. Hence, the photosynthetic apparatus of sprouts had an independent response to light. Sprouts also had an architectural and morphological poor response to light availability, suggesting a lower cost of maintenance than in saplings. In this case, sprouts and saplings of *E. cordifolia* showed a significantly different photosynthetic response to light than the sprouts *vs*. seedlings studied by Farahat & Lechowicz (2013). However, Kauppi et al. (2001) reported that stump sprouts of *Betula pubescens* and *B. pendula* showed a better photosynthetic capacity during the first growing season than seedlings. They attribute these differences to the differences observed in mesophyll structure and the nitrogen and chlorophyll content. Here, the differentiated photosynthetic capacity could be determinant for carbon balance, especially in saplings that, indeed, do not have a parental subsidy. Our estimation of daily carbon assimilation (DCA), respiration (DCRd), carbon balance (Cbal), and the ratio between DCA/DCRd suggest that saplings could be more negatively affected during cloudy days than sprouts (Table 3). This could be explained by the slightly higher maintenance cost of saplings (Table 1). This estimation just considered summer time. The carbon balance of saplings along the rest of the year is unknown. Winter assimilation and respiration rates of CO_2 were measured. As results, mean assimilation rates at PPDF 400 µmol m^{-2} s^{-1} was negative for seedlings growing at canopy openness (CO) lower than 6%, and slightly positive for >6% CO (Fig. A2). Meanwhile, the mean respiration rates at <6% CO were higher than the respiration showed under light condition, and for saplings at >6% CO lower than saplings at <6% CO (Fig. A3). However, the sapling carbon balances at >6% CO could be slightly positive (Fig. A2 and Fig. A3). Perhaps, saplings of *E. cordifolia* will have a negative carbon balance during the year, mainly because the decreasing proportion of sunny days and the increment of cloudy ones. However, it has been suggested that saplings had a high mortality due to the environmental conditions, specifically related to the PPFD incident and water demand (vapour pressure deficit; Escandón et al. 2013, Chapter I). However, carbon balance and climatic environmental conditions could be related and together perhaps explain the high mortality reported for saplings of *E. cordifolia*.

With this study we provide new knowledge about the differences between sprouts and saplings of *E. cordifolia* in terms of light interception, leaf morphology, and photosynthetic apparatus functioning. This is the first time that the leaf optical properties components are showed for both sprouts and saplings of one temperate rain forest species. The results here exposed also allow us to say that field works with *E. cordifolia* deserve an especial consideration about the origin of plan material used for analyses of shade-tolerance and forest dynamics.

5. Conclusions

Despite that saplings inhabit sites with slightly high light availability than sprouts (this Chapter), they showed architectural, morphological, and biochemical traits that potentially increase light interception efficiency at leaf level, and, therefore, could drive a higher daily crown assimilation for sunny days. This fits with the maximum rate of carbon assimilation, light compensation, and saturation points, with the cost of maintenance.

Sprouts definitely are different from saplings, but this assumption is difficult to observe with the naked eye under field conditions. Also, the differences showed herein that minimize the strategy of light capture and the non significant reduction in carbon assimilation and carbon balance should be explained with a different kind of analysis. These methods should analyze the degree of parental subsidy, or the direction of the flow flux between sprouts and parent. The use of these procedures is essential to determine the functional role of sprouts within the temperate rain forest.

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Appendix B. Supplementary material.

Table B1. Spearman correlations of architectural traits for sprouts (A) and saplings (B) of *E. cordifolia*. Coefficients are showed in columns. Probabilities are showed in rows, above the diagonal values = 1.00.

A

Sprouts	STAR	ALAC	SS	Leaf area
STAR	1	8.50E-05	1.30E-07	0.12
ALAC	-0.74	1	8.90E-05	0.47
SS	-1	0.74	1	0.09
Leaf area	-0.29	-0.14	0.32	1

B

Saplings	STAR	ALAC	SS	Leaf area
STAR	1	6.50E-06	0	0.01
ALAC	-0.76	1	9.30E-05	0.74
SS	-0.99	0.78	1	0.01
Leaf area	-0.48	0.07	0.51	1

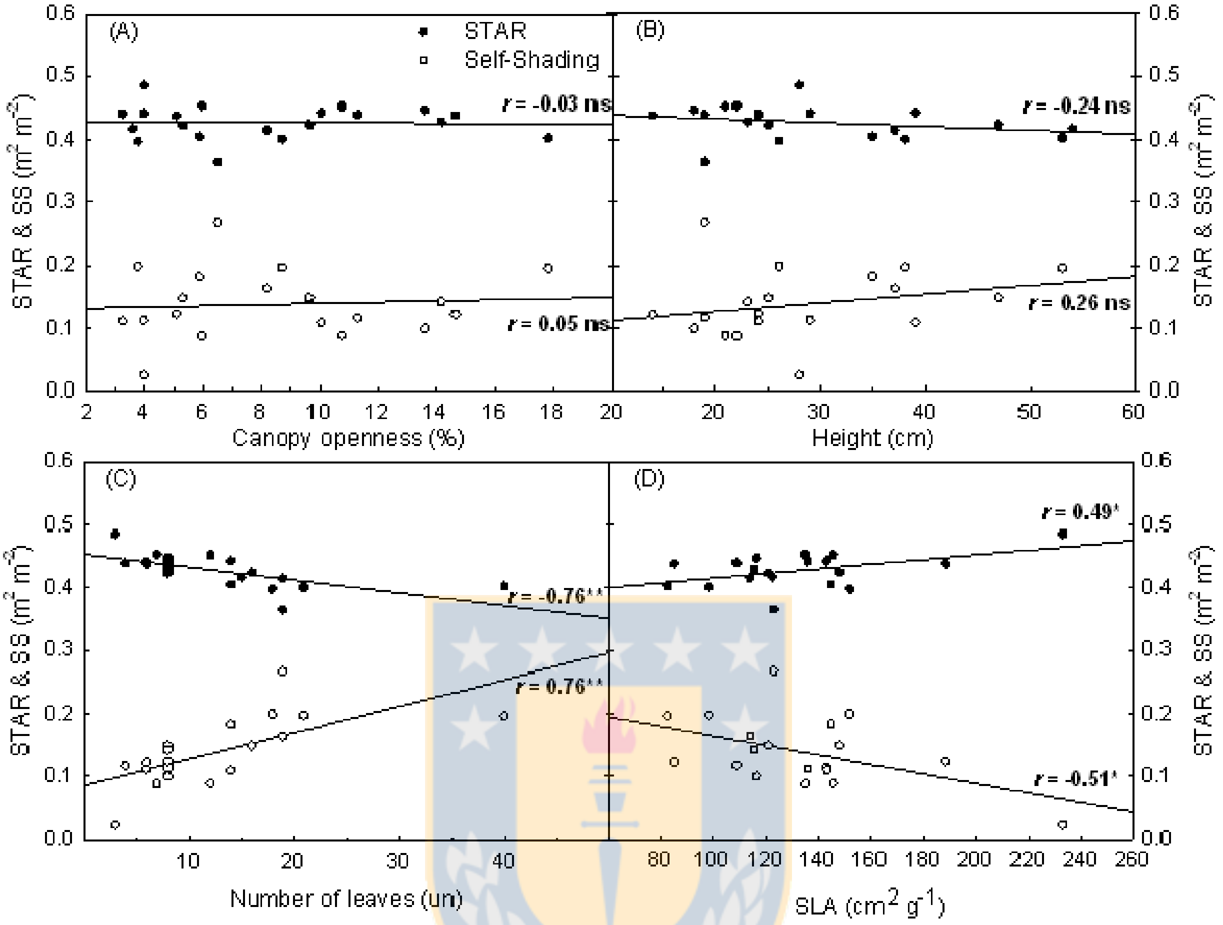

Fig. B1: STAR (filled circles) and Self-shading fraction (SS) (empty circles) of *E. cordifolia* saplings correlations with CO (A), height (B), number of leaves (C) and SLA (D). Pearson's correlation significance is as follows: **0.001<*P*<0.01; *0.01<*P*<0.05; ns *P*>0.05.

Gas exchange measurements: Measurements for seedlings were performed in July 2011 in both leaf cohorts (LC) younger (LC1; fully expanded) and older (LC2) leaves were clamped into the cuvette of an open gas-exchange system with the Li-6400 led source (Li-Cor Inc., Nebraska, USA). Given the low photosynthetic rates of these species and the small area (2 cm^2) of the leaf cuvette, the flow rate was adjusted to ensure that CO_2 differentials between the reference and the sample IRGAs were > 4 µmol mol^{-1} air.

Maximum assimilation (A_N) and dark respiration (R_d) rates were performed at a CO_2 concentration (C_a) of 400 µmol mol^{-1}. A_N consisted in 4 to six measures per seedling at 400 µmol m^{-2} s^{-1}. The Li-6400 led source with a 90% red and a 10% blue light. R_d were measured after darkening the plants for at least 30 min on the same leaf where A_N was measured.

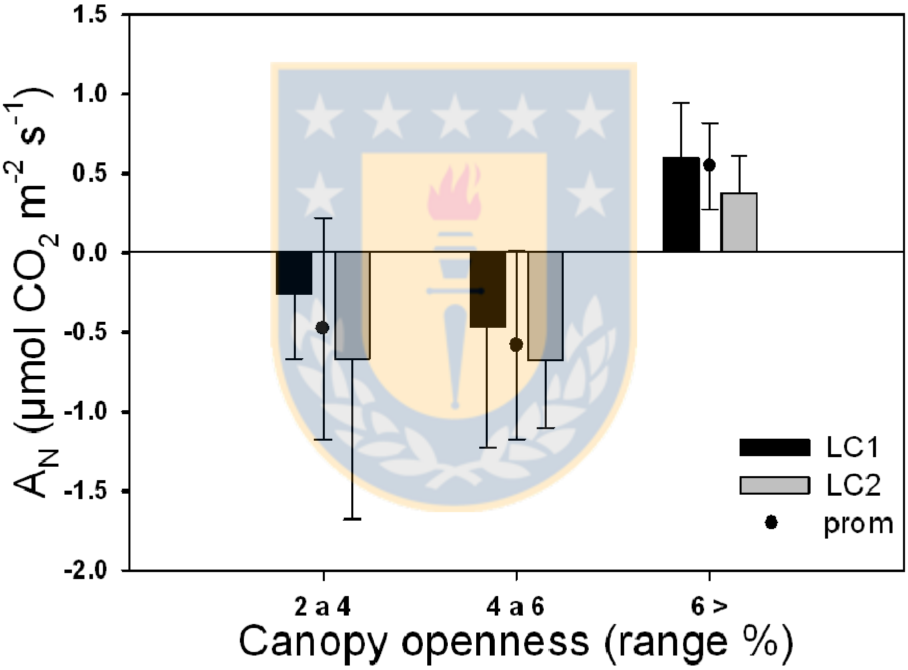

Fig. B2: Net assimilation rate of two leaf cohorts (LC) of *E. cordifolia* seedlings at different canopy openness (CO). LC1 is the older and PC2 the newest leaf cohort. Average between LCs for the three different ranges of CO are showed (filled circles). *n*=19.

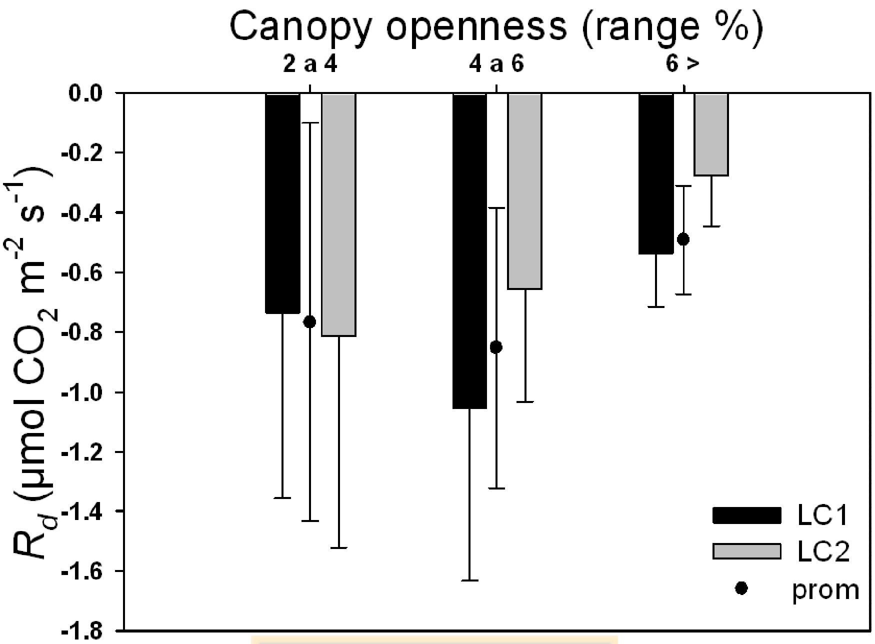

Fig. B3: Dark respiration rate of two leaf cohorts (LC) of *E. cordifolia* seedlings at different canopy openness (CO). LC1 is the older and PC2 the newest leaf cohort. Average between LCs for the three different ranges of CO are showed (filled circles). *n*=19.

Chapter III

Crown photosynthetic limitations of *Eucryphia cordifolia* Cav. plants displayed under different light and water availabilities

Escandón A.B.[2 +], Coopman R.E.[1*+], Rojas R.[1], Morales L.V.[1], Gago, J.[3], Flexas J.[3], Galmés J.[3], Corcuera L.J. [2]

[1]Forest Ecophysiology Laboratory, Conservation, Biodiversity and Territory Institute, Universidad Austral de Chile, Casilla 567, Valdivia, Chile.

[2]Laboratorio de Fisiología Vegetal, Facultad de Ciencias Naturales y Oceanográficas, Universidad de Concepción, Casilla 160-C, Concepción, Chile.

[3]Grup de Recerca en Biologia de les Plantes en Condicions Mediterrànies, Departament de Biologia, Universitat de les Illes Balears, cta. Valldemossa km. 7.5, 07122, Palma, Spain.

1. Introduction

Plants depend on the availability of heterogeneous resources during their development and life cycle in natural environments. The availability of water in the soil is affected by both soil drainage capacity and yearly oscillation of rainfall (Davidson et al. 1998, Wilson et al. 2000, Chaves & Oliveira 2004, Fisher et al. 2007). Concomitantly with the seasonal decline in soil water during the plant growing season, foliar deployment and plant growth occur throughout the season. Thus, these two events occur simultaneously. Furthermore, the spatial and temporal heterogeneity of light limits growth and development caused by either excess or deficiency. As a result of the integration of environmental factors, plants are a product of their environmental history. Therefore, the development and architectural arrangement of the crown is modified from the optimum if environmental conditions have changed significantly (Pearcy & Yang 1996, Valladares & Niinemets 2007). Hence, architecture together with the biochemical processes of the plants are modulated as to maximize profits and minimize losses (Givnish 1988, Valladares & Niinemets 2008).

Photosynthesis is a critical process for plant growth. The rate of photosynthetic carbon fixation in a plant may be limited by metabolically suboptimal abiotic factors such as temperature, and availability of water and light (Perchorowicz et al. 1981, Sharkey & Loreto 1993, Bernacchi et al. 2002, Flexas & Medrano 2002). The processes that determine carbon gain (A_N) (i.e. stomatal conductance (g_s), substomatal CO_2 concentration (C_i), electron transport rate (J_{max}), among others) reduce their rates at the leaf level during stress water. However, they may partially or totally recovered when water availability increases at ground level (Souza et al. 2004 Gallé et al. 2009). It has been observed, however, that the maximum speed of carboxylation (V_{cmax}) was not affected during water stress (Gallé et al. 2009). In addition, the variation of the relative growth rate at whole plant level tree species grown under contrasting light environments has been explained mainly by the photosynthetic rate and the proportion of plant leaves than by any other variable alone (Walters et al . 1993). The increased sensitivity of enzymatic allometric variables in juvenile *Picea asperata* was caused by water stress at low light availability (Yang et al. 2008). Therefore, growth and establishment of plants at high light and shade may be limited primarily by the available water.

The limitation of photosynthetic activity has been widely studied under different levels of water availability at the leaf level. These limitations have been quantified through the limitation by g_s, mesophyll conductance (g_m), and biochemical limitations (Grassi & Magnani 2005). The first of these is generally the earliest product of the stomatal closure induced by drought, causing the major limitation of the diffusion of CO_2 from the atmosphere to the interior of the lamina. The second is directly related to the internal resistance to CO_2 diffusion from the substomatal cavity to the site of carboxylation in the chloroplasts (Flexas et al. 2008). Finally, biochemical constraints have been described as the decrease in the capacity of carboxylation (V_{cmax}) and the regeneration of RuBP (Grassi & Magnani 2005, Gallé et al. 2009, Flexas et al. 2009). Specifically, it has been shown that water deficit affects CO_2 diffusion components while light regulates the synthesis of ATP for the regeneration of RuBP, affecting photosynthesis biochemical component (Flexas & Medrano, 2002; Flexas et al. 2002, Grassi & Magnani 2005, Gallé et al. 2009, Flexas et al. 2009). Therefore, sun plants show metabolic rates and differential photosynthetic limitations to maximize carbon gain at the crown level.

Since it is complex integrating several stressors and levels of analysis on plants during an experiment, several studies have applied short-term factors of stress disregarding leaf acclimation and climbed from a leaf level to higher levels (crown, canopy, ecosystem; Bucci et al. 2004, Santiago & Mulkey 2005). Unfortunately, this type of scaling is far from what actually occurs at the crown level. Until now, neither the photosynthetic induction time, nor the analysis and quantification limitations of photosynthesis have been considered (e.g. Lusk et al. 2011). Moreover, it has been shown that the A_N, g_s, V_{cmax} and J_{max} decrease with leaf age, which also vary along the profile of the crown according to the angle of incidence of light (Wilson et al. 2000 Xu & Baldocchi 2003 Kikuzawa & Lechowicz 2006, Allard et al. 2008, Monti et al. 2009, Whitehead et al. 2011). Therefore, the coexistence of leaves of different ages, morpho-anatomy, and structures (given the continued deployment of foliage during the growing season) would mean that different portions of the crown differentially assimilate more or less carbon according to their partial or complete acclimation to light and / or drought, and thus affecting the carbon balance from the crown. The present study shows how the limitations of photosynthesis are modulated at the crown level and what is the contribution of different foliar cohorts deployed under different light and water availability.

2. Materials and methods

2.1 Plant material and growth conditions

One-year-old *E. cordifolia* plants were grown from seeds collected at a Valdivian coastal forest under high light and water availability. Two months before bud sprouting, plants (mean ± SD height = 19.7 ± 0.3 cm) were placed in 5.8 l pots with perlite:peat moss (4:1 v/v) and 3 g/l of Basacote 3M (COMPO, Münster, Germany). Pots were maintained orientated to the magnetic north direction. To achieve this, the magnetic north was fixed with a reference point inside the nurse, and was marked on the pots using a compass. Two light treatments combined with two water availabilities were applied one month before bud sprouting, and were maintained 180 days along the growing season (Oct-Mar 2012). Light treatments were full and 7% of sunlight (hereafter high light: HL and low light: LL) reached below polyethylene greenhouse covering (52% PAR transmittance at plant canopy). Light was attenuated with three layers of neutral shade meshes. During the second week after budbreak midday PPFDs of 5 sunny days (mean ± SD) were 1108 ± 63 and 46 ± 5 µmol m^{-2} s^{-1} reaching integrated quantum fluxes (IQF) of 26.6 ± 4.2 and 1.2 ± 0.2 mol m^{-2} day^{-1} for HL and LL, respectively. These values were selected because they correspond ca. to percentile 10 and 90 of *E. cordifolia* regeneration evaluated in a 30 ha secondary coastal evergreen temperate rain forest located in South Central Chile (Katalapi Park: 41°31′8″ S, 72°45′2″ W) (Escandón et al. 2013, Chapter I). Within each light treatment a block of 80 plants was well watered (WW) and other block was subjected to moderate water stress (WS). Moderate water stress was defined as 65% decrease in g_s respect to WW plants (Flexas & Medrano 2002). g_s was determined daily within the period of its maximum (9:00-13:00 h) in 7 plants per treatment using an IRGA (LI-6400XR, Li-Cor Inc., NE, USA). Evapotranspiration was determined by weighing the pots daily at 18:00 h. WW plants were daily irrigated to field capacity. Hence, water stress intensity was controlled daily as a function of g_s and the restitution of evapotranspirated water to maintain g_s in the range of 70-80 mmol H_2O $m^{-2}s^{-1}$. In order to expand the individual level of water management, the g_s of the remaining plants were daily measured with a SC-1 porometer (Decagon Devices Inc., Washington, USA). The relation between both g_s was fitted by a polynomial regression (curve finder function, Curve Expert Professional software (v.1.2.0, http://www.curveexpert.net/,USA) allowing individual irrigation control using LI-6400XR g_s estimated values.

Air temperature, relative humidity, and PFFD were recorded every 10 s at HL and LL with meteorological stations (H21-002 HOBO, connected to S-LIA-M003 and S-THA-M0xx sensors, Onset, MA, USA). IQF was determined as the sum of PPFD during the photoperiod. With the aim of improving the description of the prevailing environmental conditions during photosynthetic functioning, daily average values of air temperature, relative humidity and vapour pressure deficit (VPD) were calculated within the photoperiod at PPFDs higher than 100 µmol m^{-2}s^{-1}, resulting at the end of the growing season in 21.2º C (±0.6), 63.2% (±3.1), and 1.1 kPa (±0.1) for HL plants, and 19.4º C (±0.5), 68.9% (±2.7), and 0.8 kPa (±0.1) for LL plants, respectively (See also Morales 2013).

E. cordifolia is an evergreen tree species, which displays leaves through all the growth season. This implies that leaves synthesis occurs at different times during the growing season and most likely under different environmental conditions. In order to evaluate differences between leaf display, cohorts, and crown level, measurements were performed in the one year-old leaf cohort (LC1) and in the subsequent two newer leaf cohorts of the same growth season (LC2 and LC3, the oldest one). All determinations were started immediately after each cohort reached full elongation (See below). The growing period during each leaf displays occurred were 20-65 and 49-91for LC2 and LC3 expressed as days after bud brake. Additionally, the above procedure avoids differential ontogenetic effects between treatments, such as foliar senescence and self-shading.

2.2 Plant architecture capture

Measurement protocols required for YPLANT software and the equations of the basic simulation model detailed by (Pearcy & Yang 1996) were followed. Plant crown architecture of *E. cordifolia* plants was mapped and then reconstructed with a FASTRACK digitizer connected to TX4 magnetic field source (Polhemus, Vermont, USA) and driven by the Floradig program developed by Hanan and Wang (2004). The digitazing system involves a magnetic signal receptor and pointer, which allows to record spatial coordinates (*x*, *y*, *z*) of a whole-plant within three meters of a magnetic hemispher diameter. Hence, we created a three-dimensional plant model which reflects the spatial locations of leaves, petioles and stems. Diameters of the stem, branch, and petiole segments were measured with a 0.01 mm digital caliper (Mitutoyo, Tokio, JP). The standardized leaf shape was obtained by scanning 25 points, at a uniform distance on the leaf perimeter of a totally expanded leaf. The size of each digitalized leaf was then scaled using the same standardized shape according to each leaf length. Virtual plants (Fig. 4) were created from R software (R Development Core Team 2010), package YplantQMC (Duursma & Cieslak 2012), which one uses the same "plant" and "leaf" files that YPLANT (Pearcy & Yang 1996). Light capture efficiency averaged over the entire sky hemisphere (STAR), crown density (AL/AC) (expressed as leaf area divided by the area that wrap the crown), mean leaf angle weighted by leaf area, were obtained accordingly to Duursma et al. (2012).

Crown capture was conducted over 14 plants of each treatment (see above) in three times during leaf display determined by days after budbreak (0, 58 and 87 days). Therefore, three leaf cohorts displayed were captured. During the digitizing proccess, each numbered node-leaf was asigned to a corresponding leaf-cohort along the whole-crown for future prospects.

2.3 Architectural and daily crown assimilation modelling

The corresponding leaf-cohort was achieved throughout R-software functions. Thus, each leaf were correctly asigned to a determined cohort. This allow us to separate the whole-crown for the architectural and daily crown assimilation modelling. Architectural traits and daily crown assimilation modelling requieres the same inputs than YPLANT (Pearcy & Yang 1996). This is, leaf and plant file (.l and .p), climatic variables averaged of each digitizing time, geographic location, .CAN file for daily PPFD data obtained from hemispherical photograph, WINPHOT for proccessing data (Tropenbos Foundation, Wagenigen, The Netherlands, ter Steege 1997) (Chazdon & Field, 1987), and photosynthetic parameters obtained from CO_2 concentration leaf response (A-C_i curves, see appendix).

Daily crown assimilation was obtained with Farquhar et al. (1980) model of photosynthesis for C_3 plants, as described by Medlyn et al. (2002), coupled with a few choices of Ball-Berry type stomatal conductance models (Medlyn et al. 2011). To use this model, there are four required parameters: V_{cmax}, J_{max}, R_d, and the slope of the conductance model (see Medlyn et al. 2011); many others optional parameters could be set. In Yplant-QMC package, the Farquhar et al. (1980) model was taken directly from MAESTRA (more information in http://bio.mq.edu.au/maestra/manual.htm).

2.4 Crown quantitative photosyntetic limitations

Quantitative photosynthetic limitations at leaf level were scaled to crown level accordingly with leaf area of virtual plants. To do this, leaf-cohorts were taken separetely and grouped by their corresponding photosynthetic parameters obtained from A-C_i curves. Farquhar et al. (1980) model of photosynthesis and Wilson et al. (2000) equations were used to achieve the quantitative analysis of stomatal (S_L) and non-stomatal (NS_L) limitations. Briefly, The rate of change of assimilation is estimated in function of the stomatal conductance to CO_2 (g_{sc}) and the derivate of the variation of maximum velicity of carboxilation rate (V_{cmax}). For each treatment, the relative sensitivity to stomatal conductance to CO_2 (l_g) was determined. Finally, relative (%) and absolute (mmol CO_2 plant^{-1} day^{-1}) limitations can be obtained for both S_L and NS_L respect to the WWHL plants (control).

2.5 Biomass, shoot elongation and mortality measurements

Biomass was measured at the end of the experiment. Mass of leaves, stems, and roots of the three different cohorts were separated, dried and quantified, using analytical weighing scales. Shoot elongation was measured using a measuring tape on the newer stem growth. Mortality was measured by dichotomy. Cumulative mortality was recorded by range of days between 50 and 200.

2.6 Statistical analyses

Normality and homogeneity of variance were evaluated by Shapiro-Wilk ($P < 0.05$) and Levene ($P < 0.05$) tests. For continuous variables, differences between light and water availability were compared by 2-way ANOVAs and the post hoc Duncan-test ($P < 0.05$). Leaf area, crown volume, and crown density (AL/AC) did not achieve normality after transformations. Therefore, Kruskal-Wallis test was conducted ($P < 0.05$).

3. Results

3.1 Stomatal conductance kinetics

Well watered plants showed a higher stomatal conductance (g_s) than water stressed ones. The fit curve of the g_s kinetic of all plants showed values ca.200 mol H_2O m^{-2} s^{-1} at the beginning of growth season (Fig. 1). 30 days after of budbreak, WSHL plant reached the desired g_s.

Meanwhile in WSLL plants took ca. 55 days to achieve the same value. In LL conditions, WW plants showed a reduced g_s compared with WWHL. As a general result, the g_s kinetic along the growth season of the displayed leaf cohorts of WW plants showed a natural decay towards the end of the season. Temporally, this decay occurs first under LL than HL conditions (Fig. 1A, B).

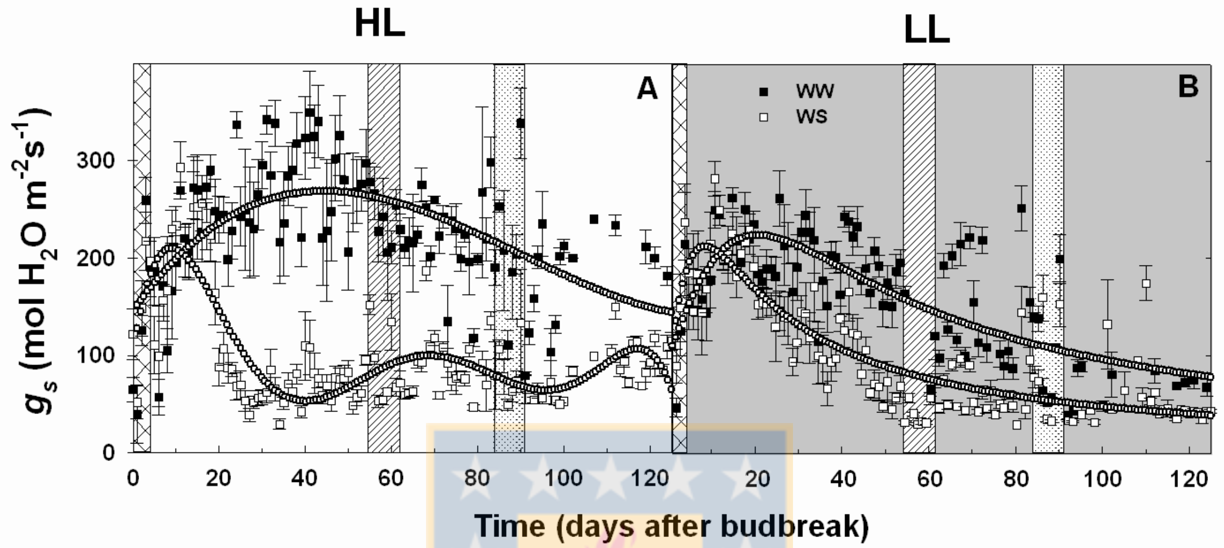

Fig. 1. g_s (mol H_2O m^{-2} s^{-1}) measured before budbreak (LC1, diamond bars), in a subsequent one generated after budbreak (LC2, diagonal lines bars), and 30 days after this latter (LC3, gridded bars) of *E. cordifolia* plants grown under different light and water availabilities. Light treatments were full and 7% of sunlight reaching integrated quantum fluxes of 26.6 ± 4.2 and 1.2 ± 0.2 mol m^{-2} day^{-1}. Water treatments were well watered (WW) and moderate water stress (WS) defined as 0 and 65% decrease in g_s with respect to the control. Mean values ± S.E. are shown. Data were fitted by: WWHL (Polynomial regression in the form of $y = a+bx+cx^2+dx^3$ ($R^2 = 0.61$)); WSHL (Polynomial regressions in the form of $y = (a+bx)/(1+cx+dx^2+ex^3fx^4+gx^5+hx^6)$, ($R^2 = 0.73$)); WWLL (Rational model $y = (a+bx)/(1+cx+dx^2)$, ($R^2 = 0.82$)); WSLL (Rational model $y = (a+bx)/(1+cx+dx^2)$, ($R^2 = 0.86$)), ($P< 0.001$ for all).

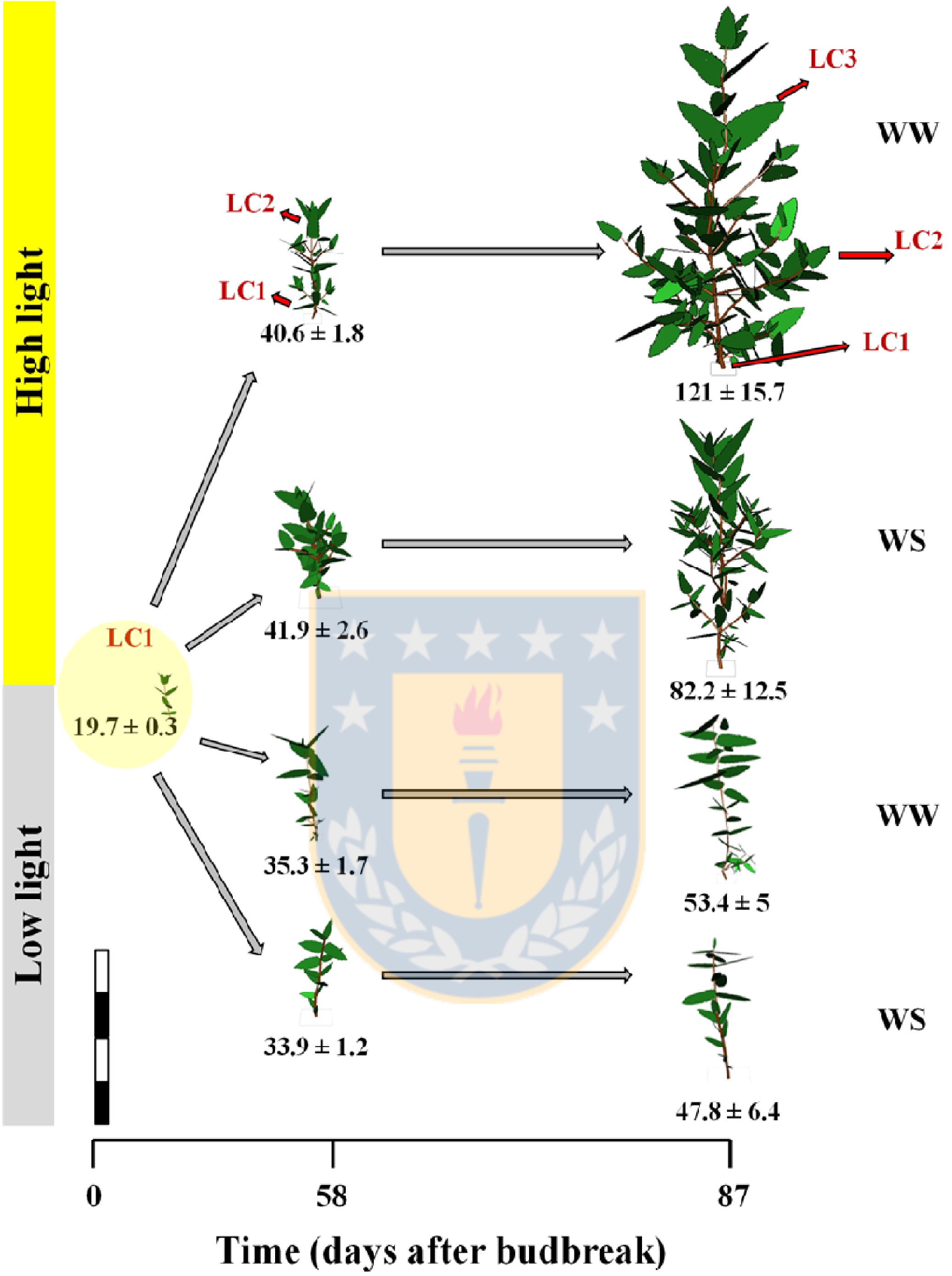

Fig. 2. Virtual crown level acclimation of *E. cordifolia* plants subjected to different light (left coloured bars) and water availabilities (right column) during ontogeny. Mean ± SE height are showed below each plant image. Comparisons between light and water availabilities were analyzed with Kruskal-Wallis test ($P < 0.05$). Red arrows indicate the proximity of the leaf cohort (LC) respect to plant height. Each segment of the scale showed at the left = 20 cm.

3.2 Crown level

3.2.1 Crown architecture and daily CO_2 assimilation

Plant height did not show significant differences at 0 days after budbreak ($P = 0.12$). 58 days after budbreak HL plants were significant taller than LL ones ($P = 0.02$). HL Plants did not show differences between WW and WS ($P = 0.43$). Similar results were obtained for LL water treatments ($P = 0.11$). 87 days after, there were differences between WWHL and LL treatments ($P < 0.0001$). Differences were also found between WW and WS for HL ($P = 0.022$) and LL plants ($P = 0.013$). During this time, the total reduction in height was 32, 56 and 60% for WSHL, WWLL and WSLL relative to WWHL, respectively.

In terms of the architectural traits, higher differences were observed towards the end of the growing season during the ontogenetic trajectory (Fig. 3). With respect to leaf area, the effect of water stress in high light conditions reduced it 69%, but did not show statistical differences against WWHL plants. Plants grown under LL conditions were reduced significantly to ca. 94%. However, under LL there was no difference attributable to a WS effect (Fig. 3A). 58 days after of budbreak, crown density (AL/AC) was significantly reduced (39%) by WS in HL compared with WW. Under LL, AL/AC was significantly reduced 47 and 44% for WWLL and WSLL. This trend was maintained 87 days after budbreak. AL/AC showed differences between all treatments.

Crown volume decreased 50% by the effect of WS in plants grown under HL respect of well watered ones. However, 87 days after budbreak showed a tendency to increase (Fig. 3C). By other hand, crown volume under LL conditions showed a reduction of ca. 95%, and was constant during the ontogeny for LL plants.

Plants of all treatments did not show significant differences in light interception efficiency (STAR) before the budbreak ($P = 0.069$) (Fig. 3D). STAR was highly reduced in WWHL plants. Compared to this latter, WSHL plants showed an ontogenetic decrease of the reduction in STAR. However, they showed a trend to decrease STAR with ontogeny trajectory towards the end of growth season. Under LL, plants showed an almost constant STAR (P-values > 0.05) (Fig. 3D). 87 days after budbreak HL plants showed significant differences ($P < 0.0001$).

Daily crown assimilation (DCA) was significantly differentiated towards the end of growth season. WS in HL conditions provoked a 67% of reduction in DCA. Under LL conditions the reduction of DCA was practically 100% during ontogenetic trajectory (Fig. 4A). Therefore, crown assimilation was limited severely (see below).

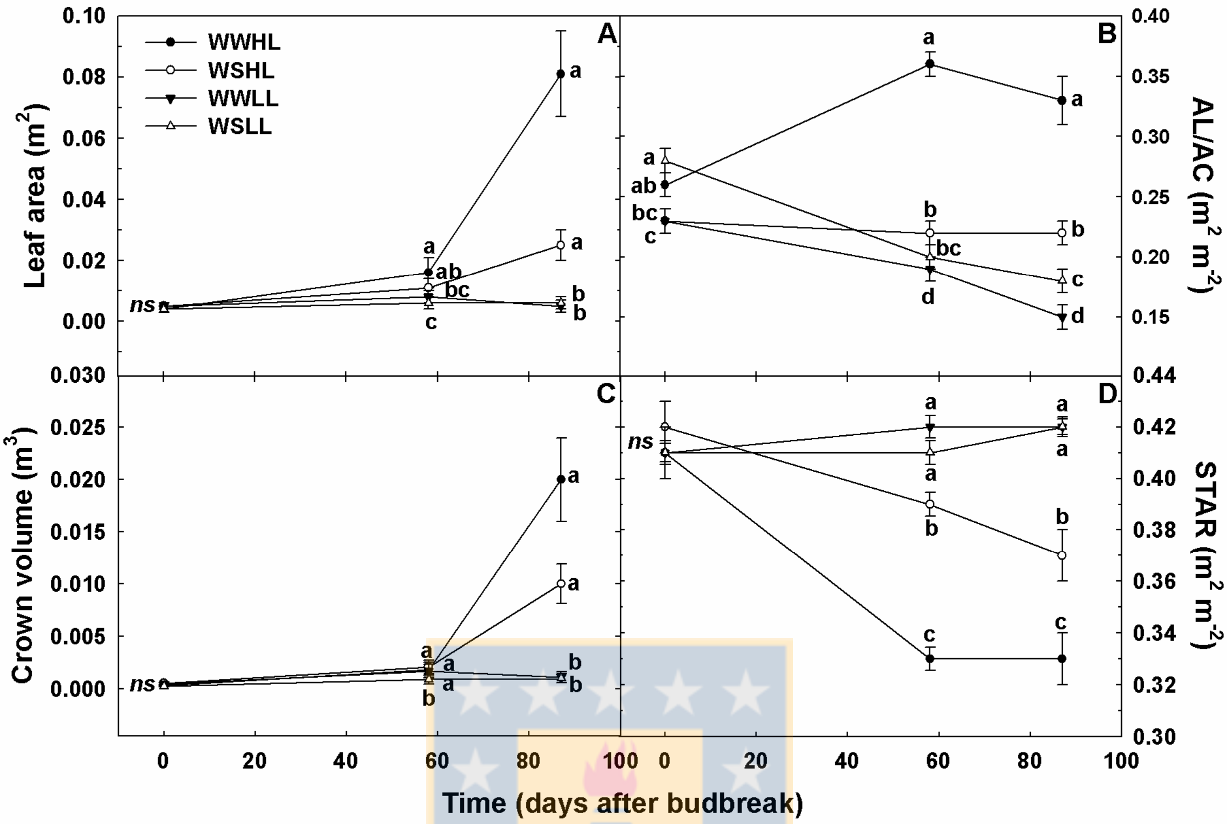

Fig. 3. Leaf area (A), crown density (AL/AC) (B), crown volume (C), and STAR (D) during ontogeny of *E. cordifolia* plants subjected to different light and water availabilities. Mean ± SE are showed. Different letters indicate statistical differences. Variables A, B and C were compared between treatments within each time with Kruskal-Wallis test ($P < 0.05$). STAR was compared with Duncan test ($P < 0.05$).

The aerial biomass 87 after budbreak was 3 folds higher than the root biomass within all treatments. Thus, WSHL, WWLL and WSLL showed reductions of total biomass (shoot+root) of 37, 81, and 87%, respectively. Root:shoot ratio was lower for LL than for HL plants. Regardless these differences, a common ratio of 0.38 was found for all treatments with a high correlation (see Fig. A7).

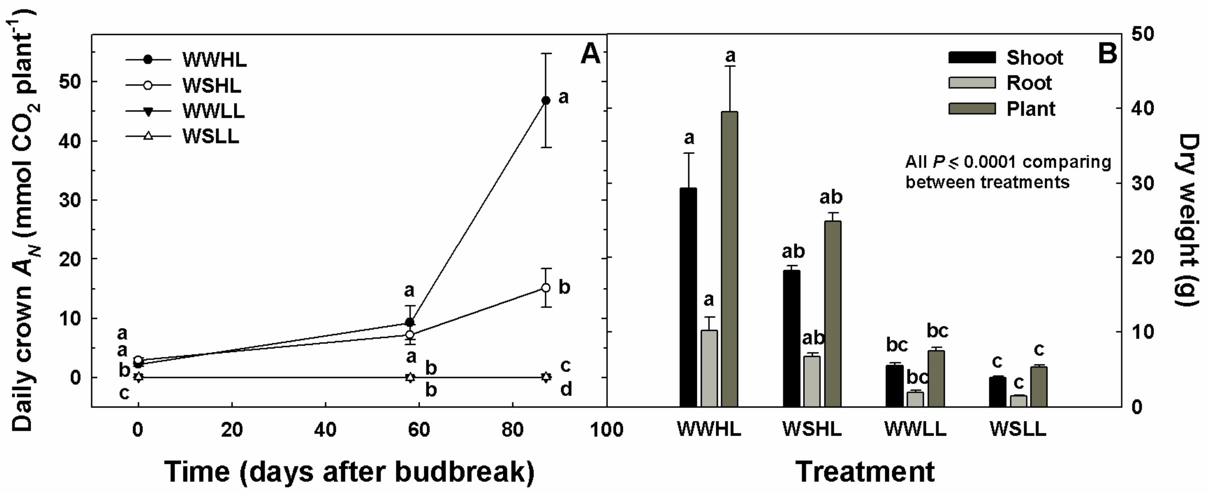

Fig. 4. Daily crown assimilation (DCA) plan-1 day-1 (A) during ontogeny and plant biomass 87 days after budbreak of *E. cordifolia* plants subjected to different light and water availabilities. Mean ± SE are showed for both DCA and plant biomass. Different letters indicate statistical differences with Duncan test for DCA ($P < 0.05$). Dry weight of plant biomass was compared with Kruskal-Wallis test.

3.3. *Crown differentiation by leaf cohorts*

3.3.1 Crown density (AL/AC)

In order to evaluate the contribution of leaf cohorts (LC1, LC2 and LC3), we separated AL/AC, due to that is one of the architectural variables that mainly explain the variation on STAR (Duursma et al. 2012), which could modulate the DCA at crown level. Obviously, STAR does not was determined deferentially by leaf cohorts, because to take out some leaves affects the crown STAR. LC1 showed a trend of reduction in AL/AC during the ontogeny, independently of the light and water availabilities (Fig. 5). When plants had two leaf cohorts (LC1 and LC2), the contribution of LC2 to AL/AC was higher than LC1 under HL, but decreased by WS (Fig. 5A, B). Under LL conditions (Fig. 5C, D), the LC2 contribution was lower than LC1. 87 days after budbreak, HL plants showed a LC3 contribution similar to LC2. Meanwhile under LL LC3 contribution to AL/AC was lower than LC2, and thus, lower than LC1 for both WW and WS (Fig. 5C, D).

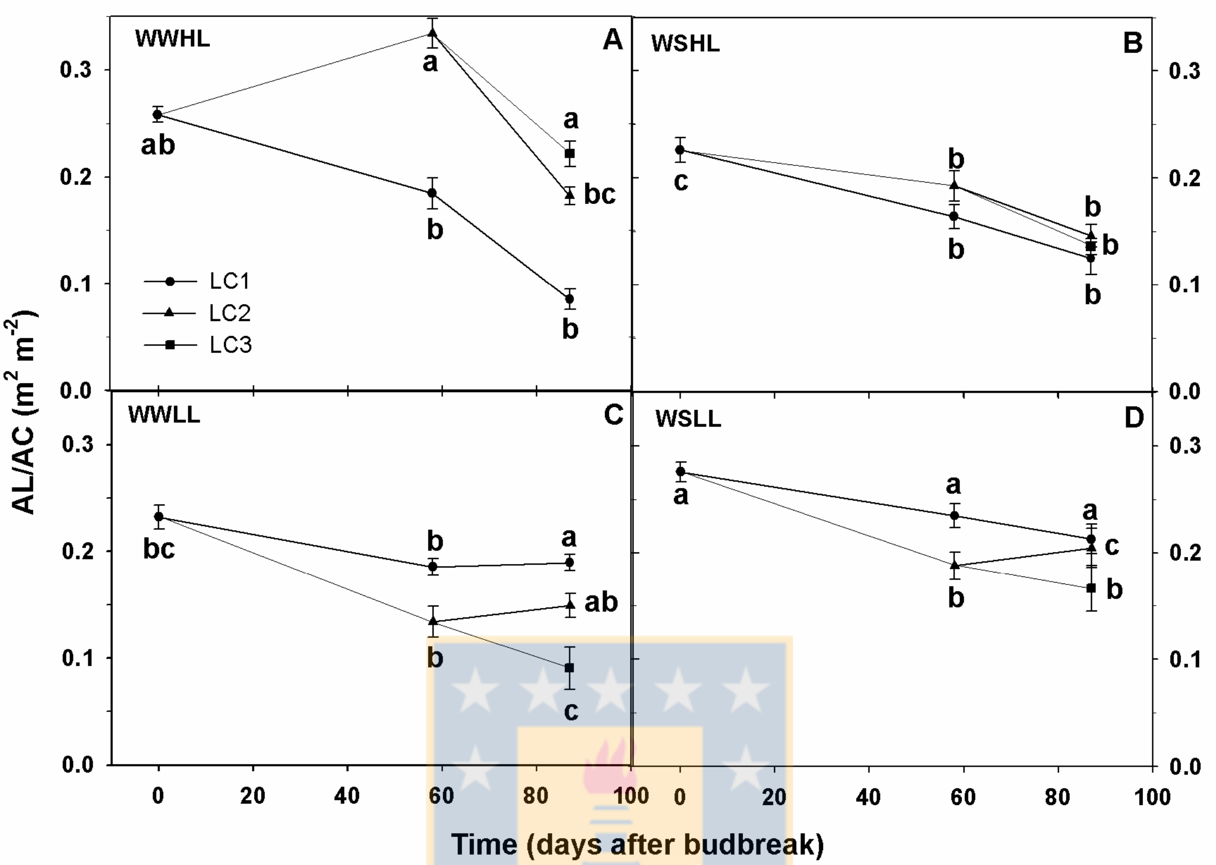

Fig. 5. Crown density contribution by leaf cohort during ontogeny of *E. cordifolia* plants subjected to different light and water availabilities. The contribution of AL/AC leaf cohort was compared between treatments by time. AL/AC was considered the trait that most explain STAR accordingly to Duursma et al. (2012).

3.3.2 Daily crown assimilation

Higher statistical differences were observed between HL and LL treatments (Fig. 6). Despite the high reduction in DCA by WS effect in HL, plants maintained a positive carbon balance. The contribution to DCA of LC2 and LC3 was higher than that of LC1. This latter showed an ontogenetic decrease (Fig. 6B). Under LL conditions, the contribution of LC1 was negative 58 days after budbreak for both WW and WS. This means that respiration was higher than carbon gain. However, at the same time LC2 was positive. 87 days after budbreak, LC1 showed a slight increment on DCA, closer to a neutral carbon balance. LC2 and LC3 were slightly positive (Fig. 6C, D).

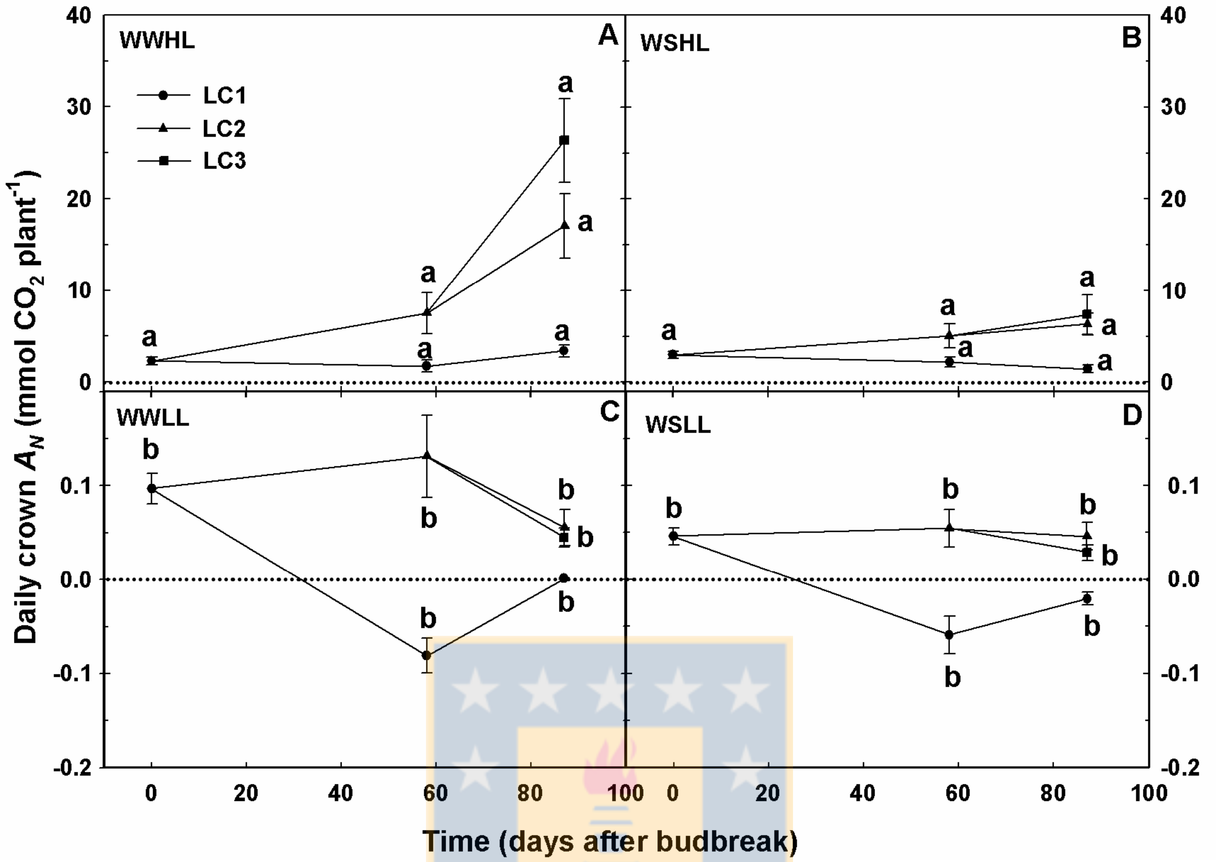

Fig. 6. Leaf cohort contribution on daily crown assimilation (DCA) during ontogeny of *E. cordifolia* plants subjected to different light and water availabilities. The horizontal dotted line denotes values equal to zero for HL (A and B) and LL (C and D) on DCA.

3.3.3 Crown photosynthesis limitations and leaf cohort contributions

Photosynthetic limitations were determined for each leaf cohort at 0, 58, and 87 days after budbreak (DAB). Accordingly, plants grown under WWHL conditions were considered as the control. LC1 at 0 DAB showed no limitations for WSHL. 58 days after budbreak WSHL plants does not showed limitations (Fig. 7, mid, LC1). However, limitations appeared after 87 days of budbreak (Fig. 7, base, LC1). Relative stomatal limitation (S_L) was relatively higher in WSLL than WWLL. WWLL plants showed a higher contribution of non-stomatal limitation (NS_L) (Fig. 7, top). The total relative limitation (i.e. S_L+NS_L) in LL plants was ca. 100%. Under LL conditions, both WW and WS plants showed the same pattern as LC1 at 0 DAB. However, the total relative limitation was higher than 100%, indicating a negative carbon balance (Fig. 7, mid LC1). During this ontogenetic time, LC2 of WSHL plants showed exclusively S_L (ca. 30%). LC2 showed a similar contribution on limitations than LC1, with a slight increment in S_L in WSLL plants, and a total ca. 100%. At 87 DAB, WSHL plants showed both S_L and NS_L, with a higher S_L contribution respect the total. In these plants, LC2 and LC3 were exclusively limited by S_L, showed an increased relative contribution ca. 66 and 73%, respectively. For LL plants, LC2 and LC3 similarly to that shown before, had a higher contribution of NS_L (WWLL) and S_L (WSLL). In summary, S_L was relatively important in crown limitation of water stressed plants. Meanwhile, for WWLL, NS_L was more important.

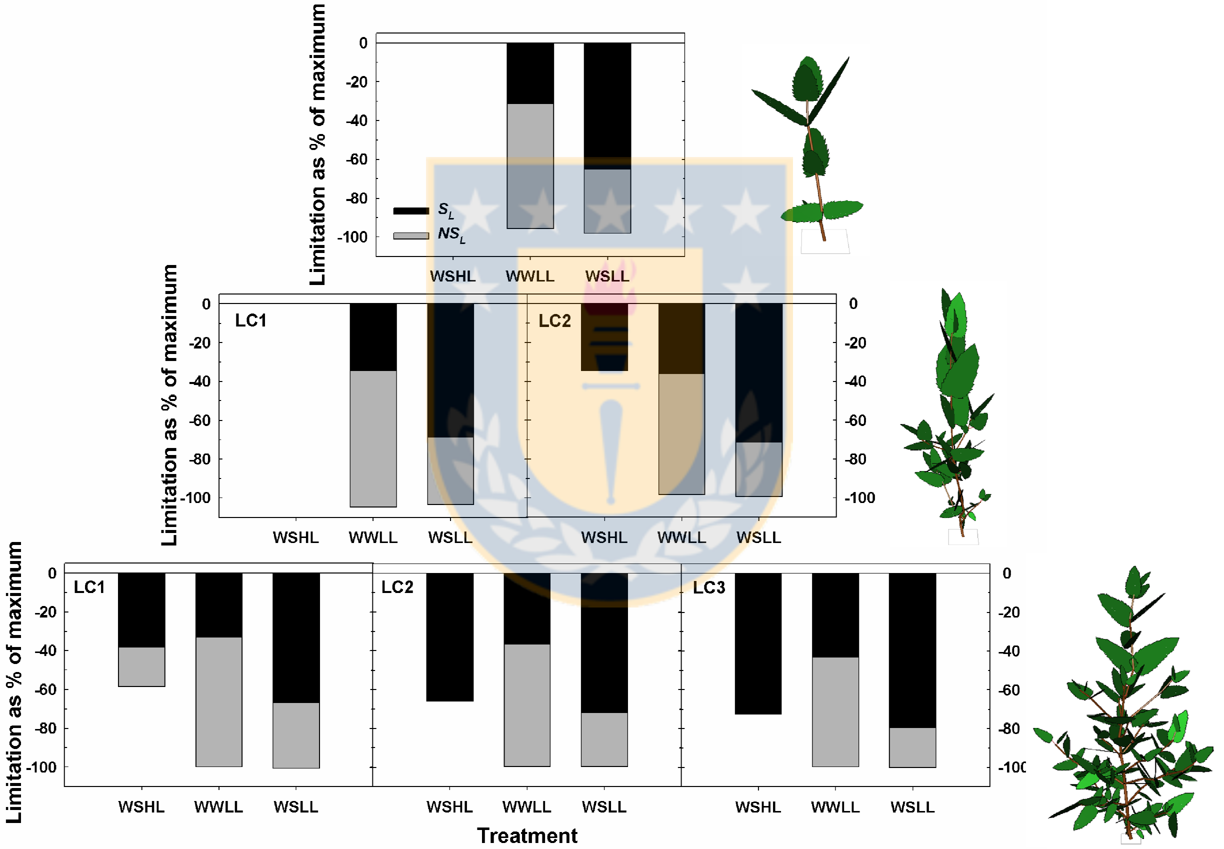

Fig. 7. Relative stomatal (S_L) and non-stomatal (NS_L) limitations of photosynthesis (%) contributed by leaf-cohorts during the ontogeny of *E. cordifolia* plants subjected to different light and water availabilities. The pyramidal organization of the top, mid and base showed 0, 58, and 87 days after budbreak, respectively. 0 had LC1; 58 had LC1 and LC2; 87 had LC1, LC2, and LC3. Photosynthetic limitations were calculated against WWHL plants. Total relative limitation is the sum of S_L+NS_L.

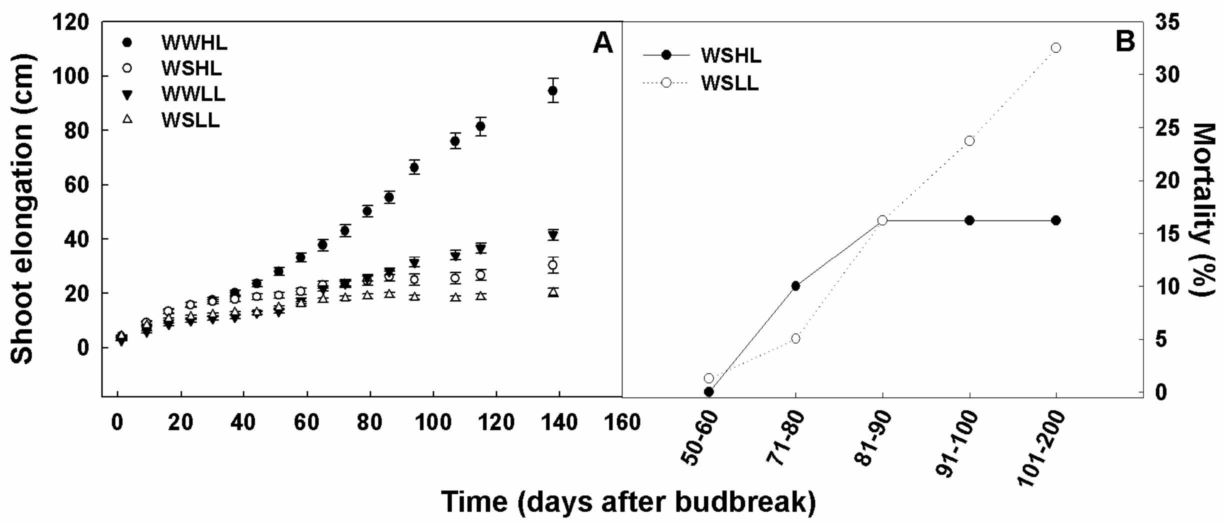

Fig. 8. Shoot elongation (main stem) after budbreak during ontogeny of *E. cordifolia* plants subjected to different light and water availabilities. Mean ± SE are shown.

Shoot elongation, was considered as the elongation of the newer portion of main stem (Fig. 8A). This elongation was ca. 3 folds diminished by the effect of WS in HL and for both WW and WS under LL conditions. Therefore, the rate of elongation was regulated by the availability of light and water. Mortality was quantified from 50-60 DAB to over 101 DAB. Only in water deficit treatments mortality occurred, where under HL reached a steady state around 15%. However, under LL plant mortality increased gradually until ca. 35% (Fig. 8B).

4. Discussion

We handled plants for crown display in a way similar to what occurs in natural conditions, specifically with respect to light and water availability. The integration of environmental conditions could determine development and crown architecture arrangement (Fig. 2). There is agreement that crown modifications converge to an optimal use of resources in order to maximize gains and minimize losses (Givnish 1988, Pearcy & Yang 1996, Valladares & Niinemets 2007, Valladares & Niinemets 2008). Leaf water status monitored through stomatal conductance determined differences on plant leaf area displaying. This could modulate several other interrelated crown traits (e.g. AL/AC, crown volume, and STAR, Fig. 3) and the ontogenetic leaf display, branching, shoot elongation, carbon gain and balance (Fig. 2, 4 and 8), and leaf rolling (Fleck et al. 2003). Based in our results, the higher total leaf area observed in control plants

(WWHL) produced an increment in crown volume and density (AL/AC), which modulates the decrease of the light interception efficiency index (STAR), which is concomitant with the increment of self-shading (SS) because of the increment of leaf overlap, for this species and many others (Fig. 3, see Chapter II, e.g. Farque et al. 2001, Lusk et al. 2011). WSHL reduces enormously the total leaf area towards the end of growth season. Consequently, it provokes a reduction in crown volume and AL/AC, likely influencing the lower daily crown assimilation (Fig. 3 and 4A). Moreover, water stress generated a decrease of ontogenetic reduction in STAR (i.e. compared with WWHL), thus, affecting a structural photoprotective mechanism under excessive light (see Valladares & Niinemets 2007 and references therein). The aforementioned means a higher STAR in WS than WW plants in HL condition, which could be one of the first reports of water stress effect on STAR and plant architecture traits. In turn, shading of plants mostly reduces leaf area increasing the STAR, which is in agreement with previous reports (Delagrange et al. 2006). The lower leaf area displayed by LL plants could be explained mainly by the effect of light scarcity than by water availability, allowing maximizing the STAR during the ontogenetic development for both WW and WS plants. The lower capability of CO_2 assimilation due to the lower light availability determined a lower investment in plants structures (e.g. leaves and stem). In this sense, it has been showed that shaded seedlings had a reduced total leaf area and leaf number, but the individual leaf area was increased. Therefore, STAR of shaded plants was higher than sunny ones, perhaps because the horizontal leaves orientation (Farque et al. 2001). In general, the resources availability modulation found in STAR at HL and LL agrees with previous literature reports (Falster & Westoby 2003, Delagrange et al. 2006; also see Valladares & Niinemets 2007). STAR can be modulated by size and leaf angle under HL conditions (Falster & Westoby 2003). The increment in self-shading could reduce incident PPFD, carbon assimilation, daily light interception and assimilation. Despite the high STAR in LL conditions, carbon assimilation was critically reduced during the ontogenetic leaf display across the growing season (Fig. 3D and 4A). However, in our study, the reductions in daily carbon assimilation (DCA) on WSHL plants were a consequence of water stress and its effect on stomatal conductance (Fig. 1), concomitantly with some of the architectural traits aforementioned. Under LL condition, DCA was reduced might by the decrease of the metabolic-light dependent machinery, possibly investing more to increase light capture (i.e. light harvest complex) than light processing (i.e. reaction centers). Therefore, the first constrain of water deficit in HL plants was the reduction of stomatal conductance (Fig. 1), which occurred at the beginning of the growth season limiting the carbon assimilation. In this way HL plants showed a higher SS (considered as the inverse of STAR; see Table A1-B, Chapter II) which likely gave a higher level of structural photoprotection than in shaded plants (Valladares et al. 2005, Valiente-Banuet et al. 2010). Highest values of self-shading variations with plant ontogeny coincided with values reported for other species (Farque et al. 2001, Lusk et al. 2011). However, this pattern does not always occur, or even could be slightly reversed, at least for *E. cordifolia* (e.g. in LL plants, Fig. 3; see Fig. A1-B, Chapter II, and Lusk et al. 2011). It has been showed that the increment in SS decreases carbon assimilation expressed by area and by day (Falster & Westoby 2003). Other studies had shown an apparent lack of interspecific plasticity in efficiency in capturing light, which is probably related to a balance between architectural features and functional limitations of the plant (Pearcy et al. 2004, Pearcy et al. 2005). The specific variation in leaf display in *E. cordifolia* plants grown at LL conditions reflects the early effect of photosynthetic rates and carbon balance as well as its effect on structural investment for survival of leaves.

Independently of the crown architecture modulation driven by the availability of light and water, and also by leaf display and plant growth during the ontogeny, it is important to recognize that these latter are influenced by carbon assimilation. The simultaneous occurrence of high light and water deficit could be biochemically critical due to the impossibility to drive more light energy at leaf level and the impairment of the CO_2 internal metabolic processes, as assimilation, fixation, and uses of carbon. In this sense, it is well known that the first constrain occurs at stomatal aperture level (e.g. Flexas & Medrano 2002). Accordingly, our results showed that stomatal conductance (g_s) decreased faster with WS in HL than in LL plants (Fig. 1). Also, it suggests a natural decay of g_s during the ontogenetic trajectory, specially demonstrated by WWHL plants g_s kinetics (Fig 1, HL). The maintaining of a lower g_s in water stressed than well watered plants during ontogenetic leaf display provoked a decrease in daily crown assimilation for plants under HL and LL conditions, which was especially expressed at the end of the growing season (Fig. 4A). In fact, the reduction of the potential DCA caused by water stress in HL condition was over 67%. It reached 100% for LL plants. These DCA reductions drove the differences on carbon gain and balance at crown level, and possibly the differences on shoot elongation kinetics, plant dry weight, and mortality (Fig. 4A, B and Fig. 8A, B). Quero et al.

(2006) observed that the impact of water deficit on maximum photosynthetic capacity was higher in *Quercus* species cultivated in HL, which is in agreement with our results. Meanwhile, the influence of shade on photosynthetic rates was more pronounced when water was not limiting. Our results showed that light response curves of LL plants were reduced ca. 50% in relation with WWHL plants. The lower light availability (see materials & methods) was extreme. Thus, LL plants showed the lowest DCA. Perhaps, the LL DCA could be more affected by the photosynthetic induction time (Fig. A6). In fact, LC1 needed more time to reach its maximum assimilation rate than LC3, where in this latter the faster induction occurred in WSLL. This photosynthetic induction pattern was not showed by WSHL leaf cohorts.

Nevertheless, during the growing season plants displayed several leaf cohorts simultaneously with environmental changes and water availability determined by g_s (Fig. 1 and Fig. 2). The contribution of leaf cohorts (LC) to DCA was achieved. Remarkably, differences in leaf cohort contributions on DCA were observed between HL and LL plants (Fig. 6). In HL plants, WS showed a reduced DCA towards the end of growth season especially on LC2 and LC3, which could be mainly explained by the stomatal limitation (S_L) and the reduced crown architecture components (see above; Fig. 8 and Fig. 3). However, LC1 also showed photosynthetic limitations explained by both S_L and non-stomatal (NS_L). Based on these results, we could infer that LC2 and LC3 showed some kind of physiological acclimation, specifically on the maximum electron flux (J_{max}) and the maximum velocity of carboxylation rate (V_{cmax}) which could be directly related with the amount of activated Rubisco (Table A1 and Fig. 7). It has been previously described the plasticity of leaves formed at different periods during the growing season when water stress was applied at mild and moderate intensity. These studies show that NS_L was relatively more important (Galmés et al. 2007, Gallé et al. 2009, Limousin et al. 2010, Misson et al. 2010, Gallé et al. 2011). Despite the slight reduction in V_{cmax} in WSHL LC3 (see Table A1), the resulting analysis determined an imperceptible NS_L. Therefore, only S_L was observed in plants under WSHL treatments in LC1. LC2 and LC3 of WSHL plants showed a similar contribution to DCA (Fig. 6B), but lower than the potential maximum where the LC3 showed the higher contribution in WWHL plants (Fig. 6A). LC2 showed a 2 fold increment in S_L from 57 to 87 days after budbreak (DAB). Meanwhile LC1 showed an ontogenetic reduction in CO_2 assimilation during the growth season, which is in agreement with other study (e.g. Kikuzawa & Lechowicz 2006). The leaf cohort ontogenetic reduction of AL/AC (LC1 and LC2) due to WS could show the reductions in leaf area during the plant growth and development, which, apparently, did not influence the decrease in assimilation contribution of leaf cohorts (Fig. 5 and Fig. 6). Leaf cohorts of LL plants showed a DCA contribution closer to zero, but positive carbon balance (Fig. 6C and D). Specifically, LC1 showed daily respiration and also a slightly higher AL/AC, compared to the other leaf cohorts under the same light condition (Fig. 5C and D). Possibly, LC1 under LL may have shown a better long-term acclimation (87 DAB) to light than to water stress, which could be determinant for crown carbon balance. LC2 and LC3 showed positive carbon balance, being higher for LC2 for both WW and WS in LL plants. This lower contribution of LC3 to DCA could be explained by the lower contribution to AL/AC (Fig. 5 and 6). LL plants showed a constant absolute (ca. 100%) photosynthetic limitation, with a higher contribution of NS_L in WW and mainly S_L on water stressed plants. This pattern occurred independently of the leaf display and ontogenetic development (Fig. 7). However, the DCA modeling of LC1 at 58 DAB showed more than 100% of photosynthetic limitation, which occurred concomitantly with the resulting negative carbon balance (Fig. 6 and 7). In general, the decreasing assimilation of LC1, the oldest leaves, could be explained because the photosynthetic parameters, such as A_N, g_s, V_{cmax} and J_{max}, decrease with the leaf age, even with incident light angle (Wilson et al. 2000, Xu & Baldocchi 2003, Kikuzawa & Lechowicz 2006, Allard et al. 2008, Monti et al. 2009, Whitehead et al. 2011). Similarly, it has been described that leaves displayed in successive growing seasons show a progressive acclimation to long-term drought suffered changes in leaf area index, leaf mass per area, and leaf chemical composition, instead of modifications of physiological parameters (Limousin et al. 2010). Also, similarly to our results, it has been reported that leaves of different layers subjected to water stress were differentially photosynthetically limited, where the basal leaves showed a proportional co-limitation between S_L and NS_L, meanwhile the uppers ones were mostly S_L limited (Cano et al. 2013). Therefore, our results confirm the assumption that under low light availability, a deficiency of other resources such as water, would have a lower impact on plant yield (Canham et al. 1996), but a higher impact on plant survival. The decreasing of DCA for HL and LL plants were not explained by photoinhibition, because there was no effect measured (Fig. A7). In fact, the effect of water stress in HL is in part similar to that found under low light, but mortality was found exclusively in plants subjected to water stress (Fig. 8B). This result puts in evidence that water stress could be mostly critical on survival under LL than HL conditions (Abrams & Mostoller 1995, Valladares & Pearcy 2002), regardless of leaf area looses by leaf senescence.

With this study, we provide new knowledge on architectural traits of plants subjected to simultaneous light and water availabilities. We also provide an approach to the determination of daily crown assimilation and limitation of photosynthesis. Our results allow us to deduce that regeneration of species in the understory could be negatively affected by the predicted decrease in rainfall for South-Central Chile, also evidenced in the last 100 years. Further decreases would affect it even more. This is highly important because most species in the Chilean temperate forest are originated in the understory.

Nevertheless, several questions remain unanswered. For instance, the effect on daily carbon assimilation in plants growing under water stress of: leaf shape during leaf display (regardless that not differences were found across temperate rain forests tree species; e.g. Lusk et al. 2012), photosynthetic induction times, leaf temperature, and night respiration (Lusk et al. 2011). Therefore, it is necessary to integrate more variables in order to achieve a more accurate plant performance under different environmental conditions. Only thus, improved predictions on the physiological consequences for plant development and growth will be reached.

5. Conclusions

It was demonstrated that limitations of photosynthesis affect differentially plants growing under different light and water availabilities. In addition, *E. cordifolia* plants were susceptible to long-term water stress. Despite mortality occurs, WSHL plants showed an apparent acclimation of V_{cmax} reflected by the absence of NS_L. However, there was an apparent decrease of V_{cmax} with the ontogeny, specifically in the oldest leaf cohort (LC1).

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Appendix C. Supplementary material

Instantaneous gas exchange measurements: Gas exchange measurements were performed during the morning (9.00 - 13.00) on fully expanded apical leaves. We used a Li-Cor 6400 IRGA-XT with an integrated fluorescence module (Li-Cor 6400-40, Li-Cor Inc., Nebraska, USA). The atmosphere inside the tank was maintained at room temperature and humidity (16 to 25 ° C range and 49-63% RH), and block temperature of 20 °C. The flow was adjusted to 300 ml min^{-1}. Photosynthetic response curves to light (A_N-Q) were made to [CO_2] environment (C_a) of 400 mol mol^{-1} PPFD varying from 2000-0 mol m^{-2} s^{-1} (10 and 90% of blue light and red) in 13 levels. The light saturation point (LSP) was calculated with Photosyn Assitant 1.1 software (Dundee Scientific, Dundee, UK). From these curves were obtained photosynthetic parameters required for modeling. Photosynthetic response curves [CO_2] (A_N-C_i) were performed to LSP PPFD of each treatment plus 20%. Changes in [CO_2] consisted of 13 levels, from 50 to 2000 ppm of CO_2 according to the protocol used by Galmés et al. (2011). Leakage to and from the cell were obtained according Flexas et al. (2007) for CO_2 assimilation corrections. Cuticular conductance (g_c) was measured in excised leaves to correct C_i and g_s (Flexas et al. 2002). Since *E. cordifolia* leaves are hipostomatic, g_c was measured by sealing the abaxial face with silicone grease and a polyethylene film to prevent stomatal gas exchange. g_c was determined under the same conditions as A_N-C_i curves. Mitochondrial respiration (R_n) was quantified after darkening the whole plant for 120 minutes. The non-photorespiratory CO_2 (R_d) in light was calculated as R_n * 0.5 (Niinemets et al. 2005, Gallé et al. 2011). From the model of Farquhar et al. (1980) settings for A_N-C_i curves, speed carboxylation (V_{cmax}) and maximum electron flux (J_{max}) apparent were calculated. Rubisco kinetic parameters in response to temperature were considered (Bernacchi et al. 2001). Leaf reflectance and transmittance was quantified with a spectroradiometer EPP-HR (StellarNet Inc., FL, USA) according to Gago et al. (2013).

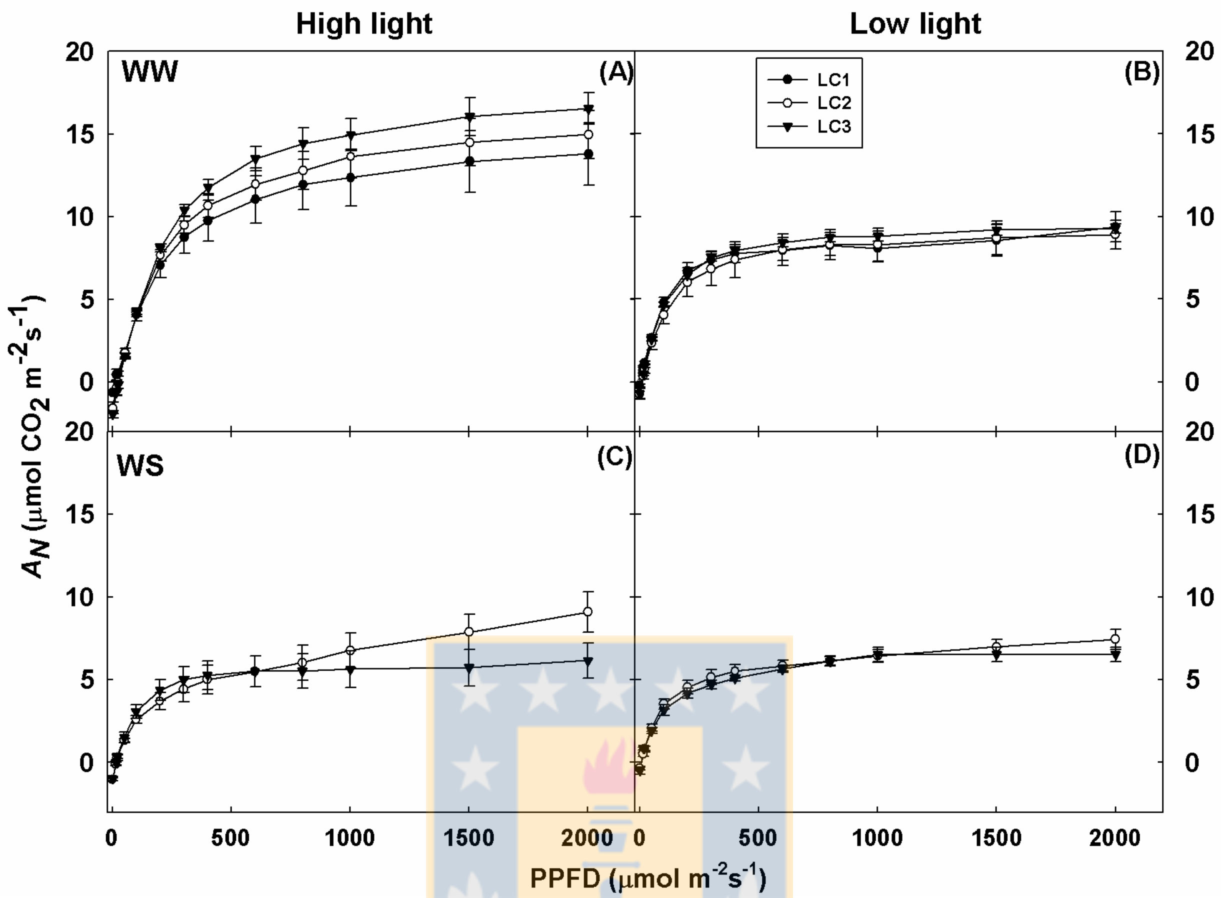


Fig. C1. Response of net photosynthesis (A_N) to light intensity (Q) measured in the older leaf cohort (LC1) and in a subsequent newer ones (LC2 and LC3) of *E. cordifolia* plants grown under different light and water availabilities. Light treatments were full and 7% of sunlight reaching integrated quantum fluxes of 26.6 ± 4.2 and 1.2 ± 0.2 mol m^{-2} day^{-1}. Water treatments were well watered (WW; A, B) and moderate water stress (WS; C, D) defined as 0 and 65% decrease in g_s with respect to the control. Values are averages ± SE of six to seven replicates depending on treatment.

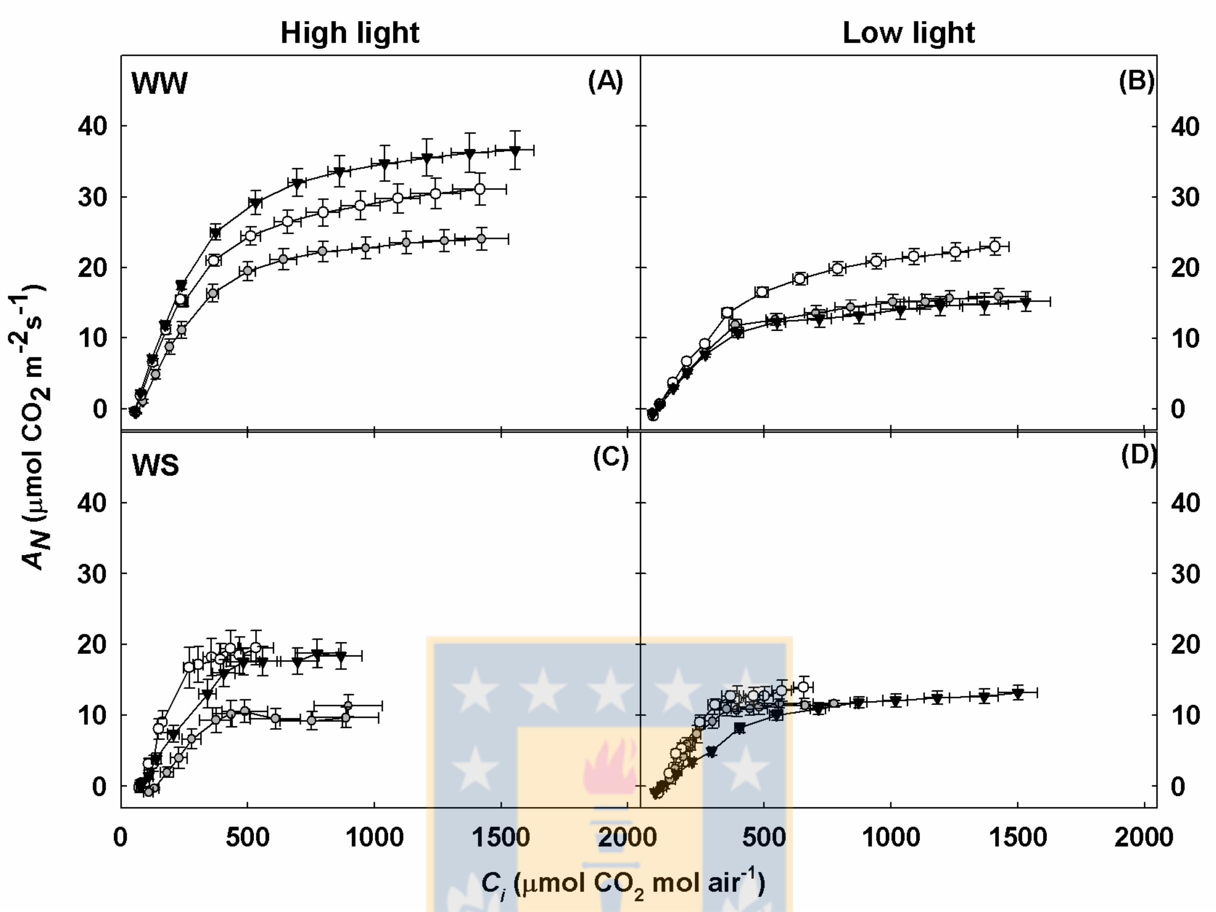


Fig. C2. Response of net photosynthesis (A_N) to sub-stomatal CO$_2$ concentrations (C_i) measured in the older leaf cohort (LC1) and in a subsequent newer ones (LC2 and LC3) of *E. cordifolia* plants grown under different light and water availabilities. Light treatments were full and 7% of sunlight reaching integrated quantum fluxes of 26.6 ± 4.2 and 1.2 ± 0.2 mol m^{-2} day^{-1}. Water treatments were well watered (WW) and moderate water stress (WS) defined as 0 and 65% decrease in g$_s$ with respect to the control. Values are averages ± SE of six to seven replicates depending on treatment. Legends are equal than in Fig. A1.

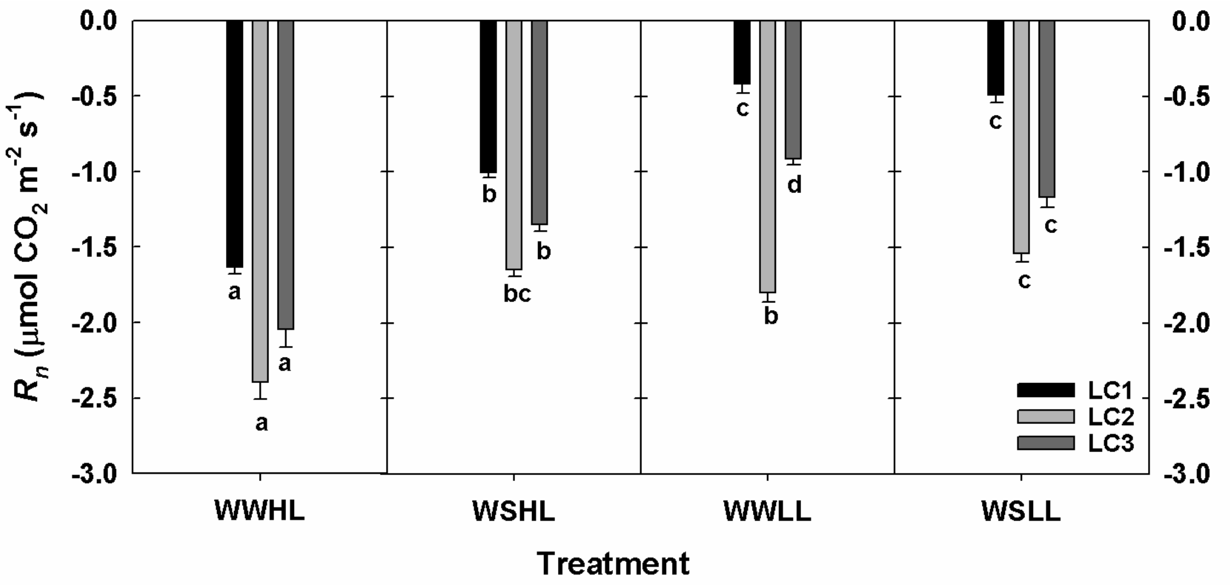


Fig. C3. Mitochondrial respiration (R_n) measured in the older leaf cohort (LC1) and in a subsequent newer ones (LC2 and LC3) of *E. cordifolia* plants grown under different light and water availabilities. Light treatments were full and 7% of sunlight reaching integrated quantum fluxes of 26.6 ± 4.2 and 1.2 ± 0.2 mol m^{-2} day^{-1}. Water treatments were well watered (WW) and moderate water stress (WS) defined as 0 and 65% decrease in g_s with respect to the control. Values are averages ± SE of six to seven replicates depending on treatment. Different letters indicate significant differences after Kruskal-Wallis test ($P < 0.05$) between treatments by leaf cohorts (LC).

Leaf temperature measurement: Leaf temperature at crown level was registered during 48 h. was registered using wire thermocouples type T (0.05 mm of diameter) each 10 s with a measurement time of 110 ms (60 Hz rejection) through Personal DAQ/56, USB data acquisition system (IOTECH, Cleveland, OH, USA) connected to a desktop personal computer for recording. A total of 40 thermocouple wires were attached to the middle portion of each leaf on the abaxial leaf surface with aluminum foil tape (Grantz & Vaughn 1999). In order to not affect the leaf angle and architecture, thus to keep intact the STAR, a woody structure was used to maintain the thermocouple wire on the air (Fig. C4). Finally, was able to contrast both leaf and air temperature (Fig. C5).

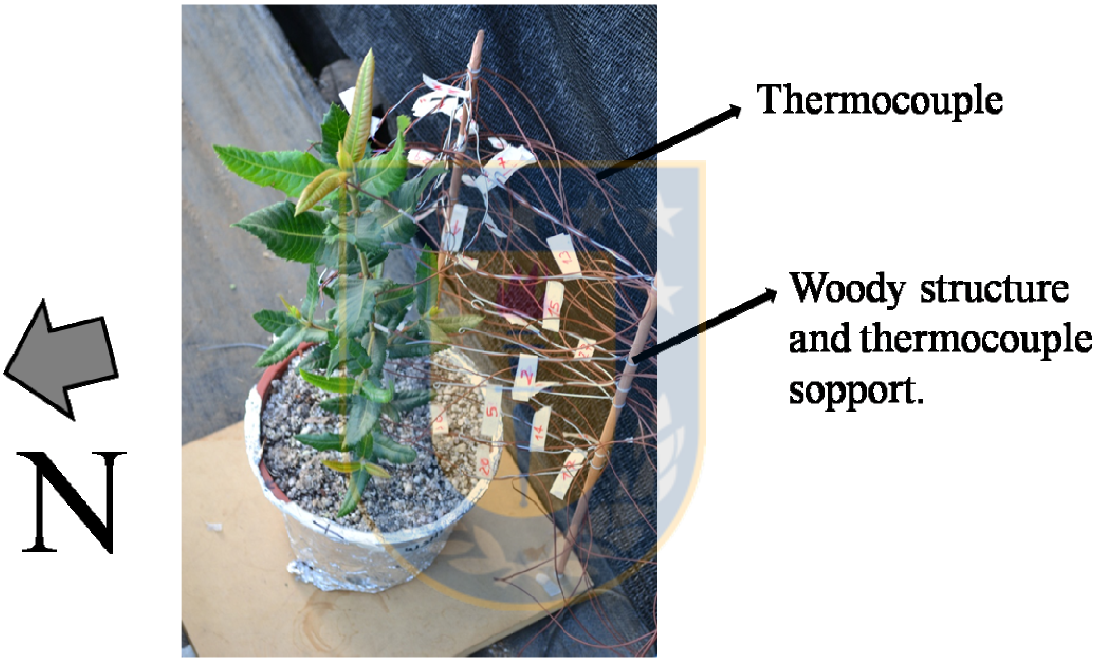


Fig. C4. Leaf temperature measurement using thermocouples on *E. cordifolia* leaves. Magnetic north is indicated.

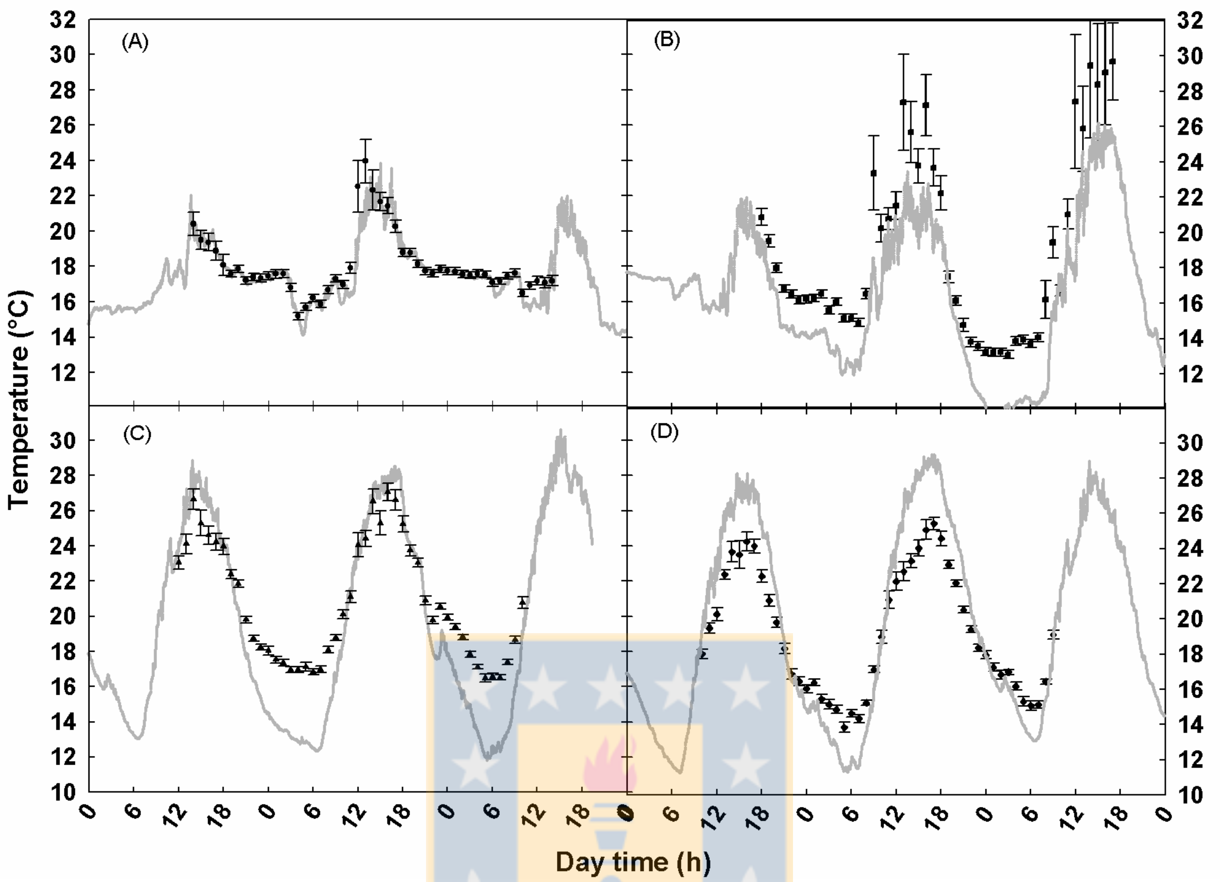


Fig. C5. Leaf (black circles) and air (gray lines) temperature kinetics of *E. cordifolia* plants subjected to different light and water availabilities during 48 h. (A) WWHL, (B) WSHL, (C) WWLL, and (D) WSLL. Leaf temperature was registered in different days.

Photosynthetic inductions: Photosynthetic induction curves were performed during the morning on the three leaves cohorts displayed during the growing season in plants of the four treatments. Previously, plants were obscured during the night. The first data recorded were at PPFD 0 µmol m^{-2} s^{-1} (respiration). Then, leaves were exposed to saturating light intensity (Urban et al. 2007), cuvette flow, relative humidity, and block temperature, equally to that used for A-C_i curves. CO_2 concentration was 400 µmol mol^{-1}. Between 3 and 4 leaves were considered to each leaf cohort. The maximum time of induction was 150 min. The photosynthetic induction time will be used to correct the daily crown assimilation.

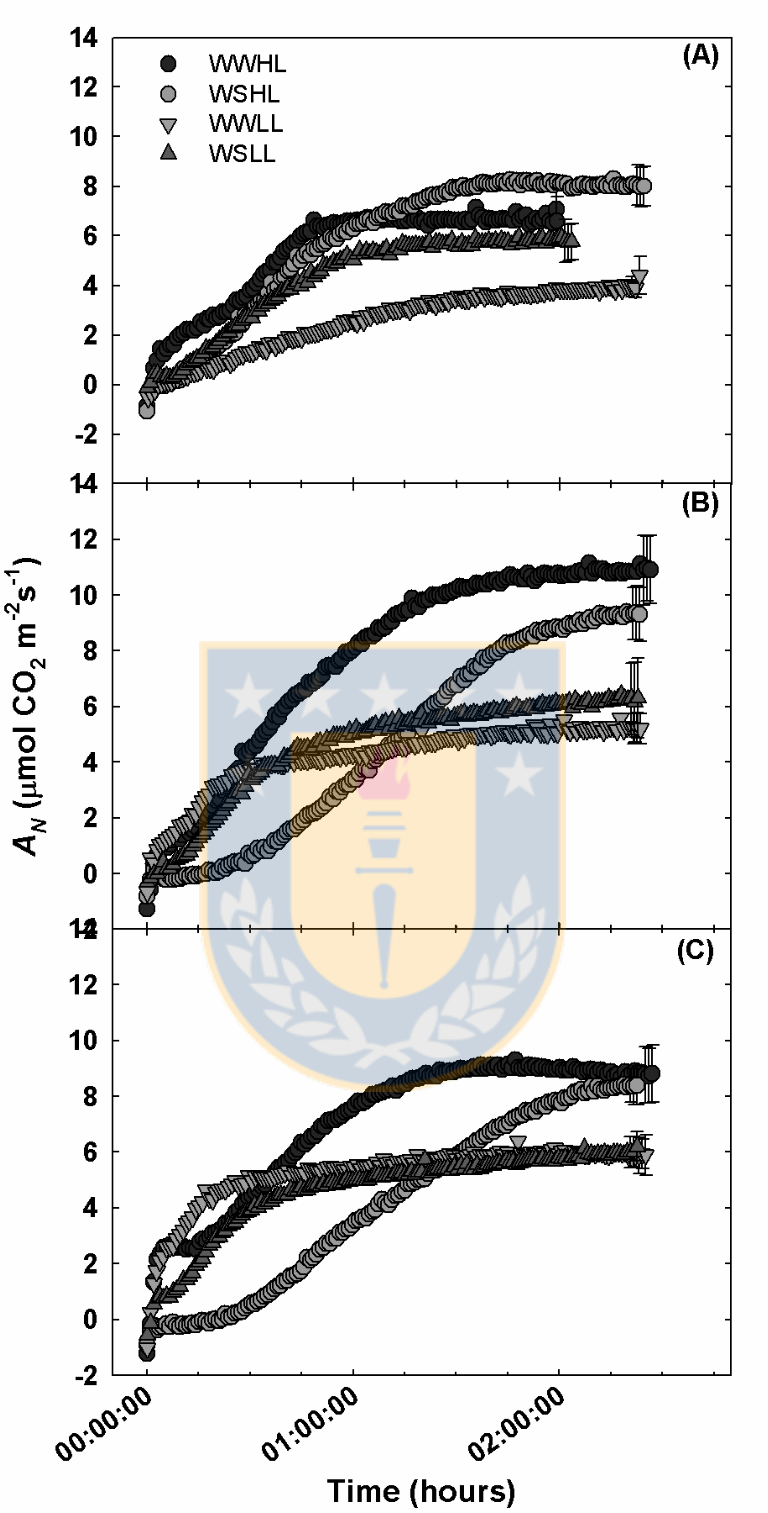
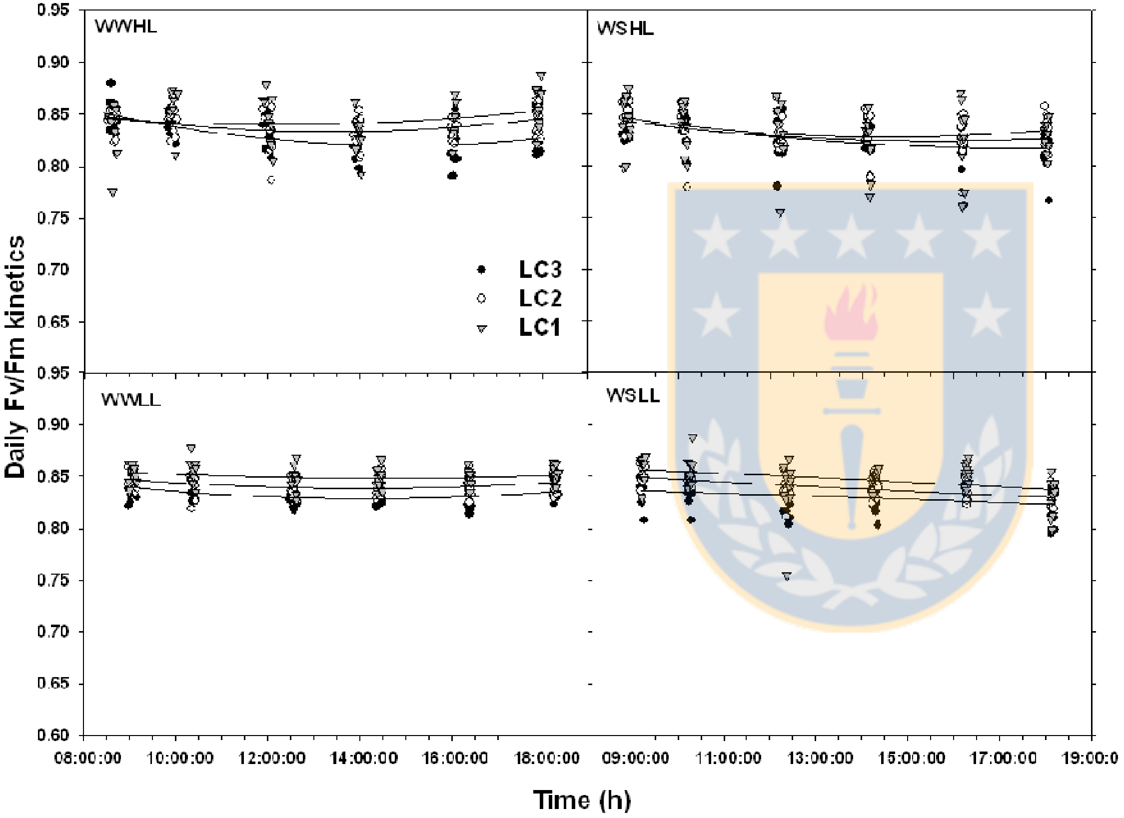


Fig. C6. Photosynthetic induction of three different leaf cohort of *E. cordifolia* displayed under different light and water availabilities. (A), LC1; (B), LC2; (C), LC3.

Daily photoinhibiton kinetics



Daily environmental kinetics during photoinhibiton measurements

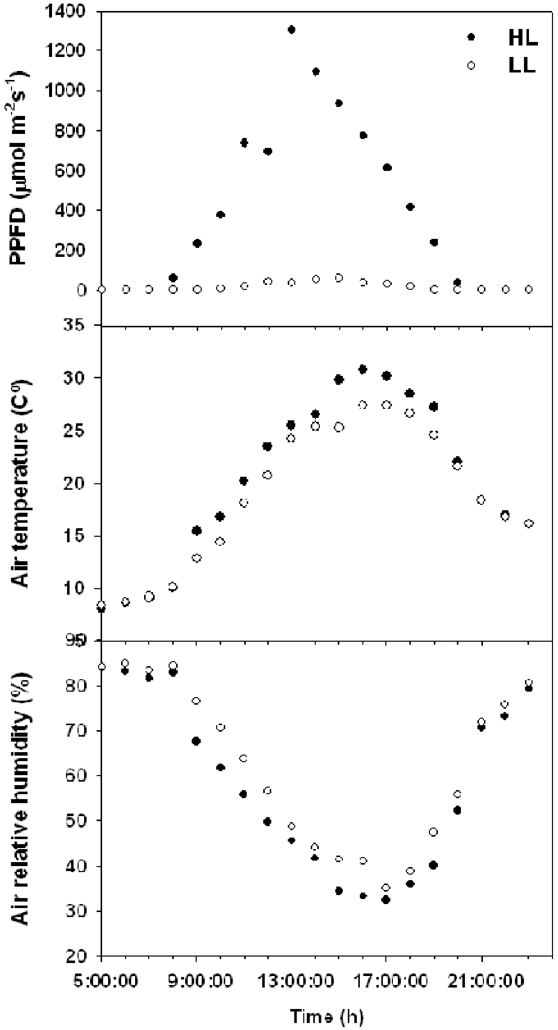


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Fig. C7. Daily photoinhibition (left) and environmental condition (right) kinetics on three leaf cohort (showed as T in the legend) of *E. cordifolia* plants subjected to different light and water availabilities.

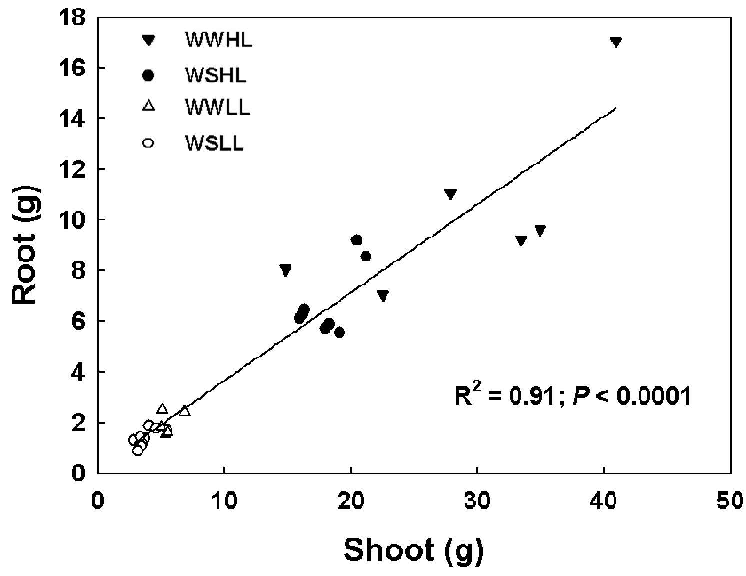

Fig. C8. Root:shoot ratio after 90 days after budbreak of *E. cordifolia* plants subjected to different light and water availabilities. Non-linear regression equation is f=y0+ax. The common ratio for all treatments was 0.38.

Table C1. Climatic and photosynthetic parameters used for modeling daily crown assimilation of *E. cordifolia* plants subjected to light and water availabilities.

LC & TRAT	setMet*			Mechanistic**			
	T_{min}	T_{max}	PAR_{day}	V_{cmax}	J_{max}	G1	R_d
LC1							
WWHL	10.8	30.6	26	42.6	100.5	5.5	1.6
WSHL	10.8	30.6	26	42.6	100.5	4.8	1
WWLL	11	26.7	1	26.5	62.7	5.4	0.4
WSLL	11	26.7	1	26.5	61.3	6.6	0.5
LC2							
WWHL	10.8	30.6	26	61.6	125.5	5.3	2.4
WSHL	10.8	30.6	26	67.2	124.9	4.8	1.7
WWLL	11	26.7	1	42.2	89.2	6.7	1.8
WSLL	11	26.7	1	30.7	67.2	5.3	1.5
LC3							
WWHL	12.3	28.1	26	59.5	147.1	4.3	2
WSHL	12.3	28.1	26	35.8	87.8	5.2	1.4
WWLL	12.5	25.5	1	26.8	50.2	7	0.9
WSLL	12.5	25.5	1	18.1	50.5	4.3	1.2

*setMet: set of environmental variables logged with HOBO micro stations (HOBO dataloggers & devices, MA, USA). **Mechanistic: Data obtained from the fit of curves *A*-*Ci* using the Farquhar et al. (1980) model of photosynthesis. Abbreviations: **T_{min}**, **T_{max}**: minimal and maximal air temperature; **PAR_{day}**: Photosynthetic active radiation; **V_{cmax}**: maximum carboxylation velocity; **J_{max}**: maximum electron flux; **R_d**: dark respiration rate.

Table C2. Spearman correlations ($P < 0.05$) between daily crown assimilation (DCA) and several architectural traits of *E. cordifolia*. Treatments (WWHL, WSHL, WWLL, WSLL) and leaf cohorts are shown. Architectural nomenclatures are shown in Table A3.

DCA	crownvol	crownsurf	nleavesp	leaflen	meanleafang	wmeanleafang	Xellipsoid
WWHL-LC1	0.9781 p=.000	0.9875 p=.000	0.1847 p=.527	0.8648 p=.000	-0.5384 p=.047	-0.5968 p=.024	0.5747 p=.032
WSHL-LC1	0.9357 p=.000	0.9533 p=.000	-0.2072 p=.477	0.9396 p=.000	-0.1962 p=.501	-0.1682 p=.565	0.1513 p=.606
WWLL-LC1	0.9777 p=.000	0.9794 p=.000	-0.4046 p=.170	0.949 p=.000	-0.1302 p=.672	-0.2953 p=.327	0.1917 p=.530
WSLL-LC1	0.9487 p=.000	0.9597 p=.000	-0.0349 p=.906	0.9505 p=.000	-0.0439 p=.881	0.0547 p=.853	0.0016 p=.996
WWHL-LC2	0.9988 p=.000	0.9961 p=.000	0.4368 p=.118	0.9832 p=.000	0.0632 p=.830	0.293 p=.309	-0.0772 p=.793
WSHL-LC2	0.9659 p=.000	0.9762 p=.000	0.4762 p=.085	0.9301 p=.000	0.1172 p=.690	0.0716 p=.808	-0.1358 p=.643
WWLL-LC2	0.7836 p=.001	0.7201 p=.004	0.4678 p=.092	0.5219 p=.056	-0.3097 p=.281	-0.2895 p=.315	0.4121 p=.143
WSLL-LC2	0.0871 p=.767	0.1223 p=.677	0.5252 p=.054	-0.0161 p=.956	0.3543 p=.214	0.4437 p=.112	-0.3595 p=.207
WWHL-LC3	0.9572 p=.000	0.9685 p=.000	-0.524 p=.054	0.9802 p=.000	-0.4484 p=.108	-0.3967 p=.160	0.4417 p=.114
WSHL-LC3	0.9846 p=.000	0.9776 p=.000	0.3109 p=.279	0.7768 p=.001	0.5552 p=.039	0.4616 p=.097	-0.5772 p=.031
WWLL-LC3	-0.1004 p=.733	-0.1126 p=.702	0.0537 p=.855	-0.1282 p=.662	0.0866 p=.768	0.4427 p=.113	-0.1424 p=.627
WSLL-LC3	-0.2518 p=.385	-0.2733 p=.344	-0.1249 p=.670	-0.253 p=.383	-0.1158 p=.693	-0.1149 p=.696	0.0718 p=.807

Table C2. Continued.

DCA	Ek	Ek2	Ok	disp	disp2	stemsurf	stemvol	stemdiam
WWHL-LC1	0.9337 p=.000	0.9179 p=.000	0.74 p=.002	-0.0888 p=.763	-0.0616 p=.834	0.853 p=.000	0.7943 p=.001	0.264 p=.362
WSHL-LC1	0.9357 p=.000	0.9156 p=.000	0.853 p=.000	0.1133 p=.700	0.1353 p=.645	0.7783 p=.001	0.6541 p=.011	-0.2221 p=.445
WWLL-LC1	0.9789 p=.000	0.9696 p=.000	0.9152 p=.000	0.2308 p=.448	0.219 p=.472	0.8763 p=.000	0.8686 p=.000	0.4799 p=.097
WSLL-LC1	0.9731 p=.000	0.9619 p=.000	0.9659 p=.000	0.0097 p=.974	0.0368 p=.901	0.8909 p=.000	0.8342 p=.000	-0.0892 p=.762
WWHL-LC2	0.9848 p=.000	0.9841 p=.000	0.9696 p=.000	-0.3958 p=.161	-0.3232 p=.260	0.9306 p=.000	0.8877 p=.000	0.3982 p=.159
WSHL-LC2	0.9622 p=.000	0.9513 p=.000	0.9304 p=.000	0.1583 p=.589	0.2767 p=.338	0.9119 p=.000	0.8762 p=.000	-0.2164 p=.458
WWLL-LC2	0.6045 p=.022	0.6046 p=.022	0.3825 p=.177	-0.3778 p=.183	-0.3769 p=.184	0.6066 p=.021	0.6729 p=.008	0.4641 p=.095
WSLL-LC2	0.043 p=.884	0.0432 p=.884	-0.1369 p=.641	-0.485 p=.079	-0.5498 p=.042	-0.0395 p=.893	-0.2589 p=.371	-0.2565 p=.376
WWHL-LC3	0.9716 p=.000	0.9708 p=.000	0.971 p=.000	0.6714 p=.009	0.6654 p=.009	0.8308 p=.000	0.7491 p=.002	0.0401 p=.892
WSHL-LC3	0.8366 p=.000	0.8162 p=.000	0.7629 p=.002	0.2493 p=.390	0.3373 p=.238	0.9558 p=.000	0.9232 p=.000	0.5779 p=.030
WWLL-LC3	-0.1529 p=.602	-0.1566 p=.593	-0.1493 p=.610	-0.0168 p=.955	0.0111 p=.970	0.0214 p=.942	0.02 p=.946	0.1358 p=.643
WSLL-LC3	-0.2869 p=.320	-0.2835 p=.326	-0.2452 p=.398	0.246 p=.397	0.278 p=.336	-0.2581 p=.373	-0.2287 p=.432	0.2332 p=.422

Table C2. Continued.

DCA	meanpath	sdpath	totlen	cw	cl	htot	htotcm	cshape
WWHL-LC1	0.8368 p=.000	0.8113 p=.000	0.885 p=.000	0.9841 p=.000	0.9291 p=.000	0.9115 p=.000	0.9115 p=.000	0.3133 p=.275
WSHL-LC1	0.7644 p=.001	0.7558 p=.002	0.7738 p=.001	0.9637 p=.000	0.826 p=.000	0.8673 p=.000	0.8673 p=.000	0.1561 p=.594
WWLL-LC1	0.9011 p=.000	0.8363 p=.000	0.7531 p=.003	0.948 p=.000	0.8499 p=.000	0.8924 p=.000	0.8924 p=.000	0.3963 p=.180
WSLL-LC1	0.9054 p=.000	0.8829 p=.000	0.9081 p=.000	0.9919 p=.000	0.9229 p=.000	0.9304 p=.000	0.9304 p=.000	-0.1905 p=.514
WWHL-LC2	0.9244 p=.000	0.9046 p=.000	0.9554 p=.000	0.9618 p=.000	0.9446 p=.000	0.9526 p=.000	0.9526 p=.000	-0.4169 p=.138
WSHL-LC2	0.9559 p=.000	0.909 p=.000	0.9227 p=.000	0.9839 p=.000	0.9507 p=.000	0.9525 p=.000	0.9525 p=.000	0.0657 p=.824
WWLL-LC2	0.4588 p=.099	0.6335 p=.015	0.5915 p=.026	0.7139 p=.004	0.6746 p=.008	0.6293 p=.016	0.6293 p=.016	-0.0701 p=.812
WSLL-LC2	0.0437 p=.882	0.2167 p=.457	0.4693 p=.090	0.1568 p=.593	0.18 p=.538	0.1856 p=.525	0.1856 p=.525	0.05 p=.865
WWHL-LC3	0.925 p=.000	0.8299 p=.000	0.839 p=.000	0.9667 p=.000	0.8919 p=.000	0.8971 p=.000	0.8971 p=.000	-0.1647 p=.574
WSHL-LC3	0.8939 p=.000	0.9268 p=.000	0.9136 p=.000	0.9551 p=.000	0.9163 p=.000	0.911 p=.000	0.911 p=.000	0.2161 p=.458
WWLL-LC3	-0.2307 p=.428	-0.1764 p=.546	-0.114 p=.698	-0.0345 p=.907	-0.1877 p=.520	-0.183 p=.531	-0.183 p=.531	-0.2726 p=.346
WSLL-LC3	-0.2124 p=.466	-0.3919 p=.166	-0.2872 p=.319	-0.3039 p=.291	-0.3114 p=.279	-0.2793 p=.333	-0.2793 p=.333	-0.0341 p=.908

Table C2. Continued.

DCA	LA	meanleafsize	STAR	ALAC
WWHL-LC1	0.9998 p=0.00	0.8473 p=.000	0.1623 p=.579	-0.4742 p=.087
WSHL-LC1	0.9999 p=0.00	0.9706 p=.000	0.2104 p=.470	-0.2101 p=.471
WWLL-LC1	0.9741 p=.000	0.9488 p=.000	-0.0286 p=.926	0.0202 p=.948
WSLL-LC1	0.9182 p=.000	0.9461 p=.000	0.3148 p=.273	0.1423 p=.627
WWHL-LC2	0.9997 p=0.00	0.9957 p=.000	0.2323 p=.424	-0.2687 p=.353
WSHL-LC2	0.9989 p=.000	0.9332 p=.000	0.3078 p=.284	-0.0917 p=.755
WWLL-LC2	0.7956 p=.001	0.6674 p=.009	-0.283 p=.327	0.1003 p=.733
WSLL-LC2	0.2465 p=.396	-0.0873 p=.767	-0.1713 p=.558	0.3668 p=.197
WWHL-LC3	0.9994 p=.000	0.9683 p=.000	0.0119 p=.968	0.1476 p=.615
WSHL-LC3	0.9998 p=0.00	0.716 p=.004	0.359 p=.208	-0.0472 p=.873
WWLL-LC3	-0.1006 p=.732	-0.1133 p=.700	0.1936 p=.507	0.1049 p=.721
WSLL-LC3	-0.265 p=.360	-0.2544 p=.380	-0.1338 p=.648	-0.0794 p=.787

Table C3. Architecture nomenclature.

Abbreviation	Trait (units)
LA	Total leaf area (m^2)
meanleafsize	Mean leaf size (cm^2)
nleavesp	Number of leaves
leaflen	Mean leaf length (cm)
meanleafang	Mean leaf angle (deg)
wmeanleafang	Mean leaf angle weighted by leaf area (deg)
Xellipsoid	Ellipsoidal leaf angle dist. par.
crownvol	Crown volume (convex hull) (m^3)
crownsurf	Crown surface area (convex hull) (m^2)
ALAC	Crown density (AL/AC) (m^2 m^{-2})
cw	Crown width (m)
cl	Crown length (m)
htot	Total height (m)
cshape	Crown shape index (-)
stemsurf	Stem + branch surface area (cm^2)
stemvol	Stem + branch volume (cm^3)
stemdiam	Stem base diameter (mm)
meanpath	Mean pipe length (mm)
sdpath	Standard deviation of pipe length (mm)
totlen	Total woody segment length (mm)
Ek	Expected distance to 5 nearest leaves (no edge corr.)
Ek2	Expected distance to 5 nearest leaves (with edge corr.)
Ok	Observed distance to 5 nearest leaves
disp	Dispersion parameter (no edge corr.)
disp2	Dispersion parameter (with edge corr.)
STAR	Light interception efficiency as the ratio of displayed to total leaf area (m^2 m^{-2})

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General discussion

Regrowth is an effective strategy especially in stressful environments and high disturbance, where the establishment of sapling is negligible (van Groenendael et al. 1996). Our results and field observations suggest that the statement by van Groenendael et al. (1996) is also valid for slightly disturbed environments in temperate rainforest. Regrowth seems to be a common feature in tree species of tropical and temperate forests. However, regrowth by root suckers occurs in a limited number of tree species (Jeník 1994). Specifically, regrowth constitute an advantage to species regeneration when regeneration by seed is not successful. This is the case of the long-lived species *Eucryphia cordifolia*, species that showed a not-randomly distribution in relation to the available light in the secondary forest. However, saplings of this species between 50 and 200 cm height in an old-growth forest have shown a random distribution with respect to light environments in the forest (Lusk et al. 2006). Regrowth of *E. cordifolia* extended its regeneration niche, because the canopy opening ranges occupied by sprouts and saplings did not completely overlapped (Chapter I). In fact, the light gradient overlap between the two recruits was less than 50%. Therefore, by grouping the types of recruits, the niche breadth is greater. Hence, regeneration of *E. cordifolia* via sprouts in a secondary temperate rain forest may have a high importance in the colonization of the horizontal and vertical space. Muñoz & Gonzalez (2009) showed that seed plants of *E. cordifolia* in a primary forest did not survive after open spaces were formed upon *Chusquea quila* dieback. Sprouts were more successful than seed plants in the use of resources, allowing a rapid growth in height of this type of regeneration and formation of new vegetative regrowth. However, saplings were heavily concentrated in the darkest places of the forest, while the sprouts grew towards the more lighted portion (Chapter I, Figure 2), fulfilling the first hypothesis proposed. Despite this, both recruits shared a portion of the light environment available in the secondary forest. Interestingly, sprouts that co-existed with saplings under shady environments (ca. 5% canopy openness) did not show an architectural configuration of a shade plant (Chapter II) reflected by the trend of differences against the saplings. These sprouts showed a light interception efficiency (STAR) lower than saplings, which was determined by a higher crown density (AL/AC) and self-shading (SS). It has been showed that increased STAR has important consequences for light capture and carbon gain (Pearcy & Yang 1998, see also Valladares et al. 2002), which could be crucial in dark environments. In this sense, the saplings growing under shade in a nursery condition showed throughout the growing season the highest STAR compared to plants growing in full sun either hydrated or under water stress (Chapter III). Concomitant with the lower STAR, sprouts showed a lower photosynthetic capacity in response to light than saplings. Their light saturation point was lower and, consequently, light compensation point also. This suggests that sprouts present a lower metabolic cost of maintaining than the saplings. However, the aforementioned could be explained in part because the sprouts were found under a slightly more somber atmosphere that the saplings, although climatic and light conditions are almost constant (Chapter I). In addition, the photosynthetic response of *E. cordifolia* sprouts differs from the proposed intrinsic property of resprouting shoots, which shown an improved photosynthetic activity (Goorman et al. 2011). By other hand, the response to light of saplings in the field was similar to that showed by water stressed plants under low light (WSLL, nursery), regardless of leaf cohort (Chapter I, Fig. A1, and Chapter III, Fig. A1-D). Interestingly, saplings gas exchange was measured on fully expanded leaves displayed towards the end of the growing season, equally that on nursery plants (fortunately, under similar PPFD, air temperature, and VPD; Fig 1, Chapter I; and materials and methods, Chapter III). Therefore, could be possible that leaves from field plants showed a degree of acclimation to water stress.

In spite of the different photosynthetic capacity in response to light level shown by both sprouts and saplings at leaf level, calculations at crown level (DCA; multiplying the assimilation (A_N) by the leaf area (LA) from both recruit types) showed that the CO_2 uptake of saplings was slightly higher (but not significantly) than sprouts in sunny days and significantly lower in cloudy days. This is similar to the trends shown by Lusk et al. (2011). In addition, the ratio between daily crown assimilation and respiration (DCA and DCRd, respectively) on a sunny day was equal in the different types of recruits. While on a cloudy day, this proportion was two times higher in sprouts compared to saplings (Chapter II, Table 3). This result suggests that despite the lower carbon uptake of the sprouts, carbon gain is equal to or higher than in saplings. Therefore, the respiratory rate determines the daily carbon balance. Possibly, the subsidy from parent plant to the sprout could further benefit the establishment of the sprouts under unfavorable environmental conditions for saplings, thus compensating for the inefficient architecture for light interception and capture. However, the environmental conditions in open sites could also exert a carbon imbalance (Chapter I). Thus, saplings are strongly constrained to occupy the actual niche within of a secondary forest. Furthermore, in more open sites, they would have to compete with the sprouts of other species and *E. cordifolia*, which have shown a higher yield compared to saplings to increased light availability (see Muñoz & González 2009). In agreement with Lusk et al. (2011), the saplings would show some dependence on their carbon balance during the season regarding status of clear or cloudy sky. Therefore, the proportion of clear and cloudy days could be relevant on the carbon balance and on the probability of survival over the growing season in the study area. In this regard, it was found that in the tropical forest, a dense cloud cover reduces the irradiance and limits photosynthesis in leaves fully exposed to light (Pearcy 1987, Mulkey et al. 1996, Zotz & Winter 1996). It has also been shown that it could affect the carbon capture at forest level (Hollinger et al. 1994, Chen et al. 1999, Kellomäki & Wang 2000). In addition, Lusk et al. (2011) showed that light-demanding species tends to earn more carbon that shade-tolerant on clear days, but this pattern was not observed on cloudy days. However, light-demanding species tended to gain more carbon than shade-tolerant ones under clear and cloudy days (Lusk et al. 2011). In addition, to quantify the carbon gain of different leaf cohorts of plants growing in shade is essential to quantify the carbon balance of the crown. This is because it is possible that some leaf cohorts show negative carbon balance (Chapter III). All the above indicates that the second hypothesis does not comply with the assumption that sprouts and saplings shown similar crown architecture. Instead, the carbon balance of sprouts and saplings in sunny day was statistically the same, while in cloudy days was greater for sprouts. This is, in short, sprouts and saplings differ in their architecture and photosynthetic capacity, which could be explained by the regrowth subsidy received from the mother plant versus the autonomy of the saplings.

The dependence on environmental variables and light availability (PPFD) shown by saplings of *E. cordifolia* in the field and the results shown in the nursery for one year old plants under shade condition, suggests that the low daily light availability and water availability determine the survival for this species. Survival in the shadow was determined by the crown level carbon balance, the contribution of each leaf cohort, and the high relative limitation of photosynthesis. In fact, to survive periods of low carbon gain and growth, shade-tolerant species under shade would allocate more photosynthates to reserve than light-demanding (Kobe 1997, Poorter & Kitajima 2007). Under this light environment, there were no architectural nor carbon gain differences at crown level. Thus, not fulfilling the fourth hypothesis. Apparently, a slight decreasing in water availability under such lighting conditions could result in death of the plant due to carbon starvation caused mainly by stomatal limitation of photosynthesis (Chapter III). This was also observed in high availability of light, with a high stomatal limitation (S_L) and insignificant non-stomatal limitation (NS_L). However, the mortality of plants growing under water stress was smaller than in shadow (15 *vs*. 35%) at the end of experiment. By contrast, plants grown with good water supply showed no mortality. It was observed that carbon gain of *E. cordifolia* is directly related with leaf area displayed, and is strongly affected by the light availability. Therefore, mortality of saplings of *E. cordifolia* in the field under shade condition could be attributed to indeterminate periods of water deficit during the growing season, concomitantly with carbon starvation caused by low light availability, and possibly by direct radiation photoinhibition. Differences in assimilation among cohort were not significant, although LC3 (the newest leaf cohort) at full sun showed a greater contribution than LC2 (newer leaf growth cohort of the season). However, in shade condition, LC3 contributed less than LC2. Therefore, at this level, morpho-physiological acclimation on leaf cohorts is not entirely clear. However, the photosynthetic constrains analysis allowed to observe an apparent acclimation of Rubisco activity (V_{cmax}), relative to the control, especially in conditions of high light availability. Thus, the third hypothesis is true, while the fourth and fifth are only partially valid.

Overview

According to the future scenario of reduced precipitation (Fuenzalida et al. 2006), which fits well with the current decreases near the study area (Morales et al. Manuscript in preparation), and the results of this study, it is suggested that field plants each year will be faced with higher water demands and less water availability in the soil, possibly increasing mortality of saplings. Thus, the regenerative dynamics from seed plants may be seriously affected. Therefore, the efficient colonization of the sprouts along with its rapid growth, would allow the persistence of the species in this forest type. Thus, possibly, it is explaining the scarcity and absence of seedlings within some old-growth forests (Aravena et al. 2002, Christie & Armesto 2003, González et al. 2002). However, this may entail greater inbreeding depression, reduced genetic wealth, and greater risks against pests and diseases (Honnay & Bossuyt 2005).

Also, estimating carbon gain at crown level and further specifying the effect of simultaneous stresses on the photosynthetic response during different times of the year is important to expand our knowledge on the dynamics and limitations of regeneration of *E. cordifolia* in these forests. Furthermore, the accuracy in modeling will help predicting the effects of global climate change on regeneration, and also to know the responsiveness of the species in the temperate rainforest diversity against weather conditions. For this it is necessary to make modeling software easier, without affecting the incorporation of more variables (eg induction of photosynthesis - little studied in temperate forest species, leaf temperature compensation, between others) that are determinants of carbon gain per day. Enlarge this annual scale modeling would be helpful in understanding the dynamics of regeneration of different species of these forests.

The understanding and knowledge of daily crown carbon uptake and carbon balance could allow to the foresters to establish guidelines over the management of plants subjected to nurse conditions. The pruning of the oldest leaf cohort during the displaying of the second growth leaf cohort could benefit the crown carbon balance for plants growing under shade. Then, a similar cultural activity could be applied in the understory of the forest, which could benefit the establishment of seedlings and saplings.

General conclusions

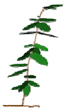
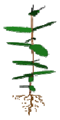
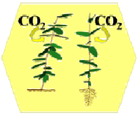
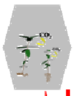
1. Regrowth capacity shown by *E. cordifolia* extends the regeneration niche, especially to where the regeneration of plants from seed is not successful. This happens primarily towards higher canopy openings, compared with saplings.
2. The crown architecture differs between sprouts and saplings. Sprouts showed an architecture which tends to look like a sun plant. The same applies to most foliar attributes studied here. Saplings respond photosynthetically better than sprouts to light and that at crown level is higher (ca. 30%) in saplings during a sunny day. However, less available light in cloudy days determines significantly lower carbon balance than in the sprouts. Sprouts could benefit from the subsidy of water, sugars, and nutrients from the mother plant. Nonetheless, this still requires specific studies to quantify such support given to the sprouts.

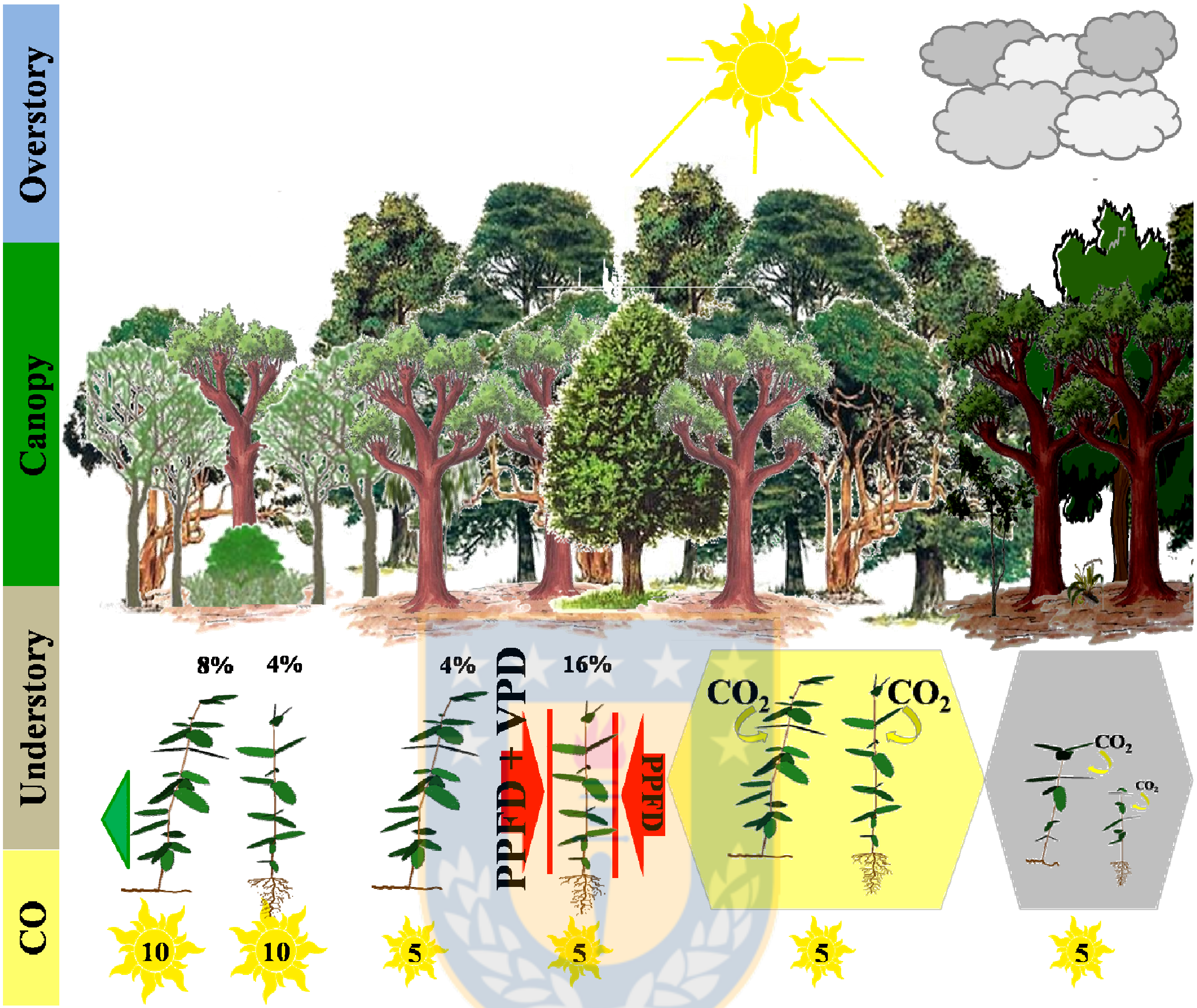
3. The foliar cohorts deployed during decreasing water availability (LC2 and LC3) in full sun (WSHL) showed an apparent acclimation of Rubisco activity (V_{cmax}) because photosynthetic limitation was mainly due to stomatal closure. However, in plants grown in the shade V_{cmax} was much lower than in sun plants. Therefore, it had a greater contribution to the limitation of photosynthesis, especially in plants well hydrated (WWLL).
4. Under low light conditions, both well watered and water stressed plants showed a similar leaf area and STAR, meanwhile crown volume and crown density (AL/AC) showed differences during the growth season. The modeled carbon gain showed differences towards the end of growth season. Water stress provoked a lower daily crown assimilation under low light.
5. Despite that plants under high light showed a higher contribution from the last leaf cohort produced, this does not occur under low light condition. Also, the higher contribution showed in HL could be explained by the increased leaf area of this leaf cohort, which was higher than LC2, independently of some degree of acclimation response to environmental conditions.

Based on the results and conclusions the following models are proposed:

Model 1**:** The first model represents the field situation of the regeneration niche of sprouts and saplings of *E. cordifolia* with respect to light availability (plants without colored background), and daily crown assimilation during a sunny and cloudy day (plants with yellow and grey colored background, respectively) within a secondary temperate rainforest of south-central Chile (Katalapi Park). The *y* axis shows the overstory (including clear and cloudy skies) and the profile of the heterogeneous forest ecosystem (including the canopy and understory). Light availability (canopy openness, CO) is represented according to the canopy density. The lower canopy density allows more light penetration and, inversely, a higher canopy density decreases light penetration across the forest canopy. The *x* axis represents the percentage of CO, where 5 and 10% CO are shown inside a sun with different sizes (the smaller sun for the lower light availability), accordingly to the peaks found for both recruits types (see Chapter II, Fig.2). The regeneration niche of the saplings occurs preferentially at ca. 5% CO, while for sprouts at ca. 10%. High PPFD and VPD (big letters) constrain the regeneration niche breadth of the saplings to the more lighted sites, meanwhile a low PPFD (small letters) does it towards the darker sites. Sprouting allows to extend the regeneration niche within a secondary rainforest towards the more lighted sites (green triangle at the left side of 10% CO sprout).The differences between CO_2 assimilation rates at crown level (DCA) for sprouts and saplings at ca. 5% of CO are represented by the sizes of plants and sizes of CO_2. This is, sprouts and saplings have a similar DCA during a sunny day, but a decreased one during cloudy days, being higher in sprouts (bigger size of plant and CO_2) than saplings(smaller size of plant and CO_2).

Legend of the first model

	Secondary forest profile (Perfil del bosque secundario; representación)
Overstory	Sobre el dosel
Canopy	Dosel
Understory	Sotobosque
CO	Canopy openness (abertura de dosel)
	Sprouts (rebrote root sucker)
	Sapling (planta de semilla)
☀	Sunny day (día soleado)
☁	Cloudy day (día nublado)
	Daily crown assimilation of sprouts and saplings during a sunny day
	Daily crown assimilation of sprouts and saplings during a cloudy day
➡	Environmental constrain
◀	Sprout extend the regeneration niche of *E. cordifolia*



Model 1. Regeneration niche of sprouts and saplings of *E. cordifolia* respect of light availability (canopy openness, CO), and the daily crown assimilation during a sunny day and cloudy day within a secondary temperate rainforest of south-central Chile (Katalapi Park). The density of the plant distribution across the light gradient (CO; suns) within Katalapi Park is represented by the percentages above the plants, which it is relative to the sampled recruit type (see Chapter I, Fig. 2; e.g. the percentage above 5% CO saplings is relative to the total of sampled saplings). Daily crown assimilation is represented for sunny and cloudy days for both recruit types. Note the differences in size between sprouts and saplings in cloudy days, and also in sunny days.

Model 2**:** Crown photosynthetic limitations of *Eucryphia cordifolia* Cav. plants displayed under different light and water availabilities at nursery conditions. The *y* axis shows the light conditions at which plants were grown. The upper *x* axis (ulmo leaves) shows the number of leaf cohorts at two different times after budbreak expressed as days (green cells upon the ulmo leaves). The inferior *x* axis shows the water regime to which plants were subjected during the ontogenetic display, where light blue indicates well watered plants and the transparent light blue the water stressed ones. The plant inside the yellow circle with the scale of 20 cm at the center of the model represents the plants at the beginning of the experiment, showing only one leaf cohort (one year-old). The yellow arrows that start from the yellow circle show the way to the different ontogenetic displays caused by the treatments. Light treatments were full and 7% of sunlight reaching integrated quantum fluxes of 26.6 ± 4.2, and 1.2 ± 0.2 mol m^{-2} day^{-1}. Water treatments were well watered and moderate water stress defined as 0 and 65% decrease in g_s with respect to the control. Horizontally, all the differences shown in the Table 1 are between the light treatments. Also, the contribution magnitude (note the different sizes) from leaf cohorts 2 and 3 accordingly to the trait are represented with the orange (higher contribution) and red (lower contribution) arrows. Vertically, some attributes are shown according to the water treatments. The apparent Rubisco activity acclimation comes from photosynthetic limitation analyses.

Legend of the second model

	3D virtual plants of different light and water regimes (plantas virtuales 3D de diferentes tratamientos de luz y agua)
High light	Alta disponibilidad de luz
Low light	Baja disponibilidad de luz
Well watered	Alta disponibilidad de agua
Water stressed	Baja disponibilidad de agua
Number	Days after budbreack (días después del rompimiento de yemas)
LC1	Leaf cohort 1 (cohorte foliar más vieja, 1 año de edad)
LC2	Leaf cohort 2 (segunda cohorte foliar, la primera desplegada de la temporada, totalmente aclimatada a la luz, parcialmente a disponibilidad hídrica)
LC3	Leaf cohort 3 (cohorte foliar más nueva, desplegada después de la LC2, totalmente aclimatada a luz y agua)

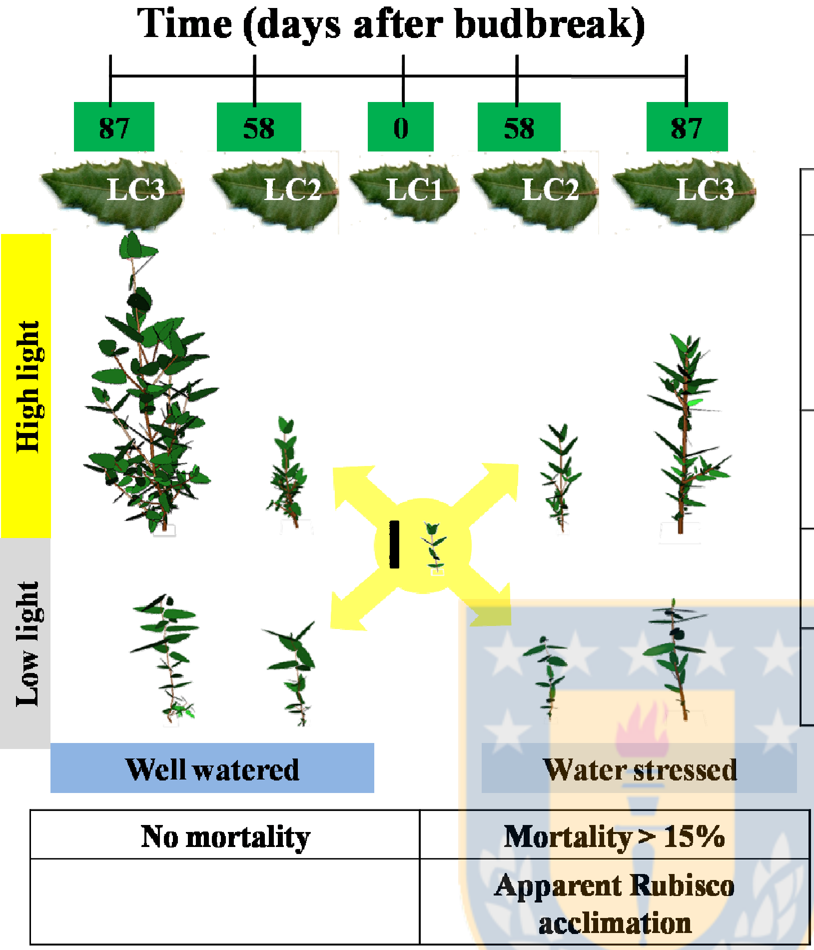


Table 1. Comparison between light treatments and LCs

LC	LA	STAR	DCA	*Vcmax*
3	↑	↓	↑	↑
2	↑	↓	↑	↑
3	↓	↑	↓	↓
2	↓	↑	↓	↓

Model 2. Crown photosynthetic limitations of *Eucryphia cordifolia* Cav. plants displayed under different light and water availabilities at nursery conditions. Architecture is represented from virtual plants captured at several times during the ontogenetic display during a growing season. Differences between light treatments and leaf cohorts are shown in Table 1 inserted at the right of the model. LC: leaf cohort; LA: leaf area; STAR: light interception efficiency; DCA: daily crown assimilation; V_{cmax}: maximum velocity of carboxylation.

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