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ECOPHYSIOLOGY OF ROOT SUCKERING OF TWO TEMPERATE RAINFOREST TREE SPECIES WITH CONTRASTING SHADE TOLERANCE

Tesis presentada a la Facultad de Ciencias Naturales y Oceanográficas de la Universidad de Concepción para optar al grado de Doctor en Ciencias Biológicas, Área Botánica

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Dedicatoria

A todos los estímulos que nos rodean
Porque hacen todo posible

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General Abstract

In this thesis, we addressed to determine the ecological role of root suckering through the ontogeny in tree species as a functional mechanism that shapes the regeneration light niche and allows them to share resources among ramets in a temperate rainforest understory. We evaluated changes and differences of the light regeneration niche, physiological responses to light, functional traits at leaf, stem and crown levels, and their relationships with individual performance of root suckers and saplings using two tree species of differing shade-tolerance that combines vegetative and sexual reproduction mechanisms (hereafter suckers and saplings, respectively): *Embothrium coccineum* (light-demanding) and *Eucryphia cordifolia* (shade-tolerant). Light availability above young recruit-types of each species and understorey were determined to evaluate niche selection and both interespecific and intraspecific (between recruit types) differentiation. Biomass allocation was used to calculate functional traits related to light capture and carbon balance (architectonic, leaf and whole plant traits) and water supply (i.e. stem trai) to infer performance of recruits. Both recruit-types of *Eucryphia* from similar light environment were digitized for crown carbon balance estimations. Recruit-types of both species were measured during one year period and harvested for allometric calculations and dual carbon-nitrogen content and isotopic composition analyses. Leaf carbon and nitrogen concentration and isotope composition were also analyzed along the light gradient. Pulse labeling with carbon ($^{13}CO_2$) was performed in the field to quantify resource transferring between young interconnected

suckers of *Embothrium*. Our results were conclusive in that i) saplings showed functional traits that allow them to minimize water loss by maximizing carbon gains in shaded microsites, whereas opposite trends were displayed by suckers; ii) although suckers and saplings differed in the functional responses during the early stages of ontogeny for both species, root suckering extends the regeneration niche towards open and illuminated microenvironments regardless of their shade-tolerance; iii) root suckers of both species are water and nitrogen subsidized along the light gradient, but light-demanding ramets gain carbon for their own in the shaded-advanced forest succession when the parent tree is in senescent stage. Our results stress that suckers contribute to both the regeneration and persistence of the species in the evergreen temperate forest understorey, likely promoting the coexistence-by-persistence of early and later tree species. To what proportion suckers are subsidized with water, nitrogen and carbon along environmental gradients are still poorly clear, thereby more studies are needed to statement parental support.

General Introduction

Clonal growth and its functionality

For plants, clonal growth is the commonest, cheaper, and quicker propagation way (Klimeš et al. 1997, Vallejo-Marín et al. 2010, Barsanti and Gualtieri 2014), hence this mechanism can provide advantages for regeneration in a given ecological scenario. It is characterized by the formation of new individuals (ramets) from the parental genotype (genet) (Harper 1977, Vallejo-Marín et al. 2010). The genet corresponds to an individual composed of multiple ramets physiologically interconnected, able to share resources, growth and reproduce (Vallejo-Marín et al. 2010). The resulting ramets from the vegetative reproduction, even, can growth independently and clonally propagate after the disconnection from the parent plant (van Groenendael et al. 1996). Hence, clonal growth provides a successful alternative for local regeneration and dispersion when the seedling establishment is low or null, functioning as a multiplicative system of plant population (Jones and Raynal 1986, Koop 1987, Pennings and Callaway 2000, Del Tredici 2001, Wiehle et al. 2009, Long and Mock 2012, Escandón et al. 2013, Lucena et al. 2015).

The clonal growth is an important strategy for regeneration in ecosystems prone to high-intensity and frequent disturbances (van Groenendael et al. 1996, Clarke et al. 2005, Clarke et al. 2013, Pausas et al. 2016), but also when disturbances are low in intensity and frequency, or even imperceptibles (Muñoz and González 2009, Shang et al. 2015). The clonal growth by root suckering, this is the generation of new-secondary stems from shallow lateral roots (Del Tredici and Orwig 2017, Martínková et al. 2018), has shown at least three different ecological functions:

1) Population persistence, through the vegetative regeneration, allowing to the plant individual inhabit a site for longer time (e.g. Long and Mock 2012).
2) Resource acquisitions and sharing between ramets within a genet, especially in sites with heterogeneous distribution of resources (Roiloa et al. 2014 and references therein).
3) Colonization, as the process of occupation of a disturbed site, exposed and without vegetation, from external propagule sources (e.g. Grashof-Bokdam and Geertsema 1998, Chapin et al. 2011, Pausas et al. 2016).

Clonal growth typically shows high survival rates of ramets during the establishment under suboptimal conditions for sexual regeneration, due to resources sharing, provoking the persistence of a population. For instance, even extreme, *Populus tremuloides* clones, "Pando", in the Rocky Mountains of the central United States are estimated to be of Pleistocene age as a result of root sprouting (Lambers et al. 2008), being the largest organism in existence (Grant et al. 1992). Also, due to the persistence, clonal growth functions as a mechanism of both expansion and recruitment beyond the mother plant and population growth (Bond and Midgley 2003, Lucena et al. 2015), often at expenses of the parental support. As the failure of sexual recruitment would be due by inadequate environmental conditions and/or resource scarcity, the regeneration niche of a species that combine the reproduction mechanism must be shaped in relation to the differential abundance and distribution of recruit-types (i.e. clonal and seed origin plants) under such given microsite (Grubb 1977, Poorter 2007).

In trees, root suckers can be placed until 40 m faraway from mother plant, allowing for species expansion towards new different light environments (Bond and Midgley 2003, Wiehle et al. 2009). However, also can occur grouped and near ~8 m from parent tree (Jones and Raynal 1986). At first sight, it seems that regeneration mechanisms are spatially differing distributed. But, what about environmental conditions (i.e light, temperature, water among others) in those new microsites occupied by suckers compared to that occupied by sapings? Although a couple of demographic studies of suckers (clonal origin) and saplings (seed origin) in deciduous forest does not demonstrated distribution trends (*Fagus grandifolia*, Beaudet and Messier 2008; *Asimina triloba*, Hosaka et al. 2008), other has showed that root suckers of *Populus tremula* (light-demanding tree species) were distributed mainly inside large gaps or at the edges of canopies, but they did not compare against seed origin plants. One study in a temperate evergreen rainforest showed light niche differentiation and extension by suckers in the shade-tolerant tree species *Eucryphia cordifolia* (Escandón et al. 2013). Therefore, saplings and suckers would differ in their occurrence in light gradient, driving the shape of the regeneration light niche more markedly in coexisting species from light-limited forests. Hence, to verify to what extent changes in light niche between suckers and saplings depend on the species shade-tolerance, it is needed to consider its effective recruiting.

Effects of environmental heterogeneity and parental subsidy on plants

As consequence of the environmental heterogeneity and variable distance from the parent, ramets could establish under different climatic-resource conditions than parent and other ramets. In this scenario, resource acquisition depends of the quality of the microsite and the biomass allocation to specialized organs for its capture (Stuefer 1998, Roiloa et al. 2007, Hutchings and Wijesinghe 2008). Often, physiological connected ramets of herbs show increments in total foliar area and biomass when light is the abundant resource when compared to severed ramets or those that growth under homogeneous conditions, and also to saplings (Stuefer et al. 1996, Roiloa et al. 2014, Escandón et al. 2018). Thus, a first effect of parental subsidy is the differential allocation biomass of ramets and seed origin plants. This effect has been well supported and explained due to water, sugars, and nutrients translocation between ramets, resulting in total biomass increments and survival probability (Schmid 1990, Oborny and Bartha 1995, Stuefer et al. 1996, Roiloa and Retuerto 2007, Roiloa et al. 2014). Besides, in trees is still hard to assess other ecological roles of ramets. Together with the long-time connection between ramets and parents (at least 17 years, Jones and Raynal 1986), parent tree can supply resources, keeping new ramets alive in shaded understory microsites at a low cost (Kowarik 1995, Peterson and Jones 1997).Water sharing between ramets of

Populus balsamifera (shade-intolerant tree) is also governed by water abundance, which allows to rooting connected-ramets grown under low water availability to maintain high leaf gas exchange rates and water potentials, although each one of the connected ramets had an own root system (Adonsou et al. 2016). Thus, it is expectable that clonal growth contributes to regeneration and/or persistence and/or resource acquisition in harsh environments for seedling establishment, resulting in extension and growth of plant populations (Wiehle et al. 2009, Pinno and Wilson 2014). However, this later-second effect still requires more empiric evidence from the forest.

Based on the previous, root suckers could contribute proportionally to the mentioned ecological functions (see avobe) in accordance with spatial heterogeneity in resource availability. For instance, in a temperate rain climate such proportion would be determined by light availability, as a limiting factor for carbon gain (e.g. Lusk 2002) necessary for plant establishment, growth and survival. A root-parental connected sucker inhabiting in high light would reach high levels of photochemistry efficiency and chlorophyll content (Roiloa and Retuerto 2007) and would gain carbon enough to cover its maintenance costs and share some carbon to other suckers and/or to the parent. Contrary, a sucker inhabiting under shade would show different or opposite traits, reflecting a regeneration mechanism rather than resource acquisitive one, being supported by the parent or other suckers which crowns can access to more light (Hartnett and

Bazzaz 1983, Pearcy et al. 1987). In that way, it is possible to highlight the ecological role of suckers in trees.

Additionally, root suckers had showed grater rates of growth than saplings in similar light conditions (González et al. 2002, Beaudet et al. 2007, Beaudet and Messier 2008, Muñoz and González 2009). However, Farahat and Lechowicz (2013) cannot explain it from some leaf -worldwide –traits linked to net carbon gain, suggesting that it might result due to parental support. But this idea still needs to be empirically tested. Therefore, a third effect is that parental support of suckers can determine differences in the physiological and morpho-architectonical responses with saplings, invoking variation in plant fitness, which shapes the niche of the species.

Ecological strategies and parental subsidy: from shade-tolerance point of view

Root suckering could be an important strategy in temperate evergreen forest than deciduous forests (e.g. Beaudet and Messier 2008), in terms of number of species in which it is present and its relative abundance. Specifically, in a temperate rainforest, suckers can be present in 60% of the species composition of a forest (see Table GA1) and reach a 100% of abundance (González et al. 2002). Those tree species differ in shade-tolerance, being suckering important for *Laureliopsis philippiana*, *Eucryphia cordifolia*, *Gevuina avellana* and *Embothrium coccineum* (ordered from very shade-tolerant to shade-intolerant) (González et al. 2002, Muñoz and González 2009).

Differences in shade-tolerance are based on functional traits displaying mainly in response to light availability (Valladares and Niinemets 2008). Functional traits in plants are defined as any morphologic, physiologic and/or phenotipic characteristic measurable at individual level, which determines its response capacity to external factors, influencing its performance (Violle et al. 2007, Valladares et al. 2007). Moreover, functional traits global patterns along environmental gradients are mainly based in woody species (Poorter and Remkes 1990, Villar et al. 2004, Klimešová et al. 2015). Some traits are related with the regeneration niche of plant species at the seedling stage (Poorter 2007). In a resource gradient, plants with clonal growth would show functional traits adjusted to the resource quality of the microsite (see Hutchings and Wijesinghe 1997, He et al. 2011, Sterck et al. 2011), allowing them maximizing the resource acquisition efficiency (Stuefer 1998, Roiloa et al. 2014). Therefore, functional traits have been successfully used to explain shade-tolerance differences between plant species. Then, studying functional traits of suckers comparatively to saplings will show us how the functional strategies can change along a resource gradient (Westoby and Wright 2006).

Functional traits can be measured at shoot, root and whole plant level. In seed origin plants, at leaf level, the specific leaf area (SLA), leaf chlorophyll content and leaf size influence light interception efficiency and capture for photochimestry in relation with leaf construction and maintenance defining the carbon gain. While, at whole plant level, the leaf area ratio (LAR) and leaf mass fraction are determinant of displayed leaf area for light capture, longevity and toughness in relation with the total plant biomass. Moreover, at shoot level, the specific stem density (SSD) and slenderness are related with the security in the water transport to leaves and evidence a strategy for shade avoidance. SLA and LAR are commonly negatively correlated with light intensity, but positively with the relative growth rate (RGR) (Poorter and Remkes 1990, Lusk 2002, Villar et al. 2004). These traits vary with the light availability and ontogeny, defining the species shade-tolerance (Lusk 2002, Lusk et al. 2008), thereby RGR is specifically modulated (Lusk and Jorgensen 2013). However, functional traits of suckers can differ from those of seed origin plants even under similar light availability (Escandón et al. 2013, Farahat and Lechowicz 2013, Escandón et al. 2018), reflecting otherwise ecological function. Until now, there are no studies that evaluate comparatively suckers and saplings functional responses along a light gradient on species differing in their shade tolerance.

Isotopic approach to assess the early functional role of suckers

Early and late successional tree species can combine clonal and sexual reproduction in both second and old-growth temperate forests (Jeník 1994, Del Tredici 2001, Deiller et al. 2003), likely promoting the species coexistence and resource capturing. As already said, the higher RGR of suckers compared to saplings was practically not related to leaf-level functional traits (Farahat and Lechowicz 2013), as occur in herbaceous plants (Klimešová et al. 2015). In seed origin plants, the water use efficiency (WUE) increase with decreasing water availability and increasing temperature along a light gradient, due to the stomatal closure. Stomatal closure induces the reduction of intercellular CO_2 concentrations within the leaf (due to consumption) and an isotopic fractionation mediated by RuBisco: less discrimination against the heavier ^{13}C (O'Leary 1981, Farqhuhar et al. 1982, Ehleringer et al. 1986). After that, positive correlations have been found between natural abundance isotopic composition of ^{13}C ($\delta^{13}C$) and light availability in C3 plants (Zimmerman and Ehleringer 1990, Broadmeadow et al. 1993, O' Leary 1995, Yakir and Israeli 1995). Otherwise, $\delta^{15}N$ have been used to differentiate the N sources for plant uptake. Although no apparent patterns in plants for $\delta^{15}N$ along a light gradient have been reported (see Heikoop et al. 1998 for other organism), it can be expected that $\delta^{15}N$ of seed plants being positively related to light availability (i.e. decreasing negative $\delta^{15}N$ values), because nitrogen from soil solution can be constrained with increasing canopy openings (positively related with light availability) in temperate forests (Godoy et al. 2001). Nitrogen availability and ectomycorrhyzal symbioses are sources of variation in $\delta^{15}N$ for plants (Hobbie and Colpaert 2003). For instance, plants that experience greater N availability may reduce their dependence on mycorrhizal fungi. This reduced dependence on mycorrhizal fungi can enrich plants by reducing the depletion associated with N transfers from mycorrhizal fungi (Högberg et al. 2011). Then, assuming hypothetically a high dependence in the roots-parental connection, carbon gain of ramets would differ in the sink strength in accordance to the resource abundance and comparatively against saplings, whereas relative to N, leaf $\delta^{15}N$ may differ both between species in accordance to the uptake strategy and suckers may do not respond to light availability as probably do saplings, because young root-lacking suckers (field observation) depends on parent subsidy.

Also, some of those interconnected ramets can act as sources when inhabiting rich-lit sites, whereas others do as sinks in poor-lit sites (Magda et al. 1988). To measure the carbon transfer direction and its amount among ramets had been possible by using pulses of a stable isotope of carbon ($^{13}CO_2$). For instance, the carbon transfer was higher when clonal, and mycorrhizal connections were present (Deslippe and Simard 2011). Other study evidenced that carbon transfer occurs among ramets towards the light-limited one (Magda et al. 1988). Generally, leaves of shade-intolerant species have higher rates of CO_2 assimilation at saturated light than shade tolerant (Niinemets et al. 1998, Lusk 2002). Accordingly, shade-intolerant species allocate more N to leaves for photosynthetic functions (Niinemets et al. 1998). By other hand, the shade-tolerant species concentrate more carbon in their leaves than shade-intolerants (Niinemets and Kull 1998). In this regard, as it is unclear whether suckers can act as a sink and/or harvester of the abundant resource, natural isotopic abundance of suckers and saplings could answer this question. Whereas sink-suckers under low light are likely subsidized by those source-suckers under less-limited light, changes in isotopic composition are expectable when pulse labeling is done over the potential source.

Approaching to the research problem

To study where root suckers are succesfully growing and how they are responding to resources availability comparatively to saplings in the forest understory is crucial for understand its importance and contribution, in relative terms, to the ecological functions and parental subsidy through the early ontogeny of species differing in shade-tolerance. For this, we choose to work in the temperate rainforest of South-central Chile, due its gap-phase dynamic means low to imperceptible perturbation scales (Yamamoto 2000, Gutiérrez et al. 2004), offering a wide light gradient although being dark (Lusk 2002, Lusk et al. 2006); and because is mainly composed by evergreen trees differing in shade tolerance that can regenerate combining sexual and clonal reproduction (see Table GA1). *Embothrium coccineum* J.R. et. G. Forster (root-cluster Proteaceae) and *Eucryphia cordifolia* Cav. (arbuscular mycorrhizic symbiont Cunoniaceae) are two evergreen tree species able to recruit both sexual (by anemochory seed dispersion) and vegetatively through root suckering (Lusk 2002, González et al. 2002). Light availability is one of the most heterogeneous resources at the understory in this forest-type (Lusk 2002, Valladares et al. 2012). At early ontogenetic stages of development, these two species show contrasting strategies of shade-tolerance, being *Embothrium* a light-demanding and

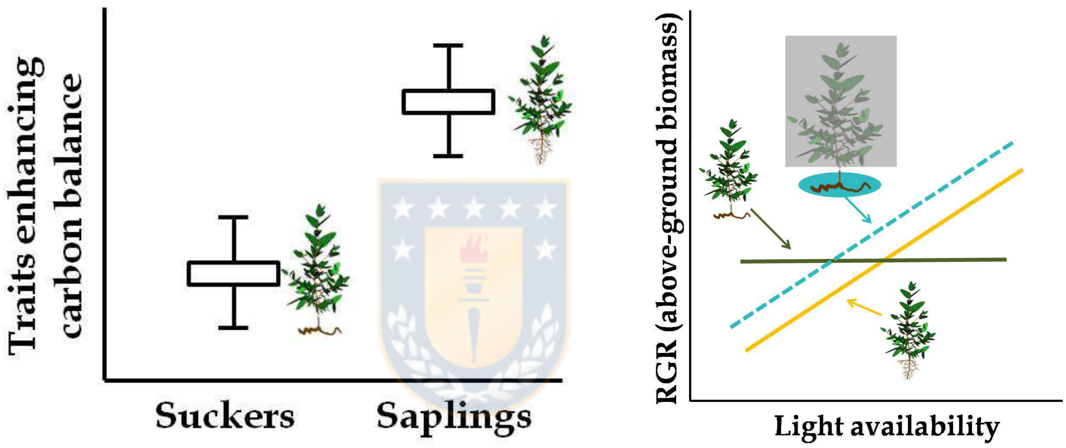
Eucryphia a shade-tolerant (Lusk 2002, Lusk and Del Pozo 2002). *Embothrium* preferably germinates in forest gaps, suffers high mortality under dark conditions, and presents high light compensation points, whereas *Eucryphia* performs in an opposite way (Figueroa and Lusk 2001, Lusk and Del Pozo 2002, Lusk 2002). Generally, leaves of light-demanding species have higher rates of CO_2 assimilation at saturated light, requiring large inputs of nitrogen and other mineral nutrients to create the pools of enzymes and pigments needed to sustain those rates than shade-tolerant (Field and Mooney 1986, Niinemets et al. 1998, Lusk 2002). Accordingly, light-demanding species allocate more N to leaves for photosynthetic functions (Niinemets et al. 1998). By other hand, the shade-tolerant species contain more carbon in their leaves than light-demandings increasing leaf life span (Niinemets and Kull 1998, Lusk 2002). These differences make to expects that clones of shade-intolerant tree species will occupy the darker side of the light gradient, whereas the opposite will occur in shade-tolerant. Also, it will be expectable strong differences in leaf C and N content and natural $\delta^{13}C$ and $\delta^{15}N$ between species. The light-demanding *E. coccineum*, because of its higher nitrogen demands in high light (for growth and photosynthetic machinery), must show a lower leaf C content, and higher content of N, $\delta^{13}C$ and $\delta^{15}N$ (due to lesser isotopic fractionation) with the increasing light availability compared to the shade-tolerant *Eucryphia*. Functional traits at different plant levels likely differ between recruit-types and species, changing the distribution pattern with the ontogenetic trajectory due to that suckers can access to the parental supply of resources, whereas saplings are independent plants. Therefore, here we are determining the light regeneration niche of two contrasting and coexisting tree species, deepening in the early and effective recruitment. Moreover, accordingly to the different carbon allocation strategies between recruit-types as a consequence of responses to light availability, it is expectable that suckers allocate more to active photosynthetic tissue if they are faith to capture more light and potentially more carbon for translocating it to shaded suckers. Alternatively, suckers could not show any trend of resource capture if they are contributing to regeneration and local persistence. Overall, we are inferring the functional role of suckers, different to regeneration as previously reported (González et al. 2002, Gutierrez et al. 2004, Muñoz and González 2009, Escandón et al. 2013).

This study is framed in the promising research field on clonal plant biology, due the booming interest on the "belowground ecology" (Klimešová et al. 2018) and the lack of studies and knowledge focused on suckering of the trees species of the temperate rainforest. Additionally, the compilations of global databases on plant functional traits have revealed the enormous lack of knowledge on clonal growth, despite its relevance on key ecological functions as on-spot persistence, space occupancy, and post-disturbance regeneration. Although recent efforts have significantly increased the information on clonal growth in herbs, much less is known about woody plants. This is especially due to the difficulty involved in using woody plants as a study system for clonal growth measurements.

Hypothesis, predictions and objectives

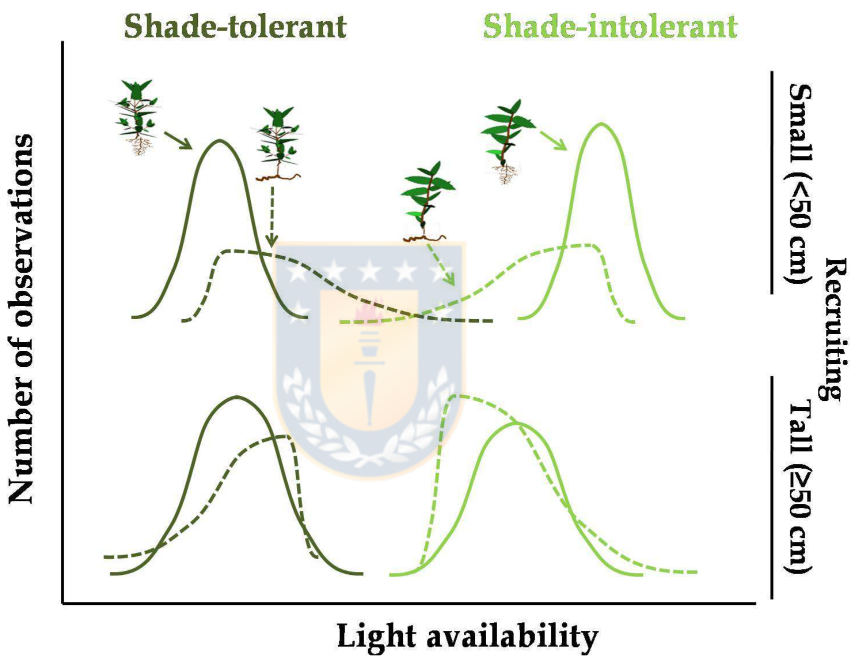
Hypothesis 1: As parent subsidy likely modulates the physiological and architectural adjustments of ramets, saplings will have traits that enhance the daily crown carbon balance, whereas above-ground growth will be more limited by light and water than that of root suckers.

Predictions:



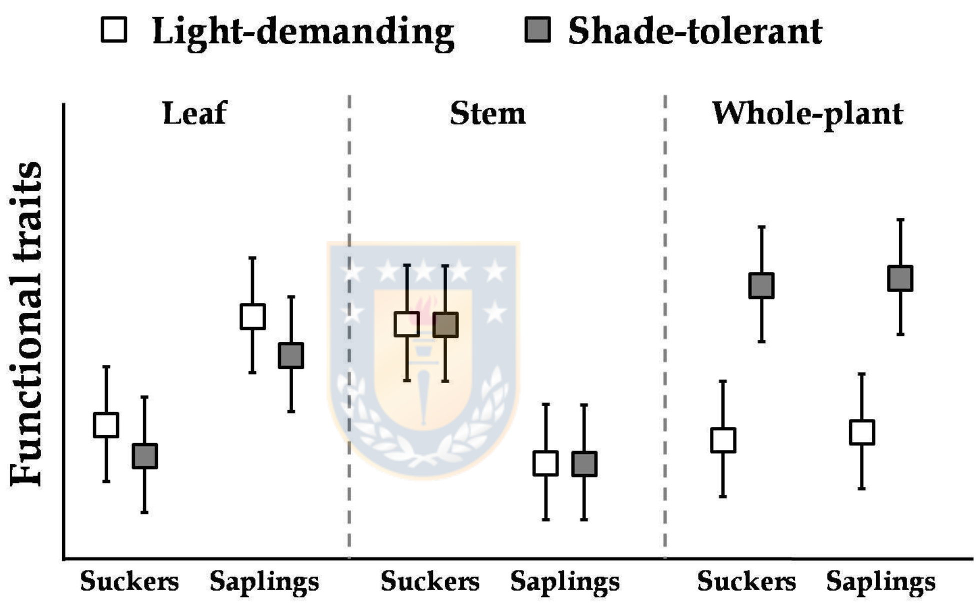
Hypothesis 2: The recruits of the two species selects a part of light gradient of the forest, thereby the suckers shape the niche extending it towards suboptimal conditions for sexual regeneration, which will be notably in very young plants.

Predictions:



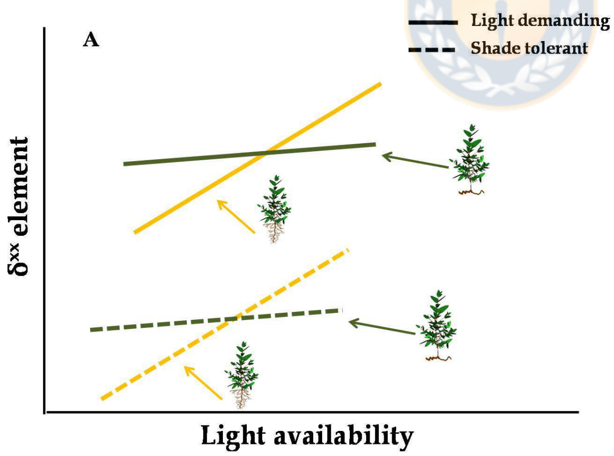
***Hypothesis* 3:** Assuming carbon sharing between ramets, the variation of morphologic and biochemical traits in organs related to resource capture will be more strongly pronounced in saplings than in suckers, whereas leaf and whole-plant traits will differentiate species shade-tolerance level.

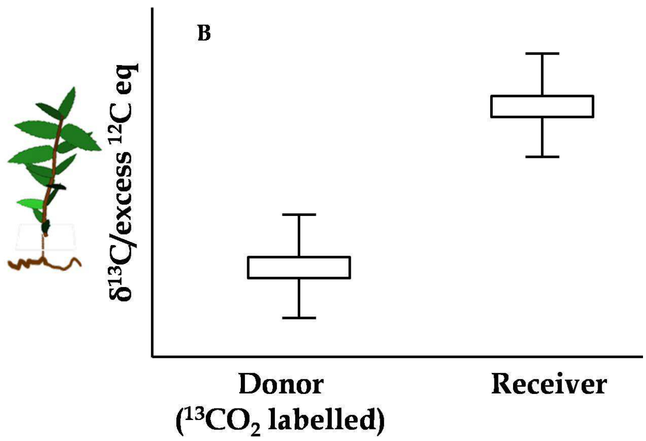
Predictions:



Hypothesis 4: Assuming resource translocation by interconnected parent-ramets and that there are differential patterns in functional traits and performances between root suckers and saplings of each species along the light gradient, root suckers will have higher $\delta^{13}C$ than saplings of each species as consequence of ^{13}C enriched translocation even across the light gradient; whereas leaf $\delta^{15}N$ must be higher in *Embothrium* root-clustered than *Eucryphia* arbuscular mycorrhizic species and more responsive to light in saplings than sucker given they differing resource sources (solution soil vs parent tree), without necessarily vary in average because intraspecifficaly they own the same resource acquisition strategy.

Predictions:




B
δ13C/excess 12C eq
Donor
(13CO2 labelled)
Receiver

General objective

To determine the ecological role of root suckering through the ontogeny in tree species of differing shade-tolerance as a functional mechanism that shapes the regeneration light niche and shares resources in a temperate rainforest.

Specific objectives

- To understand the underlying proccesses that permits the extension of the regeneration light niche of a *Eucryphia cordifolia* Cav. (H1).
- To describe the distribution pattern of suckers in the regeneration light niche of contrasting shade-tolerant tree species in a temperate rainforest (H2).
- To determine and compare the resource allocation and relative growth rate of suckers and saplings in the light regeneration niche and its trends through ontogeny (H3).
- To evaluate carbon and nitrogen transfer between interconnected ramets to understand the ecological role of suckering in woody plants (H4).

CHAPTER I

Physiological differences between root suckers and saplings enlarge the regeneration niche in *Eucryphia cordifolia* Cav.

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Keywords: climate, daily crown carbon balance, parental supply, tree ring growth, water use efficiency.

Abstract

Many clonal plants produce vegetative recruits that remain connected to the parent plant. Such connections permit resource sharing among ramets, explaining the high survival rates of vegetative recruits during establishment under suboptimal conditions for sexual regeneration. We propose that differences in the regeneration niches of sexual and vegetative recruits reflect different physiological adjustments caused by parental supply of resources to the ramets. We conducted ecophysiological measurements in saplings and root suckers of *Eucryphia cordifolia*, a tree species of the temperate rainforest of southern South America. We compared the following traits of saplings and suckers: gas exchange at the leaf level, crown architecture, daily crown carbon balance, biomass allocation to above-ground tissues (leaf-to-stem mass ratio, leaf mass area, and leaf area ratio), xylem anatomy traits (lumen vessel fraction, vessel density and size) and stem ring width. We also correlated the growth rates of saplings and suckers with relevant environmental data (light and climate). Saplings showed morphological, architectural and physiological traits that enhance daily crown carbon balance and increase water use

efficiency, in order to supply their growth demands while minimizing water loss per unit of carbon gained. The radial growth of saplings diminished under dry conditions, which suggests a strong stomatal sensitivity to water availability. Suckers have low stomatal conductance, likely because the carbon supplied by the parent plant diminishes the necessity of high rates of photosynthesis. The low responsiveness of sucker growth to temporal changes in water availability also supports the existence of parental supply. The physiological differences between sexual and vegetative recruits satisfactorily explain the ecological niche of *E. cordifolia*, with saplings restricted to more closed and humid sites.

Introduction

Many clonal plants produce vegetative recruits that remain connected, temporarily or permanently, to the parent plant. These connections permit the parent plant to share resources among ramets, in what is referred to as clonal integration (Alpert and Mooney 1986, de Kroon et al. 1996, Zhang et al. 2002, Saitoh et al. 2006, Pinno and Wilson 2014). The physiological integration among ramets explains the high competitiveness of some clonal plants with their neighbours (Otfinowski and Kenkel 2008, Roiloa et al. 2010, Liu et al. 2016). Clonal integration also permits ramets to colonize stressful microenvironments where sexual reproduction is unsuccessful (Penning and Callaway 2000, Peltzer 2002, Escandón et al. 2013). Access to parental resources during establishment possibly increases the chances of survival of clonal recruits (Kirby 1980, Hartnett and Bazzaz 1983, Wiehle et al. 2009).

Parent subsidy likely modulates the physiological adjustments of the ramets, and thus they would differ physiologically from sexual recruits of a similar developmental stage. The supply from the parent plant might enhance the water and nutritional status of ramets, leading to higher gas exchange rates (Alpert 1990, Roiloa et al. 2014). Ramets that have access to parental photoassimilatescould hypothetically down-regulate photosynthesis due to the low requirements of their carbon sinks (Alpert and Mooney 1986, Zhang et al. 2002). Low carbon assimilation at high transpiration rates would lead to lower water use efficiency in ramets compared to saplings of a similar size.

Sapling growth and survival relies entirely on the ability of the sapling to acquire carbon, water and mineral nutrients. It is to be expected that saplings will have high carbon assimilation rates in order to satisfy their high growth demands (Bond, 2000, Thomas and Winner 2002). However, if high carbon gain is attained by increasing the stomatal conductance, saplings can reach low water potentials at dry microsites. Saplings are therefore at much greater risk of hydraulic failure than are ramets connected to larger parental root systems. Because of this, saplings are expected to have high stomatal responsiveness to water availability, with the known negative consequences in terms of carbon gain (McDowell et al. 2008). Consequently, the growth and survival of saplings will be more dependent upon environmental conditions.

Eucryphiacordifolia Cav. is a long-lived tree species of the temperate rainforest of southern South America that recruits both sexual and vegetatively (Escobar et al. 2006). Vegetative recruitment consists of the formation of root suckers that usually remain connected to the genet, and tend to occupy more open and drier microsites than the saplings (Escandón et al. 2013). Water and nutrient uptake by the root suckers is entirely dependent on the parent plant because the ramets lack an independent root system. The large root system of the parent plant protects root sprouts of *E. cordifolia* from exposure to low water potentials, explaining their ability to colonize drier microhabitats (Escandón et al. 2013).

Sexual recruits of *E. cordifolia*lack prominent root systems during the sapling stage. Survival rates of saplings fall from 50 to 20% with a canopy openness factor of greater than 2%, with smaller plants suffering higher mortality. This result suggests that the higher evaporative demand of open microsites (VPD $\geq$ 0.6 kPa) promotes drought-induced mortality (Escandón et al. 2013). Accordingly, significant mortality in *E. cordifolia* saplings has been reported after reduction of 37% of the soil water content in relation to the field capacity (Morales et al. 2014). These results suggest that saplings of *E. cordifolia* are highly sensitive to water stress, explaining why they occupy less open and more humid microsites than root suckers.

In this study we evaluate ecophysiological differences between suckers and saplings of *E. cordifolia*, considering the reported differences in the niche regeneration between both recruit types, and the possible parental supply to the suckers. We predict that saplings have morphological, architectural and physiological traits that enhance the daily crown carbon balance, whereas root suckers assimilate comparatively lower amounts of carbon and have lower water use efficiency. We also expect that the growth responses to environmental variability depend on the recruit type, with the above-ground growth of saplings being more limited by light and water than ofroot suckers.

Materials and methods

Study site

This study was conducted in a 30 ha 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.). Katalapi Park hosts young regenerating forests with remnants of old-growth forest, and has been protected during the last 27 years from anthropogenic alterations (logging and cattle) to promote its natural regeneration. The most frequent tree species are *Nothofagus nitida* (Phill.) Krasser, *Laureliopsis philippiana* (Looser) Schodde, *Caldcluvia paniculata* Cav., *Eucryphia cordifolia* Cav., *Drimys wintery* J.R. et G. Forster, and several Myrtaceae and Proteaceae (Lusk and Corcuera 2011).

This area has a temperate maritime climate. Annual rainfall of ca. 1900 mm is concentrated from April to November, with a mild dry season from December to March (see details in Fig. S1, Supplementary Data available at Tree Physiology Online). During the dry season the mean air temperature reaches 15ºC, the minimum relative humidity of the air ranges between 45-55% and a 15-day-long dry period frequently occurs every summer. The maximum photosynthetic photon flux density (PPFD) ranges between 1500 µmol of photons m^{-2} s^{-1} and 7 µmol of photons m^{-2} s^{-1} at (respectively) 35% and 5% of canopy openness (i.e., the percentage of unobscured sky over a given point). For more details of the study site see Escandón et al. (2013).

Plant material and field measurements

In November 2012, we established a 50 × 50 m plot in the study site where *E. cordifolia* recruits both sexual and vegetatively. The canopy openness of the plot ranged between 0.7% and 13.4% averaging 5.3 ± 3.3% (mean ± SD), which is within the regeneration niche breadth of this species (Escandón et al. 2013). All *E. cordifolia* recruits under 1.5 m in height within the plot were identified as either root suckers or saplings. In order to classify the origin of the recruits, the root collar was carefully dug, and the root systems were observed. Specimens were identified as suckers when they were connected to a large woody lateral root, and as saplings when no subsidiary root connection or root scar indicating past connection was observed (Escandón et al. 2013). We identified 26 suckers and 29 saplings in the plot. Although it was not possible to identify the parental tree of each of the suckers (as a deep excavation could damage the recruits), we verified that all adult trees within the plot produced root suckers. This was done by digging one lateral root from each root collar until vegetative recruits were encountered.

The light environment of each recruit was estimated by means of canopy openness. A hemispherical photograph was taken over each plant apex during homogeneous, overcast conditions (Chazdon and Field 1987). The photographs were taken using a Coolpix 4500 digital camera equipped with a FC-E8 fisheye lens (Nikon, Tokyo, Japan). The camera was hand leveled and oriented so that the top of the image faced north. The photographs were analyzed for the percentage of canopy openness with the Gap Light Analyzer 2.0 software (GLA, Frazer et al. 1999). The canopy openness was slightly higher for saplings (4.6 ± 2.7 and 6.4 ± 3.3% for suckers and saplings, respectively), but it did not significantly differ among recruit types ($F_{1,\ 53}$ = 4.10, P = 0.067; response variable log-transformed). The homogeneity in the light environment allow us to compare ecophysiological measurements between the saplings and the root suckers growing within the plot.

The measurements described in the next sections were conducted in May 2013, at the end of the growing season for the study area.

Leaf gas exchange, chlorophyll content and optical properties measurements

Leaf gas exchange was measured with an IRGA Li-6400 (LiCor, Inc., Lincoln, NE, USA) during the morning (9.30 to 14.30), within the period when the maximum photosynthetic rate occurs for this species (Morales et al. 2014). We performed photosynthetic light response curves (A-Q) on fully expanded (one-year-old) leaves of both suckers and saplings growing within the study plot (three individuals per recruit type, one leaf per individual). Ten different light intensities between 1000 and 0 μmol photons m^{-2} s^{-1} were used at 400 μmol CO_2 mol^{-1} air, 17 ± 0.7°C (leaf temperature), and 65 ± 5% relative humidity within the leaf cuvette. Leaf photosynthesis was previously stimulated with ca. 200 μmol photons m^{-2} s^{-1} (light quality proportion of 85, 10, 2, and 3% red:blue:orange:yellow, respectively) using an LED lamp. Due to the low photosynthetic rates of this species and the small area (2 cm^2) of the cuvette, the flow rate was adjusted from 100 to 200 ml min^{-1} to ensure that CO_2 differentials between the reference and the sample IRGA were > 4 μmol mol^{-1} air. Photosynthesis software (Li-Cor Inc., Nebraska, USA) was used to determine the following A-Q curve parameters for each recruit: maximum net assimilation rate based on area (A_{max}), maximum quantum yield (AQE), light compensation and saturation points (LCP and LSP), and curvature factor (θ). These parameters were averaged for each recruit type and used to model the daily crown assimilation (see below).

Instantaneous light-saturated assimilation rate (A_{SAT}) and stomatal conductance (g_s) were measured at 500 µmol photons m^{-2} s^{-1} and 400 µmol CO_2 mol^{-1} air; such photosynthetic photon flux density (PPFD) is above the LSP and is not photoinhibitory (see A-Q curves, Fig. 1A). The leaves used to measure A_{SAT} were then kept in the dark for 60 minutes and dark respiration (R_d) was measured at 0 µmol photons m^{-2} s^{-1}. These measurements were conducted on 11 suckers and 9 saplings over three fully expanded one-year-old leaves, using the same leaf temperature and relative humidity within the cuvette as used to construct the A-Q curves. A_{SAT}, g_s and R_d were standardized by the IRGA leaf cuvette area (i.e., expressed on a leaf area basis). The intrinsic water use efficiency ($_i$WUE) was calculated as the ratio between A_{SAT} and g_s.

The relative chlorophyll content of three fully expanded leaves uniformly distributed along the stem was measured using an SPAD-502 Plus (Konica Minolta Optics, Inc., Osaka, Japan). Leaf optical properties (transmittance, reflectance and absorbance) were measured with the light source of the IRGA cuvette using a spectroradiometer (HR2000CG-UV-NIR; Ocean Optics Inc., Dunedin, USA) following Gago et al. (2013).

Plant crown architecture

The northern side of each recruit within the study plot was marked in order to later simulate the light environment. The recruits were carefully excavated with ca. 30 × 30 × 25 cm of soil in order to extract a substantial part of the root system. Plants were then immediately put into containers and watered to field capacity in order to maintain their architectural traits. The plants were then carried to the field station (located 500 m from the plot) to be digitized. Measurements were taken of the stem ends, both basal and apical. Petiole diameter was measured of leaves at low, middle and high positions in the crown. All measurements were taken with a digital caliper. We then created an average leaf shape on a flat surface over cartesian coordinates centered in the leaf blade base, considering at least 25 points of the leaf border. This leaf model was used to populate the nodes of each virtual plant (see below). Finally, the position (3D coordinates) of each branch and leaf node in the crown was recorded using the 3D FASTRACK®-digitizer (Polhemus, Colchester, VT, USA), with the FLORADIG software (CSIRO Entomology, Brisbane, Australia). Virtual plants were constructed with the YplantQMC package of the R software (Duursma and Cieslak 2012), which uses the same "plant" and "leaf" files as the 3D plant model YPLANT (Pearcy and Yang 1996).

The crown architecture of each virtual plant (see examples in Fig. 2) was described by means of the following variables: total plant leaf area (A_L), total surface area of the 3D convex hull wrapped around the leaf cloud (A_C), projected (A_P) and displayed (i.e., exposed) leaf area (A_D) averaged over the entire hemisphere. We calculated the self-shading (SS) as $(A_P-A_D)/A_P$ (Lusk et al. 2012) and the crown density (CD) as the ratio of A_L to A_C (Duursma and Cieslak 2012). The light interception efficiency was estimated as the "silhouette to total area ratio" ($\overline{STAR}$) that is the ratio of A_D to A_L averaged over the entire sky hemisphere. Finally, we calculated the leaf dispersion (LD) as the average ratio of the observed mean distance from each leaf to the five nearest leaves, divided by the expected value if the leaves were randomly located (see Duursma et al. 2012 for more details).

Daily crown assimilation modelling

The daily crown carbon assimilation was estimated for each recruit by means of the package YplantQMC run using the R software (Duursma and Cieslak 2012). We used the crown architecture, the hemispherical photography data, and the leaf optical properties of each recruit as inputs. As light response parameters we used the mean values of A_{max}, AQE, and θ for each recruit type, (i.e., suckers and saplings) obtained from their respective A-Q curves. For the environmental conditions, minimum and maximum temperature and daily photosynthetically active radiation (i.e., integrated quantum flux) above the canopy were set according to data registered at 4-6% of canopy openness during an average clear day (i.e., PAR>1500 μmol photons m^{-2} s^{-1}). This canopy openness represents the average value for all of the studied plants. These data were obtained from a H21-002 HOBO meteorological station connected to S-LIA-M003 and S-THA-M0xx sensors (Onset, MA, USA). Clear days were identified by the PAR recorded with a Li-1400 data logger connected to Li 250, Li 1400-104 and Li 1400-106 sensors (Li-Cor Inc., NE., USA) at 100% canopy openness. The daily crown carbon balance of each recruit was determined as the difference between the daily crown carbon assimilation and the daily R_d values (the latter values were retrieved from the A-Q curves; see above).

Xylem anatomy

After digitization, plants were harvested, and the basal portion of the stem (ca. 2 cm length) was collected in 15 suckers and 15 saplings. The samples were soaked in distilled water for 24 hours. After soaking, a Sakura Accu-Cut SRM™ 200 rotary microtome (Zoeterwoude, The Netherlands) was used to obtain thin (40µm) fresh transverse sections of the basal stems. Sections were stained with a safranin solution (0.05%) for 3 min, washed with distilled water, dehydrated in increasing concentrations of ethanol (50%, 70%, 80%, 90%, 96%, 100%; 5 min each), and permanently mounted on glass slides with Neo-mount (Merck, Darmstadt, Germany) after removal of the ethanol with Neo Clear Clarification™ (EM Science, NJ, USA). The cross sections were photographed at 40× of magnification with a digital camera (Moticam 2500 5.0 MP; MOTIC™) attached to a binocular microscope (Olympus CX21-FS1; Olympus Corporation, Tokyo, Japan).

Recruit age was estimated from the number of xylem rings. The annual ring width was measured with the MoticImagePlus 2.0 software (Motic China Group Co., Ltd, Xiamen, China). The cross section photographs were converted to binary (black and white) images, and the xylem vessels of one fourth of each cross section were counted. Individual vessel lumen diameter was then determined using ImageJ 1.47q (Wayne Rasband/NIH, Bethesda, MD, USA) software. The percentage of the xylem area occupied by total vessel lumen area, the number of vessels per unit of xylem area, and the average vessel diameter (hereafter vessel lumen fraction, vessel density and vessel size, respectively) were calculated. Huber values were calculated as the vessel lumen fraction divided by the total leaf area.

Biomass allocation

After crown digitization, leaves were removed and scanned. Leaf area was determined from each scanned image using ImageJ 1.47q software. Leaves and stems (including the portion of the stem remaining after the anatomical analysis) were dried in a forced air oven for 72 hours at 60º C. Leaf mass area (LMA), leaf area ratio (LAR), and leaf to stem mass (LSR) were determined. LMA was calculated by dividing leaf dry mass by leaf area. LAR is the relationship between leaf area and total above-ground plant dry weight. LSR is the ratio between leaf and stem dry mass.

Climatic data

To evaluate the effect of climate on the radial growth of both suckers and saplings, we compiled a meteorological time series from the nearest weather station with the most complete climate record for the 2004-2013period (Tepual Airport, 41°25'S, 73°05'85''W; Meteorological Office of Chile, http://www.meteochile.gob.cl/). This period includes the three years preceding the year of birth of the youngest sampled plant, until the year in which the sampling took place. Therefore, this climate time series permits us to evaluate the reported delay of the effect of climate on radial tree growth (Fritts, 1996). We compiled monthly rainfall, air temperature and relative humidity data for this period. Using this data we estimated the monthly vapour pressure deficit (VPD) according to Murray (1967). The annual values (sum of rainfall, mean temperature, and mean VPD) were calculated using the monthly records of April of a given year through

March of the following one, coinciding with the hydrological year in the region (Lara et al. 2008). We also calculated the spring (September to November), summer (December to March), and growing season (September to March) climatic values.

Statistical analyses

Variables related to biomass allocation (LSR, LMA and LAR), xylem anatomy (vessel lumen fraction, vessel density, vessel size and Huber value) and crown architecture (LD, A_LA_C, SS and) were compared between the two recruit types by means of one-way ANOVAs. Relative leaf chlorophyll content and variables related to gas exchange at the leaf level (A_{SAT}, g_s and $_i$WUE) were compared between suckers and saplings by means of linear mixed models, where the individual was included as a random factor nested within the recruit type and the three measured leaves considered as pseudoreplicates. Model fit and estimation of dispersion were conducted using an analysis of variance. Parameter estimation was obtained by means of maximum likelihood. The significance of the contribution of the recruit type on the variability of the mixed model was calculated by comparing (by means of a likelihood ratio test) the null model (including random factor only) with the alternative one that incorporated the recruit type as an explanatory variable. These analyses were conducted with the lm4 library of the R package (Bates et al. 2014). The daily crown carbon balance was compared between recruit types by means of a one-way ANCOVA, including above-ground biomass as a covariate.

We evaluated changes in growth rates between saplings and suckers by comparing the above-ground biomass between recruit types by means of a one-way ANCOVA, whereagewas considered as a covariate. We also compared changes in the cumulative ring width with age between the two recruit types by means of a linear mixed model, where the individual was included as a random factor nested within the recruit type, and the annual rings measured were considered as pseudoreplicates. We used the same type of analysis to assess gas exchange at the leaf level. We then evaluated the effect of environmental variables on growth rate. For this purpose, we first compared the effect of canopy openness on the changes of above-ground biomass with age; this analysis was conducted separately for suckers and saplings by means of two independent two-way ANOVAs. For the case of suckers, we also evaluated the effect of the parent root diameter (as a rough proxy of parental supply) on changes of the above-ground biomass with age by means of a two-way ANOVA. Finally, to evaluate the effect of the regional climate on radial growth rate, we first calculated the median annual ring width by year and recruit type. Then, for each recruit type, we cross-correlated the median of the annual ring width with the climate time series (rainfall, temperature, and VPD) of the corresponding and the three preceding years (i.e., time lag effect tested from 0 to 3 years). We conducted these timelag analyses because (at least in adult trees) carbon storage might demonstrate a delay in the relationship between climate and tree growth (Fritts 1976).

Results

Biomass allocation and xylem anatomy

Whereas suckers allocated more biomass to leaves than to stems, saplings allocated nearly equal amounts of biomass to both leaves and stems (i.e., the confidence interval of LSR included one; Table 1). However, because of the higher LMA in suckers (Table 1), the leaf area to shoot biomass ratio (LAR) was not different between the two recruit types (Table 1).

We also found differences in the xylem anatomy. The vessel lumen fraction was higher for suckers than it was for saplings (Table 1). This pattern was mostly the result of the slightly higher vessel density in suckers, rather than being due to differences in the mean vessel diameter between the two recruit types (see details on vessel size distribution in Fig. S2, Supplementary Data). The Huber value was higher in suckers than it was in saplings (Table 1), due to the higher vessel lumen fraction.

Gas exchange and crown architecture

Net photosynthetic rate measured at the leaf level (A_{SAT}) was higher in saplings (Table 2), despite their lower LMA (Table 1). The higher A_{SAT} in saplings could be explained by the higher g_s and relative chlorophyll content of saplings as compared with suckers (Table 2). For the same g_s, saplings had higher A_{SAT} and consequently showed higher $_iWUE$ than suckers (Table 2).

Leaf dispersion (LD) did not significantly differ among recruit types (Table 1). The lower quartile of LD was higher than one for both suckers and saplings (interquartile ranges: [1.06, 115] and [1.02, 1.19] respectively), indicating a leaf distribution more regular than random (Duursma et al. 2012). Saplings showed lower crown density (A_L/A_C) and self-shading (SS) than suckers, and consequently a higher light interception efficiency ($\overline{STAR}$; Table 1).

Saplings showed higher daily crown carbon balance when compared with suckers of the same total above-ground biomass ($F_{1,\ 50}$ = 23.62, P< 0.001; Fig. 1B; Table S2 in Supplementary Data), which is consistent with the higher A_{SAT} and the higher $\overline{STAR}$ of saplings compared with suckers.

Growth rate

There were no statistical differences in above-ground biomass between recruit types of the same age (P = 0.132; Fig. 3A; Table S4 in Supplementary Data). Canopy openness explained a significant portion of the variability in the above-ground biomass for saplings, but not for suckers (Table 3). However, above-ground biomass was positively affected by average root diameter of the parent roots feeding the suckers. The explained variance by parent root diameter was even higher than that by sucker age (Table 3).

The cumulative ring width for a given plant age was higher in saplings than in suckers (P = 0.049; Fig. 3B; Table S5 in Supplementary Data). On the other hand, the annual ring width of saplings was positively correlated with the spring rainfall of the corresponding year (Table 4). No significant correlation was found between temperature or VPD and annual ring width of saplings. In the case of suckers, the annual ring width was positively correlated with the mean temperature of the growing season, the spring and the summer of the corresponding year, as well as with the annual VPD of the previous year (Table 4).

Discussion

As predicted, *Eucryphia cordifolia* saplings showed morphological, architectural and physiological traits that enhance the daily crown carbon balance when compared with root suckers. Even with the same total leaf area per above-ground biomass, the crown arrangements of saplings permit a higher light interception efficiency, and the incident light is rapidly captured due to their high chlorophyll content. In addition, saplings have a higher g_s, that enhances CO_2 supply at the carboxylation site, thus increasing carbon assimilation at the leaf level (Lambers et al. 2008). Suckers showed higher LMA than saplings, which was unexpected considering their lower carbon assimilation rate (on area basis) and chlorophyll content (Wright et al. 2005, Lambers et al. 2008). Higher LMA in root suckers compared with neighbouring saplings was also reported in *Fagus grandifolia*; but contrary to our results, no differences were found in gas exchange parameters at the leaf level in that study (Farahat and Lechowicz 2013). Leaves with higher LMA and thicker palisade parenchyma were reported in *E. cordifolia* saplings exposed to high light, compared with those growing in shade (Morales et al. 2014). However, there were no significant differences in canopy openness among the studied suckers and saplings (see Materials and Methods section), discarding dissimilarities in the light environment as a cause of the differences in LMA among recruit types. One explanation of the high LMA in suckers might be related to the reported ontogenetic changes that increase LMA in trees.

These changes likely have a genetic basis that allow the leaves to cope with harsh canopy conditions like high radiation, water deficit and wind (Thomas and Winner 2002). In fact, in the tropical tree *Macaranga gigantean*, the morpho-anatomical characteristics of the leaves of stump suckers are intermediate between those of saplings and adult trees; the high LMA values of stump suckers and adults in this species are related to leaf traits providing mechanical resistance to damage, rather than enhancing carbon gain (Ishida et al. 2005). Accordingly, the higher LMA of *E. cordifolia* suckers could be due to anatomical changes that increase leaf toughness (thicker vascular and sclerenchymatic tissues), but do not enhance the rate of photosynthesis based on area (de la Riba et al. 2016).

Despite the fact that we did not directly measure carbon translocation among *E. cordifolia* ramets, our results suggest that the maintenance and construction costs of root suckers were subsidized by parent photoassimilates, similar to other studies of clonal species (e.g., Alpert and Mooney 1986, Zhang et al. 2002). Firstly, the low carbon gain (at both leaf and crown level) in the root suckers can be explained by a possible down-regulation of photosynthesis induced by the low sucker requirements if their carbon necessities were supplied by the parent tree (for down-regulation of photosynthesis by sinks see Herold 1980, Watson and Casper 1984, Paul and Foyer 2001). Secondly, whereas above-ground biomass for a given age in saplings depends on light availability, it was strongly related to parent root diameter in suckers. This suggests that the higher the parental supply, the higher the growth rate of the root suckers. Finally, despite the greater carbon balance of saplings as compared to suckers, the two recruit types did not differ in terms of biomass. The similar growth rates were achieved by different biomass allocation strategies: saplings allocated more carbon to stem radial growth and suckers to produce denser and/or thicker leaves (high LMA).

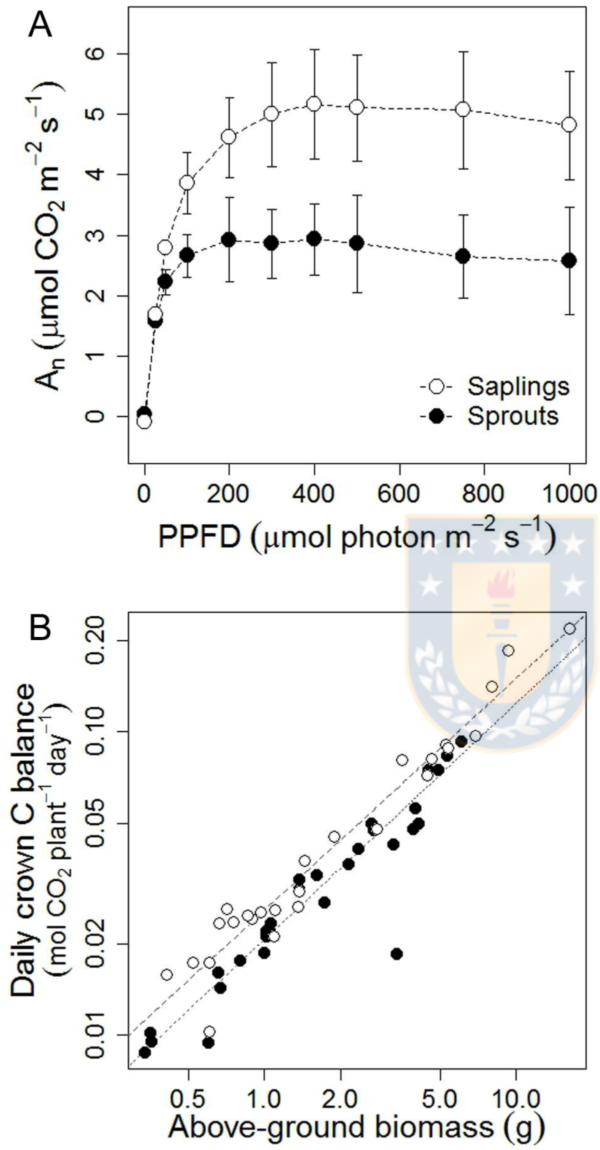
In spite of their higher g_s, saplings have a higher $_{i}$WUE, indicating that they adjust gas exchange in order to maximize the rate of carbon assimilation (to supply their growth demands), minimizing water loss per unit of carbon gained. Despite the fact that A_{SAT} was three times greater in saplings than in suckers, g_s was only twice as great. These results suggest a higher level of stomatal control in saplings than in root suckers, which is to be expected considering the high risk of hydraulic collapse due to both the small root systems of saplings and the high water potential at the turgor loss point for this species (Jiménez-Castillo et al. 2011). The high stomatal control in response to water availability can also be inferred from the climate-growth relationship. The annual ring width in saplings was significantly affected by the water availability in the spring (i.e., the lower the rainfall, the smaller the ring width; Table 4). Lower rainfall during the spring could induce stomatal closure in saplings (in order to decrease water loss by transpiration), and thus diminish carbon availability to be allocated to growth (Fritts 1976).

Root suckers are potentially able to conduct the high amounts of water supplied by the parent root system, due to their high vessel lumen fraction in relation to the transpiration surface (i.e., high Huber values). The enhanced water status of suckers due to the parental supply would explain their less efficient carbon assimilation rate in terms of water loss. Both the lower $_{i}$WUE and the weak relationship between rainfall and ring width support the hypothesis of poor stomatal control of suckers in response to environmental water availability. On the other hand, the radial growth of root suckers was positively correlated with the temperature of the corresponding year of growth. Mean temperatures were within the range of values for which increasing temperatures stimulate carbon gain, as indicated by studies conducted in a congeneric species inhabiting the Australian temperate rainforest (Hill et al. 1988). If this is the case for *E. cordifolia*, the arising question is why changes in temperature did not affect saplings in a similar way. An increase in temperature also enhances the evaporative demand (Murray 1967), and thus warmer conditions would depress the carbon gain in saplings by stimulating stomatal closure in order to save water. This gas exchange regulation in response to warmer and drier conditions probably does not occur in root suckers, which are less susceptible to water limitation due to their connection to the large parent root system. In fact, radial growth in suckers was also positively related to VPD, reflecting the positive relationship between this climate variable and air temperature (Murray 1967).

Conclusions

In the present study, we showed that root suckers and saplings of *Eucryphia cordifolia* differ in functional traits related to carbon gain and hydraulic architecture. Specifically, saplings are able to assimilate more carbon with higher water use efficiency, allowing them to supply their growth demands while minimizing water loss per unit of carbon gained. However, the stomatal response to water availability likely diminishes growth rates under dry conditions and ultimately might compromise survival, explaining why saplings are restricted to more closed and humid microsites (Escandón et al. 2013). On the other hand, suckers can maintain a low stomatal conductance, likely because carbon supply by the parent plant reduces the necessity of high rates of photosynthesis. Despite their low transpiration rates, root sucker leaves have access (through a large vessel lumen fraction) to high amounts of water, provided by the parent root system. Parental supply satisfactorily explains the low responsiveness of sucker growth to temporal changes in water availability, and their capacity to occupy more open and drier microsites (Escandón et al. 2013).

Figure 1.1.



A
A_n (µmol CO_2 m^{-2} s^{-1})
0
1
2
3
4
5
6
Saplings
Sprouts
0
200
400
600
800
1000
PPFD (µmol photon m^{-2} s^{-1})
B
Daily crown C balance
(mol CO_2 $plant^{-1}$ day^{-1})
0.01
0.02
0.05
0.10
0.20
0.5
1.0
2.0
5.0
10.0
Above-ground biomass (g)

Figure 1.2.

Figure 1.3.

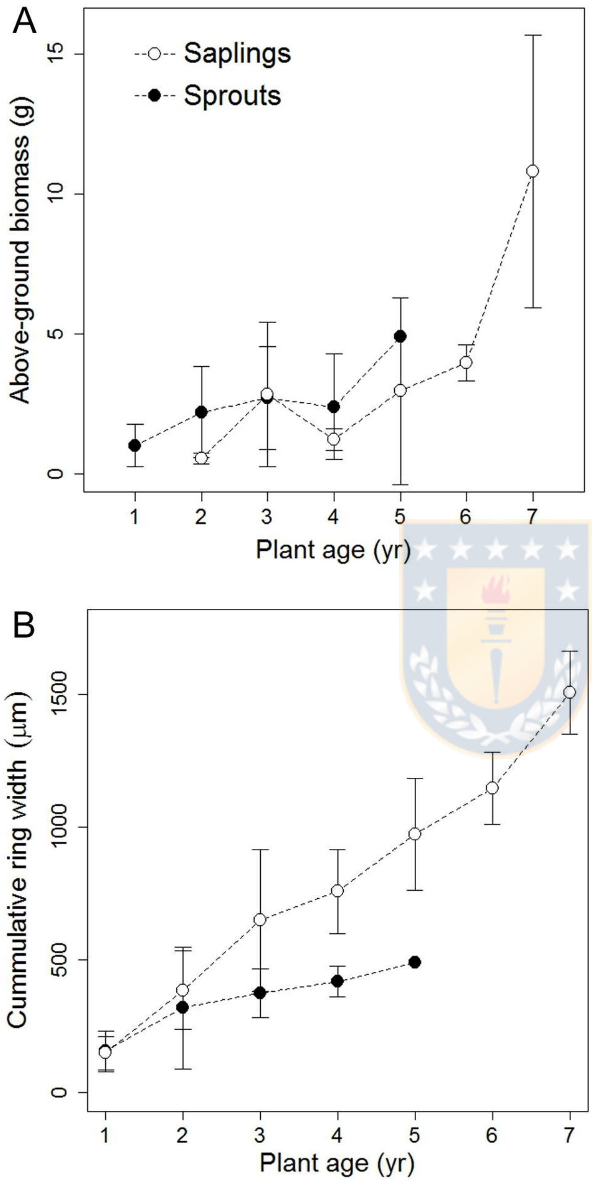

A
Saplings
Sprouts
Above-ground biomass (g)
0
5
10
15
1
2
3
4
5
6
7
Plant age (yr)
B
Cummulative ring width (µm)
0
500
1000
1500
1
2
3
4
5
6
7
Plant age (yr)

Table 1.1. Summary of the results of the one-way ANOVAs comparing variables related to the biomass allocation, xylem anatomy, and crown architecture between saplings and root suckers of *E. cordifolia*. Mean values (±SD) for each variable and recruit type are also shown. The full ANOVA results are shown in Table S1 of the Supporting Information.

Variables	ANOVA results		Saplings	Suckers
Biomass allocation[1]				
Leaf stem ratio (LSR; g g^{-1})	$F_{1,52} = 10.16$	$P = 0.002$	1.19±0.54	1.77±0.76
Leaf mass area (LMA; g m^{-2})	$F_{1,52} = 5.97$	$P = 0.018$	54.41±10.8	62.42±13.08
Leaf area ratio[2] (LAR; cm^2 g^{-1})	$F_{1,52} = 0.007$	$P = 0.933$	93.66±29.13	94.24±21.79
Xylem traits				
Vessel lumen fraction (%)	$F_{1,28} = 24.32$	$P < 0.001$	5.5□1.9	9.0□2.0
Vessel density[3] (□m^{-2})	$F_{1,28} = 3.03$	$P = 0.092$	2.7□1.4	3.7□2.3
Vessel size[3] (□m^2)	$F_{1,28} = 1.43$	$P = 0.242$	242□108	288□120
Huber value (mm^2 m^{-2})	$F_{1,28} = 5.02$	$P = 0.033$	2.4□1.3	3.6□1.7
Crown architecture				
Leaf dispersion[4] (LD)	$F_{1,53} = 1.36$	$P = 0.249$	1.11□0.13	1.15□0.14
Crown density ($A_L A_C$; cm^2 cm^{-2})	$F_{1,53} = 16.47$	$P < 0.001$	0.14□0.04	0.17□0.06
Self-shading (SS; cm^2 cm^{-2})	$F_{1,53} = 9.27$	$P = 0.004$	0.12□0.04	0.15□0.04
Silhouette to total area ratio ($\overline{STAR}$; cm^2 cm^{-2})	$F_{1,53} = 8.44$	$P = 0.005$	0.44□0.02	0.43□0.02

([1]) One outlier was discarded from the analyses. ([2]) LAR was calculated considering only the above-ground biomass. ([3]) Log-transformation prior to the analyses. ([4]) Reciprocal transformation prior to the analysis.

Table 1.2. Results of the lineal mixed models (LMM) comparing relative chlorophyll content and instantaneous gas exchange variables at leaf level between saplings and root suckers. Mean values (±SD) for each variable and recruit type are also shown.

	Likelihood ratio test							
	AIC	*BIC*	*logLik*	χ^2	*df*	*P*	*Saplings*	*Suckers*
Relative chlorophyll content (SPAD units)								
Null	1364.6	1374.8	-679.31					
+ Recruit type	1327.6	1341.2	-659.8	39.02	1	<0.001	45.6±4.5	35.5±6.1
Carbon assimilation rate at light saturation (*A_{SAT}*; µmol CO_2 m^{-2} s^{-1})								
Null	-15.041	-8.758	10.521					
+ Recruit type	-41.025	-32.648	24.512	27.98	1	<0.001	2.7±0.6	0.8±0.5
Stomatal conductance (g_s; mmol H_2O m^{-2} s^{-1})								
Null	-465.25	-458.97	235.63					
+ Recruit type	-474.89	-466.51	241.44	11.64	1	<0.001	53.3±0.6	26.6±0.5
Intrinsic water use efficiency ($_iWUE$; µmol CO_2 mmol H_2O^{-1})								
Null	401.68	407.96	-197.84					
+ Recruit type	392.64	401.02	-192.32	11.04	1	<0.001	0.054±0.016	0.028±0.015

Table 1.3. Summary of the results of the two-way ANOVAs comparing changes in the above-ground biomass (log-transformed) with age, canopy openness and the parent root diameter (the latter for root suckers only). The full ANOVA results are shown in Table S3 of the Supporting Information.

	ANOVA results		**Explained variance (%)**
Saplings			
Age (A)	$F_{1,21} = 18.65$	$P = 0.000$	39.2
Canopy openness (CO)	$F_{1,21} = 5.29$	$P = 0.032$	11.1
A × CO	$F_{1,21} = 2.62$	$P = 0.120$	5.5
Suckers			
Age (A)	$F_{1,21} = 5.52$	$P = 0.027$	16.7
Canopy openness (CO)	$F_{1,21} = 0.08$	$P = 0.781$	0.2
A × CO	$F_{1,21} = 2.40$	$P = 0.134$	7.3
Age (A)	$F_{1,21} = 6.89$	$P = 0.015$	16.7
Parent root diameter (PRD)	$F_{1,21} = 9.20$	$P = 0.006$	22.3
A × PRD	$F_{1,21} = 0.09$	$P = 0.765$	0.2

Table 1.4. Cross-correlation coefficients for the median ring width and several climatic variables for the complete year (April to March), the growing season (September to March), spring (September to November) and summer (December to February). Significant correlations at 95% confidence are indicated with an asterisk.

	Saplings				Suckers			
Lag	**Annual**	**Growing season**	**Spring**	**Summer**	**Annual**	**Growing season**	**Spring**	**Summer**
Rainfall								
-3	-0.34	-0.34	-0.12	-0.39	0.19	-0.03	-0.12	0.05
-2	0.00	-0.08	-0.31	0.04	0.67	0.38	0.12	0.63
-1	0.45	0.47	0.24	0.55	-0.75	0.34	0.71	-0.04
0	-0.64	0.30	0.78 (*)	0.07	-0.34	-0.72	-0.57	-0.76
Mean temperature								
-3	0.25	0.29	0.15	0.27	0.17	0.03	0.07	-0.01
-2	-0.09	-0.10	-0.15	-0.04	0.12	-0.39	-0.22	-0.49
-1	0.06	-0.40	-0.17	-0.50	-0.83	-0.20	-0.28	-0.05
0	-0.43	-0.15	-0.29	-0.11	0.60	0.99 (*)	0.94 (*)	0.97 (*)
Vapor pressure deficit (*VPD*)								
-3	0.38	0.37	0.28	0.39	0.01	-0.02	0.06	-0.03
-2	-0.18	-0.09	-0.15	-0.06	-0.53	-0.60	-0.39	-0.61
-1	-0.34	-0.44	-0.19	-0.46	-0.17	-0.06	-0.41	-0.02

0	-0.25	-0.23	-0.51	-0.19	0.89 (*)	0.78	0.84	0.77

Supplemental Information for "Physiological differences between root suckers and saplings enlarge the regeneration niche in *Eucryphia cordifolia* Cav."

Antonio B. Escandón, Roke Rojas, Loreto V. Morales, Luis J. Corcuera,
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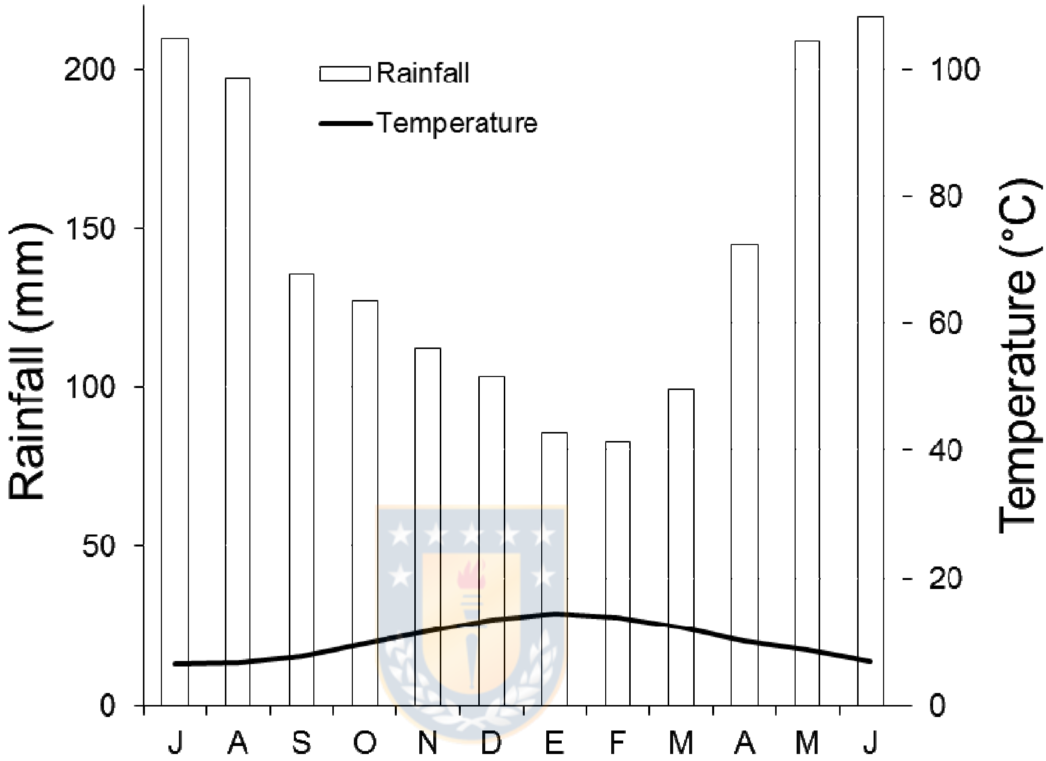

Fig. S1. Climate diagram from the nearest weather station to the study site (Tepual Airport, 41°25'S, 73°05'85''W; Meteorological Office of Chile, http://www.meteochile.gob.cl/). Monthly data are the average for the 1958-2010 period.

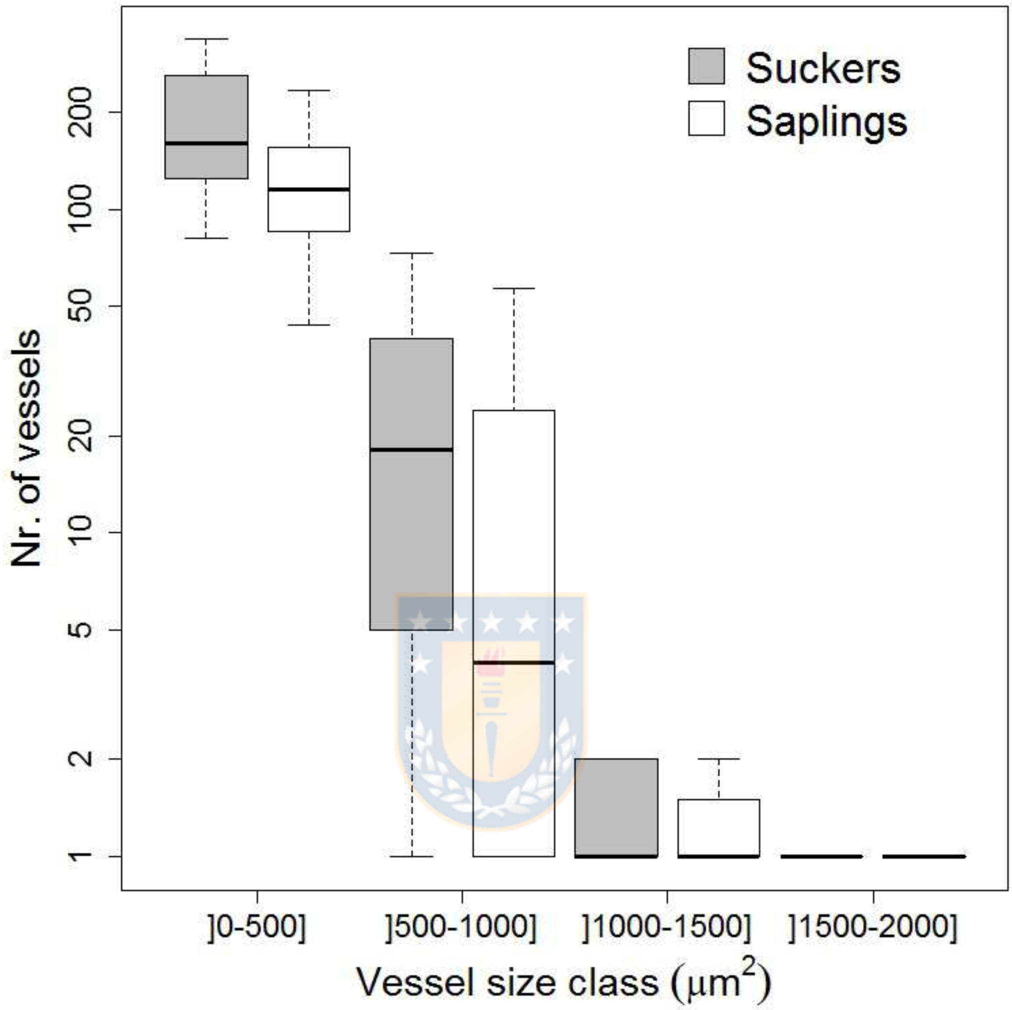

Fig. S2. Number of xylem vessels per size class in root suckers and saplings of *E. cordifolia*. The number of small vessels are significantly higher ($F_{3, 112} = 149.28$, $P < 0.001$) in both suckers and saplings (non-significant vessel class recruit type interaction: $F_{3, 112} = 0.99$, $P = 0.398$).

Table S1. Full results of the one-way ANOVAs comparing variables related to the biomass allocation, xylem anatomy, and crown architecture between saplings and root suckers of *E. cordifolia*. Mean values (±SD) for each variable and recruit type are also shown.

Variables	*df*	*SS*	*MS*	*F*	*P*	Saplings	Suckers
Biomassallocation[1]							
Leaf stem ratio (LSR; g g^{-1})							
Recruittype	1	4.44	4.44	10.159	0.002	1.19±0.54	1.77±0.76
Residuals	52	22.76	0.44				
Leaf mass area (LMA; g m^{-2})							
Recruittype	1	864.7	864.71	5.972	0.018	54.41±10.8	62.42±13.08
Residuals	52	7529.7	144.8				
Leaf area ratio[2] (LAR; cm^2 g^{-1})							
Recruittype	1	5	4.68	0.007	0.933	93.66±29.13	94.24±21.79
Residuals	52	34035	654.52				
Xylemtraits							
Vessel lumen fraction (%)							
Recruittype	1	0.009	0.009	24.32	<0.001	5.5±1.9	9.0±2.0
Residuals	28	0.011	0.0004				
Vessel density[3] (μm^{-2})							
Recruit type	1	0.14	0.14	3.03	0.092	2.7±1.4	3.7±2.3
Residuals	28	1.295	0.046				
Vessel size[3] (μm^2)							

Recruit type	1	0.055	0.055	1.43	0.242	242±108	288±120
Residuals	28	1.07	0.038				
Huber value (mm^2 m^{-2})							
Recruit type	1	10.817	10.817	5.02	0.033	2.4±1.3	3.6±1.7
Residuals	28	60.369	2.156				
Crown architecture							
Leaf dispersion[4] (LD)							
Recruittype	1	0.013	0.013	1.36	0.249	1.11±0.13	1.15±0.14
Residuals	53	0.489	0.009				
Crown density (A_LA_C; cm^2 cm^{-2})							
Recruittype	1	0.036	0.036	16.47	<0.001	0.14±0.04	0.17±0.06
Residuals	53	0.115	0.002				
Self-shading (SS; cm^2 cm^{-2})							
Recruittype	1	0.012	0.012	9.27	0.004	0.12±0.04	0.15±0.04
Residuals	53	0.07	0.001				
Silhouette to total area ratio ($\overline{STAR}$; cm^2 cm^{-2})							
Recruittype	1	0.003	0.003	8.44	0.005	0.44±0.02	0.43±0.02
Residuals	53	0.018	0.0003				

([1]) One outlier was discarded from the analyses. ([2]) LAR was calculated considering only the above-ground biomass. ([3]) Log-transformation prior to the analyses. ([4]) Reciprocal transformation prior to the analysis.

Table S2. Results of the one-way ANCOVA comparing changes in the daily crown carbon balance (log-transformed) between saplings and root suckers, including above-ground biomass as covariate.

	df	***SS***	***MS***	***F***	***P***
Biomass[1] (AB)	1	5.490	5.490	1128.53	<0.001
Recruit type (RT)	1	0.115	0.115	23.62	<0.001
AB ×RT	1	0.0004	0.0004	0.08	0.775
Residuals	50	0.243	0.005		

([1]) Log-transformed prior de analysis

Table S3. Results of the two-way ANOVAs comparing changes in the above-ground biomass (log-transformed) with age, canopy openness and the parent root diameter (the latter for root suckers only).

	df	*SS*	*MS*	*F*	*P*	**Explained variance (%)**
Saplings						
Age (A)	1	0.892	0.892	18.65	0.000	39.2
Canopy openness (CO)	1	0.253	0.253	5.29	0.032	11.1
A × CO	1	0.125	0.125	2.62	0.120	5.5
Residuals	21	1.004	0.048			
Suckers						
Age (A)	1	0.231	0.231	5.52	0.027	16.7
Canopy openness (CO)	1	0.003	0.003	0.08	0.781	0.2
A × CO	1	0.100	0.100	2.40	0.134	7.3
Residuals	25	1.045	0.042			

Age (A)	1	0.231	0.231	6.89	0.015	16.7
Parent root diameter (PRD)	1	0.308	0.308	9.20	0.006	22.3
A × PRD	1	0.003	0.003	0.09	0.765	0.2
Residuals	25	0.837	0.033			

Table S4. Results of the two-way ANOVA comparing changes in the above-ground biomass (log-transformed) with age between saplings and root suckers.

	df	*SS*	*MS*	*F*	*P*
Age[1] (A)	1	0.886	0.886	17.50	<0.001
Recruittype (RT)	1	0.119	0.119	2.34	0.132
A×RT	1	0.163	0.163	3.22	0.079
Residuals	50	2.531	0.051		

([1]) Log-transformed prior de analysis.

Table S5. Results of the lineal mixed models (LMM) comparing changes with age in the cumulative ring width (root square transformed) between saplings and root suckers.

				Likelihood ratio test		
	AIC	***BIC***	***logLik***	χ^2	***df***	***P***
Null	1158.55	1167.94	-576.27			
+ Age (A)	876.17	888.69	-434.09	284.38	1	<0.001
+ Recruittype (RT)	874.29	889.94	-432.15	3.88	1	0.049
+ A ×RT	864.71	883.49	-426.35	11.58	1	<0.001

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Conflict of interest

None declared.

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Figure legends

Figure 1. Gas exchange at leaf and crown level of root suckers and saplings of *E. cordifolia*. (A) Light response curve (mean±SD). (B) Relationship between the daily crown carbon balance and the above-ground biomass.

Figure 2. Examples of three-dimensional reconstruction of root sucker and sapling crowns of *E. cordifolia*. Scale bar = 10cm.

Figure 3. Growth rate of root suckers and saplings of *E. cordifolia*. (A) Changes in above-ground biomass (mean±SD) with age. (B) Changes in cumulative ring width (mean±SD) with age.

CHAPTER II

Root suckering promotes recruitment in two temperate rainforest trees with contrasting shade tolerance

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Abstract

The regeneration niche differentiation helps to explain plant coexistence and thus biodiversity. The study of the regeneration niche has been traditionally based on sexual recruitment, while overlooking clonal growth. Root suckering offers a successful alternative for local dispersal under suboptimal conditions for sexual reproduction. For light-limited forests, we hypothesized that: 1) root suckering would increase the regeneration niche towards high-light conditions in shade-tolerant trees and towards dark conditions in light-demanding species; 2) contrasting responses of survival and growth to light availability would explain niche differentiation of both suckers and saplings; and 3) distinct responsiveness to light among species and recruit-types would reflect differences in functional traits. We tested these hypotheses with two evergreen tree species that coexist in the temperate rainforest of southern South America: *Embothrium coccineum* (light-demanding) and *Eucryphia cordifolia* (shade-tolerant). We measured the light availability in two study plots above each recruit and along transects established in the understory. Niche selection, niche differentiation and changes in survival probability with light were inferred from the analysis of the light frequency distributions. We evaluated the effect of light on the relative volumetric change in stems over a 1-year period. Functional traits of leaves, stems, and crowns were measured in suckers and saplings growing under similar light conditions; these traits were then compared among size classes, recruit-types and species. Root suckering was the prevalent reproduction mode of both studied species, extending the light

niche towards open microenvironments only during the earliest ontogenetic stages. The poor structural strength of the leaves and wood of small *Eucryphia* saplings explains its underuse of open microsites. Neither photosynthetic assimilation nor carbon subsidy can sustain *Embothrium* suckers at the shadiest microsites. Suckering proved to increase the persistence of *Embothrium* until advanced stages of forest succession, facilitating its coexistence with the late-successional *Eucryphia.* Our study emphasizes that clonal growth is essential to understand the dynamics of temperate rainforests.

Keywords: forest dynamics; light; ontogeny; plant functional traits; performance; regeneration niche.

Introduction

A regeneration niche, defined as the set of environmental requirements needed for germination and establishment, is a keystone concept in the study of community dynamics (Grubb, 1977; Poorter, 2007). Tree diversity in forest ecosystems can be partially explained by their differing abilities to germinate and establish in diverse microhabitats (Wright, 2002; Gilbert and Lechowicz, 2004). The most conspicuous environmental heterogeneity in tropical and temperate rainforests is driven by temporal and spatial changes in understory light availability (Denslow, 1987; Valladares et al., 2012; Valladares et al., 2016). Accordingly, forest dynamics are largely modulated by the interspecific differences in the light requirements of trees during their early ontogenetic stages (Canham et al., 1994; Kobe, 1999; Lusk and Laughlin, 2017). Differences in light regeneration niches among tree species has been attributed to interspecific variability in functional traits related to the efficiency of light absorption and use under shade conditions (Lusk, 2002; Poorter, 2009). However, the role of clonal organs in forest dynamics has rarely been considered, even though they are closely related to important ecological functions, namely on-spot persistence, space occupancy or post-disturbance resprouting (Ottaviani et al., 2017; Klimešová et al., 2018a).

When considering the large variety of clonal growth mechanisms, root suckering is one of the most widespread among phylogenetic groups, growth forms and biomes (Klimešová and Klimeš, 2008; Klimešová et al., 2017; Pausas et al., 2018). Root suckers proliferate from adventitious buds located on lateral roots that spread laterally in such a way that ramets develop beyond the parent plant (Bosela and Ewers, 1997; Jones and Raynal, 1986; Pausas et al., 2018). Physiological integration among ramets permits vegetative recruitment under high competition pressure, resource scarcity and stressful conditions (Klimešová et al., 2018b). Therefore, root suckering offers a successful alternative for local dispersal and regeneration where the establishment of germinated seeds is not viable (Koop, 1987; Pennings and Callaway, 2000; Wiehle et al., 2009). In this sense, root suckering has been described as an efficient mechanism to colonize open microsites by the shade-tolerant tree *Eucryphia cordifolia*, whose sapling survival has proved to be very low under high light conditions (Escandón et al., 2013). Root suckering has also been considered advantageous for recruiting in deep shade in the light-demanding tree *Ailanthus altissima* (Kowarik, 1995). In the case of the shade-tolerant *Fagus grandifolia*, root suckering has been described as a successful regeneration mechanism under harsh conditions (Held et al., 1983; Morris et al., 2004; Takahashi et al., 2010). However, saplings and suckers of *F. grandifolia* were not segregated along the light availability gradient of an old-growth temperate forest, at least during their early ontogenetic stages (5-30 cm in height; Beaudet et al., 2008). In the case of the light-demanding *Populus tremula*, root suckers tended to be associated with the gaps of a boreal forest, although the relationship among suckers and canopy clears was very weak (Homma et al., 2003). Therefore, there is still no strong evidence supporting the role of root suckering in the extension of light niches towards suboptimal conditions: well-lit for shade-tolerant species and dark for light-demanding species. This hypothesis could be tested by evaluating niche differentiation of suckers and saplings in co-occurring tree species with contrasting shade-tolerance.

If parental subsidy weakens the response of ramets to environmental conditions (Escandón et al., 2018), functional traits will be expected to differ among coexisting recruits of sexual and vegetative origins. In temperate and tropical rainforests, shade-tolerant species have tough, persistent leaves that accumulate through plant development, providing a larger light interception surface, whereas light-demanding species exhibit the opposite suite of traits (Lusk, 2002; Poorter, 2009). The patterns described at the interspecific level are consistent with the functional differences between suckers and saplings in the temperate rainforest species *Eucryphia cordifolia*. Although saplings of this species deploy the same foliar surface as suckers, the spatial arrangement of suckers' leaves allows for greater light interception, and therefore, a potentially greater carbon gain at the whole plant level, which explains the differences in the light niche occupied by the two recruit-types (Escandón et al., 2018). We are unaware of any study regarding the interspecific variability in functional traits between suckers and saplings in light-demanding tree species. In this sense, saplings of light-demanding species show plastic responses to low light in order to increase light interception, such as elongated (slender) stems and/or large and thin leaves (Poorter and Werger, 1999; Rozendaal et al., 2006); such responses to low light would not be expected in the subsidized root suckers, in such a way that saplings and suckers of light-demanding species growing in the understory should differ in their functional traits.

The aim of this study was to evaluate the role that clonal growth plays in defining the ecological niches of tree species and to understand the underlying processes involved. Specifically, we hypothesized that the regeneration niche (in terms of light) is shaped by root suckering in tree species, with contrasting patterns depending on the species shade-tolerance, and thus on their functional traits. Specifically, we predicted that 1) suckers would occupy more closed microhabitats compared to saplings in light-demanding species, while the opposite was expected for a co-occurring shade-tolerant tree; 2) differences in the light niches of suckers and saplings would be explained by their contrasting responses to light in terms of survival and growth; and 3) distinct responsiveness to light among species and recruit types would reflect differences in functional traits of leaves, stems, and crowns. For this purpose, we studied the regeneration niches of *Embothrium coccineum* J.R. et. G. Forster and *Eucryphia cordifolia* Cav. (hereafter *Embothrium* and *Eucryphia*), two evergreen tree species that coexist in the temperate rainforests of southern South America (Lusk, 2002; González et al., 2002; Escandón et al., 2013). These two species are able to recruit by both seeds and root suckering (Lusk, 2002; González et al., 2002), but they are markedly different in their shade-tolerances: *Embothrium* mostly germinates in forest gaps and suffers high mortality under dark conditions, whereas *Eucryphia* performs in an opposite way (Figueroa and Lusk, 2001; Lusk and Del Pozo, 2002; Lusk, 2002). The results of this study will therefore contribute to understanding the dynamics of temperate rainforests, where the role of clonal growth has traditionally been overlooked.

Materials and methods

Study site

This study was carried out in the western foothills of the Andes in south-central Chile, in the Anticura sector of Puyehue National Park (40º39’ S, 72º11’ W, 350 m a.s.l.). This sector of the park is mostly covered by old-growth temperate rainforest, with some fragments of second-growth forest (ca. 50 years-old) dominated by *Nothofagus dombeyi*, *Eucryphia cordifolia*, *Caldcluvia paniculata* and *Embothrium coccineum*. Our study site was located in these fragments because they were the only places where adult trees of both *Embothrium* and *Eucryphia* were found. The study area experiences a temperate maritime climate, with 2725 mm of annual precipitation and a minimum rainfall of at least 111 mm per month during the summer months (December-March 2014-2015). The warmest and coldest months were January (14.4ºC) and July (5.4ºC), respectively (Anticura weather station of the Forestry National Corporation - CONAF; 1980 – 2016 period).

Adult trees and regeneration sampling

Two permanent plots (25 × 60 m each) were established in September 2016 (just before the growing season). Each plot was located in a different fragment of secondary forest embedded in a matrix of old-growth forest; the two plots were 400 m apart. Each plot included >5 adult individuals of the studied species and (altogether) the two plots comprised the light availability gradient described for this part of the national park (Gianoli et al., 2010; Table A.1). Within these permanent plots, we identified adult individuals of *Embothrium* and *Eucryphia*, those with trunk diameters at breast height (DBH) ≥5 cm. In the center of each plot, we established a subplot of 5 × 50 m, where all of the recruits of the studied species between 2 and 150 cm in height were identified as either root suckers (from vegetative reproduction) or saplings (from sexual reproduction). To determine whether a recruit was a sapling or a sucker, the root collar was carefully revealed, and the superficial soil was temporarily removed. Recruits were identified as root suckers when their root collars were still connected to their parental roots, and as saplings when they did not show any subsidiary root connections or root scars indicating past connections (Escandón et al., 2018). These sampling methods resulted in the identification of 280 adults, 282 root suckers and 182 saplings (Table A.1).

Light environment characterization

The light environment was determined according to the global site factor (GSF), which is defined as the light fraction that is expected to reach the forest floor at a specific site; the GSF is determined relative to the available light above the canopy (the higher the GSF value, the higher the relative light availability; Gianoliet al., 2010). Within each subplot, the GSF was measured above each recruit's apex and at 55 equidistant points distributed along 3 longitudinal transects (5 m apart within each subplot). For this purpose, hemispherical photographs were recorded under homogeneous overcast conditions using a Coolpix 4500 digital camera equipped with a FC-E8 fisheye lens (Nikon, Tokyo, Japan). The camera was hand leveled and oriented sothat the top of the image faced north. For measurements along thetransects, photographs were recorded at ca. 30 cm from the soil's surface, corresponding to the median height of the sampled recruits. The GSF was obtained after analyzing individual photographs using canopy analysis software HemiView version 2.1 (1999, Delta-T Devices Ltd, UK). In order to estimate the GSF, we specified the coordinates and elevation of the study site (recorded with a GPS Garmin 100), lens degree angle of view (from the FC-E8 fisheye lens user's manual), and solar properties (cf. Sherwood, 2015).

Growth and functional traits

The relative growth rate (RGR) of the stem volume for each recruit within the plots was measured over a 1-year period. Changes in stem volume have previously been used to understand differences in the shade-tolerance of saplings in tropical and temperate rainforests (Kohyama and Hotta, 1990;

Lusk and Jorgensen, 2013). For this aim, the basal stem diameter and stem length of each recruit were measured in September 2016 and 2017. The main stem was marked with white acrylic paint on the collar scar in the case of saplings, and just above the parental root connection in root suckers. Basal stem diameter was measured on the white mark using a 0.01 mm precision digital caliper (Mitutoyo, Tokio, JP). Stem length was measured between the white mark and the terminal bud base using a 0.1 cm precision flexible tape. Both basal stem diameter and stem length were used to calculate the RGR as follows:

$$RGR = ((\ln(l_f \times \pi\ (d_f/2)\exp2)) - (\ln(l_i \times \pi(d_i/2)\exp2))) / (t_f - t_i),$$

where t indicates the time (i.e. 1-year period) and the subscripts $_i$ and $_f$ are the initial and final measures of stem length (l) and basal stem diameter (d) (Lusk and Jorgensen, 2013).

After measurements were conducted in September 2017, 40 root suckers and 40 saplings were selected per species from the total pool of recruits (i.e. regardless of the plot) in order to harvest a similar number of individuals per recruit-type and species throughout the entire GSF gradient. Selection was conducted randomly with the “randbetween” function of Microsoft Office Excel software (Microsoft Office Enterprise 2007; Microsoft Corporation, Redmond, WA, USA). After discarding damaged recruits (mostly slashed or trampled by humans), the final number of plants analyzed was 36 suckers and 31 saplings of *Embothrium*, and 29 suckers and 39 saplings of *Eucryphia*. The GSF distribution of the selected plants did not differ from that of the complete set of sampled recruits (Kolmogorov-Smirnov test: D = 0.044, P = 0.989; data not shown). In addition, the GSF of the subset of plants did not differ for recruit-types (i.e. saplings and root suckers), species, or the interaction of these factors (Table A.2). Therefore, differences in functional traits among recruit-types and/or species could not be attributed to differences in the light environment, representing the functional response to the light environment of the study plots. Selected recruits were carefully excavated and kept under wet conditions until they were processed in the field laboratory. A set of ecologically relevant traits in relation to functional strategies dealing with variations in light availability under closed and open conditions for woody plants were measured and calculated for recruits in order to better understand plant distribution.

For leaf level traits, the chlorophyll relative content (hereafter chlorophyll concentration [chl]) was measured on one-to-three leaves per recruit using a CCM-200 *plus* chlorophyll meter (Opti Science, Inc., NH, USA). Briefly, the CCM-200 *plus* uses the ratio between % transmittance at 931 nm and 653 nm to estimate a unitless measure proportional to the amount of chlorophyll concentration present in the sample (see CCM-200 *plus* operator's manual for more details). Then, all of the leaves were counted, removed and digitized with a flat-bed scanner at 300 dpi of resolution. Total leaf area was determined from each scanned image using ImageJ 1.47q software (Wayne Rasband/NIH, Bethesda, MD, USA). Leaves and stems were separately dried in a forced air oven for 72 h at 60º C and then weighed. Specific leaf area (SLA), which indicates the leaf efficiency for light capture per unit of biomass invested (Poorter et al., 2009), was calculated by dividing the total leaf area by the total leaf dry mass (Pérez-Harguindeguy et al., 2013). Leaf size was estimated by dividing the total leaf area by the number of leaves per plant (Kraft et al., 2008). Regarding stem traits, specific stem density (SSD) was calculated as a ratio between the stem dry weight and the stem volume ratio (Kirkham, 2005). The slenderness index was calculated by dividing the stem length by the basal stem diameter; this index reflects an allometric response to shade, consisting of an increase in stem height at the expense of lateral growth (Cardillo and Bernal, 2006; Petritan et al., 2009; Barros et al., 2012; Valladares et al., 2012). As calculated, the index assumes that stems do not taper with height, but there is no evidence suggesting that this tapering would differ among recruit-types or species. For traits at crown level, the aboveground leaf area ratio (aLAR) was calculated as the ratio between total leaf area and total aboveground plant dry weight. The aboveground leaf mass fraction (aLMF) was calculated by dividing the leaf dry mass by the total aboveground plant dry mass (Poorter et al., 2012). We calculated the leafing density by dividing the total number of leaves by the stem length (cf. Niinemets and Tobias, 2019); this parameter helped indicate how the allometry of each recruit changed with ontogeny. High values of leafing density can be achieved by accumulating long-lived leaves through ontogeny (i.e. through low leaf turnover). Finally, we divided the total leaf area by the basal stem cross-section area, and used this variable as aproxy of the leaf to sapwood area ratio (A_LA_S). This ratio (A_LA_S) is the inverse of the Huber value and indicates the transpiration surface area by unit of stem water supply (Tyree and Ewers, 1991; Martínez-Vilalta et al., 2009).

Data analyses

Within the study area, the minimum light requirements of tree species increase with size, particularly when comparing plants smaller and larger than 50 cm in height of the light-demanding species (Lusk et al., 2008). Therefore, for all of the analyses, we distinguished between two size classes: small (less than 50 cm) and large (50 cm in length or more).

To evaluate light niche selection, we compared the GSF distribution of each recruit-type per species with that of the forest by using the non-parametric Kolmogorov-Smirnov (K-S) test. For each comparison, the P-value obtained in the K-S test was compared with a significance level established through the step-up false discovery rate (FDR) procedure to control for the probability of a type I error under repeated testing (Benjamini and Hochberg, 1995). When differences in the distributions were found, we identified which part of the understory light gradient was preferred or avoided by each recruit-type by comparing the GSF distribution of the understory with that of the recruits using the function “qcomhd” of the R package *WRS2* (Wilcox et al., 2014), which compares quantiles (deciles in our case) estimated from two independent distributions using a percentile bootstrap to calculate confidence intervals. This test requires at least 20 observations in each group and provides a more detailed understanding of where and how distributions differ (Mair and Wilcox, 2019). The type I error was controlled with Hochberg's method, which was implemented by defaulting the “qcomhd” function. Niche differentiation between suckers and saplings was evaluated (independently for each species) following the same procedure. All of these tests were performed separately for small (<50 cm) and large (≥50 cm) recruits in order to evaluate potential ontogenetic changes in the ecological niche (Lusk et al., 2008).

If light did indeed modulate the survival of the recruits (and considering size as a surrogate of age), it would be expected that the large recruits are underrepresented under stressful light conditions; therefore, the frequency distribution of GSF would differ between the two size classes. Consequently, to evaluate the probability of survival along the GSF gradient, we compared the frequency distribution of small (<50 cm length) and large (≥50 cm length) individuals for each species and recruit-type by using K-S tests. The significance level (α) of the K-S tests was established by means of the step-up FDR (Benjamini and Hochberg, 1995). When differences were detected, to determine where along the light gradient such differences occurred, we compared the GSF deciles distribution occupied by small and large recruits using the function “qcomhd” of the R package *WRS2* (Wilcox et al., 2014).

In order to fully understand the light preferences of recruits, we evaluated the effect of GSF on the stem volume RGR separately for each recruit-type and species. Provided that plants rarely sustain constant exponential growth, the RGR usually decreases with plant size in a non-linear fashion (Philipson et al., 2012). To further understand the ontogenetic trajectory of the RGR and how it is shaped by light availability, we adjusted a linear model with log-RGR as a function of the initial size (measured as the initial stem length) and its interaction with GSF. GSF was log-transformed to take into account the typical asymptotic response of growth to light availability (Soto et al., 2017). The normality and homoscedasticity of the residuals were evaluated (respectively) by means of the Shapiro-Wilk test and the Non-constant Variance Score (NCV) test, the latter with the "ncvTest" function available at the *car* library of the R software (Fox and Weisberg, 2018). The significance level (α) was established by means of the step-up FDR (Benjamini and Hochberg, 1995; see details describing this method above).

We evaluated differences in each studied functional trait among recruit-types, species and the two aforementioned size classes (i.e. small and large). Due to the unbalanced design, the type I sum of square (SS) ANOVA ("anova" function) was used, which partitions the variance between factors sequentially, in the same order they were included in the model; in this way, the pervasive effect of the more represented groups on the variance partitioning is removed (Zahn, 2010). The variance explained by each factor was calculated as the percentage of the SS for a given factor relative to the total SS. The significance level (α) was established by means of the step-up FDR (Benjamini and Hochberg, 1995). Least-square means was used to evaluate *posthoc* differences through the function "emmeans" of the *emmeans* R package (Lenth, 2018). To extract and see information on all pairwise comparisons, we used the function "cld" from the *multcompView* package, adjusting P-values with the Tukey method at $\alpha = 0.05$. Shapiro-Wilk tests and NCV tests were used to verify the normality and homocedasticity of the models, respectively. When needed, response variables were Box-Cox transformed to meet the normality and homoscedasticity assumptions of the ANOVA.

Results

We recorded a total of 464 recruits, of which 271 corresponded to *Embothrium* and 193 to *Eucryphia* (see also Table A.1.). Root suckering was more relevant in *Embothrium* (76.0%) than in *Eucryphia* (39.4%), as revealed in the independency test of Pearson's Chi square (χ^2=61.94, df=1, P<0.001). The percentage of suckering increased for both of the species when only large recruits were considered (77.9% for *Embothrium* and 60.8% for *Eucryphia*). Differences between species in root suckering considering only large recruits decreased, but remained significant (χ^2=4.95, df=1, P = 0.026).

Regeneration light niche

The GSF frequency distribution of small *Embothrium* suckers and *Eucryphia* saplings was significantly different from that of the understory forest (Table 1), reflecting the niche selection of these recruit-types.

Contrary to our expectations, the quantile comparison indicated that small *Embothrium* suckers were less frequent in the shaded microsites than what would be expected at random, considering the GSF distribution of the understory (GSF=[0.07 to 0.09], Fig. 1C, 2A, Table A.3). Small *Eucryphia* saplings underused both the shadiest (GSF<0.05) and brightest microsites (GSF=[0.15 to 0.18], Fig. 1D, 2B, Table A.3), reflecting this species'intermediate shade-tolerance level. The GSF frequency distribution of large *Embothrium* and *Eucryphia* recruit-types did not differ from that of the understory forest (Table 1, Fig. 1A,B).

The evaluation of niche differentiation showed that the GSF frequency distribution significantly differed between suckers and saplings for both *Embothrium* and *Eucryphia*, but only in small plants (Table 2). The quantile comparison indicated that *Embothrium* suckers were more frequent than saplings along most of the GSF gradient occupied by these recruits, except at the shadiest and brightest extremes of their light niche (Fig. 2, Table A.4). On the contrary, differences for *Eucryphia* were detected in the seventh GSF decile (Fig. 2D, Table A.4), with suckers occupying more illuminated sites than saplings, as expected. Such differences disappeared when large recruit-types (i.e. recruits ≥50 cm length) were evaluated (Table A.4).

Survival and growth

The ratio of large to small recruits for *Embothrium* suckers and saplings were respectively 0.48 and 0.41 (see sample sizes in Table A.1). These results suggest that less than the half of the small *Embothrium* recruits survive until to reach ≥50 cm length. In the case of *Eucryphia*, the ratio was 0.36 for saplings and 1.71 for suckers. The latter indicates no mortality of *Eucryphia* suckers, increasing the pool of large suckers over the time.

The Kolmogorov-Smirnov (K-S) test conducted to compare the GSF distribution between small and large recruits only supported differential survival along the GSF gradient for *Embothrium* suckers (Table 3). Specifically, differences emerged at the lower end of the GSF distribution, with a lower GSF value for large rather than small suckers in the 3rd decile, indicating high survivorship of large suckers in shaded microsites (Fig. 3, Table A.5).

As expected, the relative growth rate (RGR) in the stem volume decreased with initial plant size (measured as stem length) in a non-linear fashion, but only for *Eucryphia* recruits (Fig. 4). The ontogenetic trajectory of the RGR in *Eucryphia* saplings and suckers was modulated by the GSF, in such a way that the RGR of small plants was higher at low light (significant length × light interaction; Table 4, Fig. 4). The 95% confidence intervals of the estimated coefficients for length × GSF interaction in *Eucryphia* suckers and saplings overlapped (suckers: [-0.024 to -0.002]; saplings: [-0.022 to -0.004]), indicating that the effect of light on RGR was equivalent for the two recruit-types.

Even though the residuals of the model developed for *Eucryphia* saplings (as well as for *Embothrium* suckers) were not normal (Table 4), the results have statistical support considering that (1) the P-values for the corresponding regressions had values far from the margin of significance (i.e. the alpha value established by the FDR correction), (2) the residuals were homoscedastic, and (3) the absence of normality only had significant effects when it implied heterodasticity (Quinn and Keough, 2002).

Functional traits

Most of the variability in functional traits was explained by the size class ([chl], leaf size, SSD, A_LA_S, Slenderness index) or by the species identity (SLA, aLAR, aLMF, leafing density). In addition, a significant part of the remaining variability was attributable to the type of recruit (i.e.sucker or sapling) for traits defined at the leaf or stem level (SLA, [chl], leaf size, SSD); for crown-related traits, recruit-type only explained variability in A_LA_S (Table 5). Nevertheless, differences between recruit-types were detected for small plants (Table 5).

At the leaf level, small plants tended to show higher SLA than large plants, which was even more prevalent in *Eucryphia*. SLA was higher in small *Eucryphia* saplings than suckers (Table 5, Fig. 5A). Variability in [chl] was explained by differences between recruit-types in small *Embothrium* plants, with suckers showing higher values (Table 5, Fig. 5B). In both of the studied species, leaf size was higher in large plants and, in the small recruits, suckers showed larger leaves than saplings (Table 5, Fig. 5C). At the stem level, the same pattern described for leaf size was found for SSD, with denser stems in large plants than in small plants, and in small suckers compared to small saplings (Table 5, Fig. 5D). For the two species and recruit-types, the slenderness index was significantly higher in large recruits (Table 5, Fig. 5E). In the case of *Embothrium*, the slenderness index tended to be higher in suckers compared to saplings (significant recruit-type × species interaction; Table 5), although no significant differences were detected in the *posthoc* analysis (Fig. 5E). At the crown level, A_LA_S was higher in large plants; particularly, *Eucryphia* tended to show higher values than *Embothrium* (Table 5, Fig. 5F). For small plants, suckers showed higher A_LA_S than saplings (Table 5, Fig. 5F). Small plants had higher aLAR than large plants, and *Eucryphia* showed greater aLAR values than *Embothrium* (Table 5, Fig. 5G). Accordingly, small plants showed higher aLMF than large plants and *Eucryphia* had higher aLMF values than *Embothrium* (Table 5, Fig. 5H). Leafing density was higher in *Eucryphia* than in *Embothrium* (Table 5, Fig. 5I). Although large plants tended to have higher leafing densities than small plants (P marginally significant; Table 5), the *posthoc* only detected differences in leafing density between size classes in *Eucryphia* (Fig. 5I).

Discussion

Vegetative recruitment through root suckering was very profuse; it was even more relevant than sexual reproduction when only effective recruitment was considered. Root suckering extended the light niche towards open and illuminated microenvironments during the early stages of ontogeny for both studied species, regardless of their shade-tolerance. However, niche differentiation between suckers and saplings vanished when only established recruits were evaluated. The eco-physiological processes that underlie this pattern differ between species according to their shade-tolerance.

Suckering in the shade-tolerant species

The small saplings of *Eucryphia* underused most open conditions and the shadiest ones; this result supports the intermediate shade-tolerance reported for this species (Escobar et al., 2006; Lusk et al., 2006). On the contrary, small suckers did not express niche selection. In this way, *Eucryphia* suckers extended the regeneration niche towards open and well-lit conditions during early ontogenetic stages, supporting previous findings for this species (Escandón et al., 2013). However, niche selection of saplings disappeared when effective recruitment was considered (i.e. only large saplings). The ontogenetic shift in the ecological niche of *Eucryphia* saplings could be explained by differential mortality along the light gradient. In fact, in our study site, large saplings tended to occupy well-lit microhabitats to a greater extent than small saplings, suggesting lower sapling survival in shade. This result slightly differs from that reported in a coastal temperate rainforest located 106 km southwest from the study area, where sapling survival was less in small plants, even though survival of smaller plants increased under very closed canopies (2-4% of canopy openness; Escandón et al., 2013). The interactive stress induced by the high vapor pressure deficit and the high radiation during summer were claimed to be the underlying processes driving the lower sapling survival under more open canopies (Escandón et al., 2013). However, moisture levels in the current study area are much higher, with a difference of 155 mm in the summer rainfall averaged for the period 2015-2016 (see weather stations in the description of the corresponding study areas). Therefore, our study site was most likely less water stressed under similar canopy openness. In addition, the light gradient included in our study did not comprise deep shade (<5% of canopy openness), precluding the estimation of survival probability under very low light.

Both *Eucryphia* suckers and saplings showed an ontogenetic trajectory in the RGR of their stem volume, with greater growth in small recruits. This ontogenetic pattern has been widely described and it is satisfactorily explained by changes in the functional traits throughout plant development (Metcalfe et al., 2006; Rees et al., 2010; Philipson et al., 2012). Small *Eucryphia* recruits allocated more aboveground biomass to leaves compared to large plants (see higher aLMF in small recruits), thus potentially having higher carbon gain that could be allocated to the volumetric increase of the stem. On the contrary, large plants allocated carbon to produce more slender and denser stems, in detriment to the volumetric growth of the stem. This resource allocation pattern is advantageous when competing for light with neighboring plants; it also reduces the risks of mechanical damage (Poorter and Werger, 1999; van Gelder et al., 2006). The ontogenetic pattern of RGR in *Eucryphia* recruits was modulated by light, provided that small plants grew faster under low light conditions. This result seems counterintuitive at first, considering the high quantum yield of shade tolerant plants (Valladares and Niinemets, 2008). However, the effect of light on the stem RGR could respond to the plastic responses of stem length in shady conditions, in order to overtop neighbors and thus increase light interception (Poorter and Werger, 1999).

Contrary to our expectations, the effect of light on RGR was the same for both saplings and suckers. This result is not consistent with a previous study, where growth increased with canopy opening in *Eucryphia* saplings, but not in suckers (Escandón et al., 2018). The greater responsiveness to light of *Eucryphia* saplings was interpreted as a consequence of their higher efficiency in terms of light interception and high carbon assimilation per unit of leaf surface (Escandón et al., 2018). In the present study, the similarity of aLAR and chlorophyll concentration between suckers and saplings could explain the convergent response of growth to light.

Light niche differentiation of *Eucryphia* suckers and saplings was only detected during early ontogeny and could be related to functional differences among recruit-types that no longer exist in more advanced developmental stages. Specifically, small saplings have higher SLA and lower SSD than small suckers. The lower SLA in suckers (compared to saplings) has been interpreted as the result of the ontogenetic inertia expressed by suckers that are increasing their leaf strength with plant development in order to cope with harsh canopy conditions (e.g. high radiation, low temperature, water deficit and wind; Williams and Black, 1993; Thomas and Winner, 2002). Ontogeny could also explain the differences in SSD between small-sized suckers and saplings. Wood density increases throughout ontogeny by increasing the lumen area and decreasing the wall fraction of the xylem fibers (Osazuwa-Peters et al., 2017). In addition, the pith constitutes a large part of the stem volume in very young plants, which is markedly less dense than the xylem (Evert, 2006). Therefore, the ontogenetic decrease of the pith proportion in the stem could also help explain the lower SSD of small compared to the large plants, and that of saplings compared to suckers. In both cases, the higher the SSD the greater the resistance to harsh conditions (Chave et al., 2009). Therefore, the higher leaf and stem strength of suckers aids them in successful establishment and growth under more open microsites, which are more exposed to temperature extremes and drought compared to sites with more closed canopies (Lusk and Laughlin, 2017).

Suckering in the light-demanding species

Contrary to our predictions, the small *Embothrium* saplings did not express niche selection, occupying all of the available light gradient randomly. In this sense, it is noteworthy that the range of light availability considered in our study did not include deep shade (<5% of canopy openness), a situation in which the mortality of *Embothrium* saplings reached 50% during a 14-month period (Lusk, 2002). In fact, the similitude in the GSF frequency distribution of small and large *Embothrium* saplings suggests that survival did not change along the studied light gradient. In addition, and contrary to our expectations, small suckers underused the shaded microsites of the forest, thereby extending the regeneration niche towards more illuminated areas in the early developmental stages. The higher wood density of small suckers (compared to small saplings) could indicate greater resistance to cold temperatures and water deficits, which characterize open and well-lit microhabitats of this temperate rainforest (see Lusk and Laughlin, 2017). On the other hand, the shade-underuse by *Embothrium* suckers was unexpected, provided they have larger, chlorophyll-richer leaves than saplings, but have similar aLAR, aLMF, and leafing densities. Therefore, regardless of the potential parental subsidy, it was expected that *Embothrium* suckers would be less carbon-limited than saplings in shady conditions, unless they were functioning as a carbon source in the genet. In this sense, the low representativeness of *Embothrium* in the canopy was noticeable as was its poor sexual recruitment in the study plots (24%; see Table A.1). This suggests that we studied a senescent population of *Embothrium*, which is being replaced by shade-tolerant species such as *Eucryphia* (Escobar et al., 2006). If so, the aged adults of *Embothrium* could be acting as a strong carbon sink in detriment to growth in small suckers in the shade. In this sense, the ontogenetic shift of the GSF frequency distribution towards the lower end of the light gradient suggests that suckers could successfully establish themselves in shady conditions. Indeed, previous research conducted in the same study area showed that the great majority of *Embothrium* recruits growing at <5% canopy openness were root suckers (Lusk, 2002).

The RGR of the stem volume did not follow the expected ontogenetic trajectory for *Embothrium* suckers or saplings. Resource allocation patterns aid in explaining this result. Specifically, biomass allocation to foliage did not change throughout ontogeny in suckers or saplings (see similarities of aLAR, aLMF, and leafing densities between size classes). Such results support the high turnover rate of foliage described for this species (Lusk, 2002). In fact, light-demanding species typically produce leaves with high SLA, and thus with a fast return of the carbon invested to leaf construction under high light, but low longevity (Lusk, 2002). Therefore, the photo-assimilates of the *Embothrium* recruits could have been mainly invested in the replacement of the leaves, rather than in the volumetric increase of the stem; this allocation pattern would have hindered the detection of tendencies in RGR measured from the growth of the stem, instead from the entire plant.

Conclusions

Niche selection was only detected in *Eucryphia* seedlings, which underused well-lit conditions, and *Embothrium* suckers, which underused shade. The reduced structural strength of the leaves and wood of small *Eucryphia* saplings could explain why they are not able to occupy most open microsites, typically exposed to thermal and drought extremes. In the case of the *Embothrium* suckers, shade-underuse may not be explained by functional traits, but by the senescence of the genet in this secondary forest in an advanced state of succession. Ecological patterns detected in small plants disappeared when large plants were evaluated, which is consistent with the functional trait similarities of large suckers and saplings for both species. Changes of the stem growth rate with light availability of both species did not explain the ecological patterns found, but could explain a differential mortality along the gradient of light availability.

Overall, our results suggest that root suckering is a successful mechanism of recruitment in the two studied species, but does not expand the light niche. However, suckering did improve the persistence of *Embothrium* until advanced stages of forest succession, facilitating the coexistence with the late successional *Eucryphia*. In this sense, it is noteworthy that many tree species in the temperate rainforests of southern South America express clonal growth strategies, regardless of their shade-tolerance strategy (Paula et al., unpublished data). Therefore, clonal growth could explain why the light niche highly overlaps among most tree species of this ecosystem-type, where coexisting trees comprise a wide range of functional responses to light (Lusk et al., 2006). Consequently, a better understanding of clonal growth and its ecological meaning will significantly contribute to deepening knowledge regarding temperate rainforest dynamics and diversity.

Author's contributions

A.B.E., S.P. and A.S. planned and designed the research. A.B.E. and S.P. conducted the field work. A.B.E. and S.P. analyzed the data. A.B.E. and S.P. wrote the manuscript with contributions from A.S.

Declarations of conflicts interest

None.

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Supplementary data

Supplementary material related to this article can be found in the online version.

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Tables

Table 2.1. Kolmogorov-Smirnov test (K-S) comparing the GSF distribution of recruit-types and species with the GSF distribution of the understory. The analyses were conducted considering small and large recruits (respectively <50 cm and ≥50 cm in length). The significance level (α) was established by means of the step-up false discovery rate (FDR) procedure. Significant differences (in bold) indicate that recruits of a given species are non-randomly distributed in relation to the understory GSF gradient.

		Small recruits				Large recruits				Understory
Species	Recruit-type	D	P	α FDR	GSF IQ range	D	P	α FDR	GSF IQ range	GSF IQ range
Embothrium	Suckers	0.21	**0.0089**	0.0125	[0.11 - 0.18]	0.18	0.14	0.025	[0.10 - 0.15]	[0.08 - 0.18]
	Saplings	0.25	0.03	0.019	[0.08 - 0.13]	0.18	0.69	0.038	[0.09 - 0.15]	
Eucryphia	Suckers	0.14	0.8	0.044	[0.10 - 0.17]	0.11	0.8	0.05	[0.10 - 0.19]	
	Saplings	0.29	**0.0005**	0.006	[0.09 - 0.13]	0.17	0.48	0.032	[0.09 - 0.17]	

Table 2.2. Kolmogorov-Smirnov test (K-S) comparing the GSF distribution of recruit-types. The analyses were conducted considering small and large recruits (respectively <50 cm and ≥50 cm in length). The significance level (α) was established by means of the step-up false discovery rate (FDR) procedure. Significant differences (in bold) indicate that recruits of a given species were distributed differently along the GSF gradient.

Species	Size class	D	P	αFDR
Embothrium	Small	0.31	**0.003**	0.0125
Eucryphia	Small	0.35	**0.012**	0.025
Embothrium	Large	0.15	0.88	0.05
Eucryphia	Large	0.17	0.62	0.0375

Table 2.3. Kolmogorov-Smirnov test (K-S) comparing the GSF distribution of small and large recruits for each recruit-type and species. The analyses were conducted considering small and large recruits (respectively <50 cm and ≥50 cm in length). The significance level (α) was established by means of the step-up false discovery rate (FDR) procedure. Significant differences (in bold) indicate that small or large recruit sizes of a given recruit-type and species are distributed differently along the GSF gradient.

Species	Recruit-type	D	P	α FDR
Embothrium	Suckers	0.25	**0.008**	0.0125
	Saplings	0.23	0.45	0.0375
Eucryphia	Suckers	0.15	0.85	0.05
	Saplings	0.27	0.075	0.025

Table 2.4. Model parameters of the linear regressions conducted to evaluate the relationship of the RGR (response variable) with initial size (length) and light availability (GSF; log transformed) for each recruit-type and species. The significance level (α) was established by means of the step-up false discovery rate (FDR) procedure (see significant differences in bold).

Species and recruit-type	Regression coefficients					Model information and assumptions
	Fixed effects	Estimate	SE	*t*-value	P	
Embothrium						P = 0.078
Suckers	Intercept	0.0195	0.88	0.02	0.98	α (FDR) = 0.038
	Length (L)	0.028	0.02	1.5	0.14	Multiple R^2 = 0.049
	log(GSF)	0.39	0.43	0.91	0.36	Shapiro-Wilk test: W = 0.972, P = 0.006
	L × log(GSF)	0.01	0.01	1.12	0.27	NCV test: χ^2 = 0.371, df = 1, P = 0.54
Saplings	Intercept	-1.82	1.35	-1.35	0.18	P = 0.11
	Length (L)	-0.005	0.02	-0.27	0.79	α (FDR) = 0.05
	log(GSF)	-0.31	0.59	-0.53	0.6	Multiple R^2 = 0.14
	L × log(GSF)	-0.006	0.01	-0.72	0.47	Shapiro-Wilk test: W = 0.96, P = 0.14
						NCV test: χ^2 = 0.013, df = 1, P = 0.91
Eucryphia						P = 0.0054
Suckers	Intercept	-2.18	0.8	-2.73	0.01	α (FDR) = 0.025
	Length (L)	-0.017	0.01	-1.55	0.13	Multiple R^2 = 0.195
	log(GSF)	-0.78	0.38	-2.04	**0.046**	Shapiro-Wilk test: W = 0.967, P = 0.093
	L × log(GSF)	-0.013	0.01	-2.29	**0.026**	NCV test: χ^2 = 0.514, df = 1, P = 0.47
Saplings	Intercept	-2.21	0.58	-3.85	0	P< 0.0001

Length (L)	-0.016	0.01	-1.6	0.11	α (FDR) = 0.0125
log(GSF)	-0.84	0.26	-3.24	**0.0017**	Multiple R^2 = 0.472
L × log(GSF)	-0.013	0	-2.82	**0.006**	Shapiro-Wilk test: W = 0.961, P = 0.006
					NCV test: χ^2 = 0.023, df = 1, P = 0.88

Table 2.5. Results of ANOVAs evaluating differences in functional traits between size classes (small: <50 cm and large: ≥50 cm in length), recruit-types (root suckers and saplings) and species (*Embothrium* and *Eucryphia*). The significance level (α) was established by means of the step-up false discovery rate (FDR) procedure (see significant differences in bold). The results of the tests conducted to evaluate the ANOVA assumptions are also included (Shapiro-Wilk and NCV tests).* Box-Cox transformed functional trait.

Source	df	SS	MS	F-value	P	Var. (%)	α FDR	df	SS	MS	F-value	P	Var. (%)	α FDR	df	SS	MS	F-value	P	Var. (%)	α FDR
	Specific leaf area (SLA)*							Chlorophyll concentration ([chl])*							Leaf size*						
Size (SZ)	1	0.00043	0.00043	32.51	**<0.001**	14.1	0.009	1	0.0135	0.0135	26.67	**<0.001**	15.7	0.013	1	52.31	52.31	72.37	**<0.001**	32.3	0.005
Recruit (RT)	1	0.00021	0.00021	15.76	**<0.001**	6.9	0.014	1	0.0048	0.0048	9.52	**0.003**	5.6	0.018	1	19.70	19.70	27.26	**<0.001**	12.2	0.012
Species (SP)	1	0.00085	0.00085	63.41	**<0.001**	27.6	0.007	1	0.0022	0.0022	4.26	0.041	2.5	0.025	1	0.30	0.30	0.41	0.524	0.2	0.046
SZ × RT	1	0.00005	0.00005	3.63	0.059	1.6	0.027	1	0.0061	0.0061	12.07	**<0.001**	7.1	0.016	1	7.77	7.77	10.75	**0.002**	4.8	0.017
SZ × SP	1	0.00001	0.00001	0.65	0.423	0.3	0.044	1	0.0006	0.0006	1.16	0.285	0.7	0.039	1	2.38	2.38	3.30	0.072	1.5	0.029
RT × SP	1	0.00002	0.00002	1.42	0.236	0.6	0.036	1	0.0030	0.0030	5.92	**0.017**	3.5	0.021	1	0.03	0.030	0.04	0.844	0.0	0.055
SZ × RT × SP	1	0.00005	0.00005	3.49	0.065	1.5	0.028	1	0.0027	0.0027	5.34	0.022	3.1	0.022	1	0.79	0.79	1.09	0.299	0.5	0.04
Residuals	109	0.00145	0.00001		Total	52.6		105	0.0532	0.0005		Total	38.2		109	78.78	0.72		Total	51.4	
	Shapiro: W = 0.98, P = 0.101; NVC: χ^2 = 0.44, P = 0.506							Shapiro: W = 0.99, P = 0.521; NVC: χ^2 = 0.02, P = 0.876							Shapiro: W = 0.99, P = 0.447; NVC: χ^2 = 0.26, P = 0.611						
	Stem specific density (SSD)*							Slenderness index							Leaf-to-stem area ratio ($A_L A_S$)*						
Size (SZ)	1	1.190	1.190	270.81	**<0.001**	63.2	0.004	1	618.37	618.37	89.93	**<0.001**	42.9	0.005	1	54.79	54.79	5.93	**0.017**	2.5	0.02
Recruit (RT)	1	0.141	0.141	32.16	**<0.001**	7.5	0.01	1	0.28	0.28	0.04	0.840	0.0	0.055	1	58.85	58.85	6.37	**0.013**	2.7	0.018
Species (SP)	1	0.004	0.004	0.87	0.354	0.2	0.042	1	0.61	0.61	0.09	0.767	0.0	0.052	1	1060.14	1060.14	114.7	**<0.001**	48.1	0.002
SZ × RT	1	0.05536	0.0553	12.60	**<0.001**	2.9	0.014	1	14.45	14.45	2.10	0.150	1.0	0.033	1	18.17	18.17	1.97	0.16	0.8	0.033
SZ × SP	1	0.006	0.006	1.33	0.252	0.3	0.037	1	15.39	15.39	2.24	0.138	1.1	0.032	1	1.13	1.13	0.12	0.73	0.1	0.05
RT × SP	1	0.003	0.003	0.63	0.428	0.1	0.044	1	40.93	40.93	5.95	**0.016**	2.8	0.019	1	2.55	2.55	0.28	0.6	0.1	0.48
SZ × RT × SP	1	0.006	0.006	1.25	0.265	0.3	0.038	1	0.8	0.8	0.12	0.734	0.1	0.051	1	0.9	0.9	0.1	0.76	0	0.052
Residuals	109	0.479	0.004		Total	74.6		109	749.47	6.88		Total	48.0		109	1007.71	9.25		Total	54.3	
	Shapiro: W = 0.99, P = 0.630; NVC: χ^2 = 0.62, P = 0.430							Shapiro: W = 0.99, P = 0.781; NVC: χ^2 = 0.02, P = 0.903							Shapiro: W = 0.98, P = 0.195; NVC: χ^2 = 0.30, P = 0.582						
	Aboveground leaf area ratio (aLAR)*							Aboveground leaf mass fraction (aLMF)							Leafing density*						
Size (SZ)	1	796.98	796.98	49.78	**<0.001**	20.6	0.008	1	0.3476	0.3476	28.67	**<0.001**	10.3	0.011	1	0.02798	0.02798	4.19	0.043	2.0	0.025
Recruit (RT)	1	30.64	30.64	1.91	0.169	0.8	0.034	1	0.0010	0.00010	0.08	0.778	0.0	0.053	1	0.00222	0.00222	0.33	0.565	0.2	0.047
Species (SP)	1	1126.82	1126.82	70.38	**<0.001**	29.1	0.006	1	1.6144	1.6144	133.13	**<0.001**	47.8	0.0008	1	0.64775	0.64775	97.04	**<0.001**	45.2	0.003
SZ × RT	1	8.44	8.44	0.53	0.470	0.2	0.045	1	0.0006	0.0006	0.05	0.830	0.0	0.054	1	0.01133	0.01133	1.70	0.195	0.8	0.035
SZ × SP	1	48.13	48.13	3.01	0.086	1.2	0.029	1	0.0526	0.0526	4.34	0.040	1.6	0.024	1	0.00852	0.00852	1.28	0.261	0.6	0.037
RT × SP	1	41.44	41.44	2.59	0.111	1.1	0.031	1	0.0024	0.0024	0.20	0.658	0.1	0.049	1	0.0017	0.0017	0.25	0.615	0.1	0.048
SZ × RT × SP	1	75.54	75.54	4.72	0.032	2.0	0.023	1	0.0116	0.0116	0.96	0.329	0.3	0.041	1	0.00576	0.00576	0.86	0.355	0.4	0.043
Residuals	109	1745.18	16.01		Total	54.9		111	1.3460	0.0121		Total	60.1		109	0.7276	0.00668		Total	49.2	
	Shapiro: W = 1.00, P = 0.927; NVC: χ^2 = 0.02, P = 0.880							Shapiro: W = 0.99, P = 0.828; NVC: χ^2 = 1.40,P = 0.236							Shapiro: W = 0.99, P = 0.261; NVC: χ^2 = 0.25, P = 0.614						

Figure legends

Figure 2.1. Global site factor (GSF) frequency distribution for the understory (grey area) and the recruits (lines) of the two studied species, shown separately for large and small individuals (≥50 cm and <50 cm in length, respectively). Continuous lines correspond to root suckers and discontinuous lines to saplings. Symbols along the *x*-axis in panels C and D indicate the deciles of the GSF distribution of the understory, different symbols show the results of the GSF decile comparison as shown in Fig. 2A (*Embothrium*) and Fig. 2B (*Eucryphia*): black symbols in the corresponding GSF decile indicate significant differences between the understory and the root suckers (triangles) or saplings (squares); crosses were used otherwise. Circles along the frequency distribution lines denote the corresponding GSF deciles, different colors show the results of the GSF decile comparison as shown in Fig. 2C (*Embothrium*) and Fig. 2D (*Eucryphia*): black circles correspond to those deciles differing between small suckers and small saplings; white circles were used otherwise.

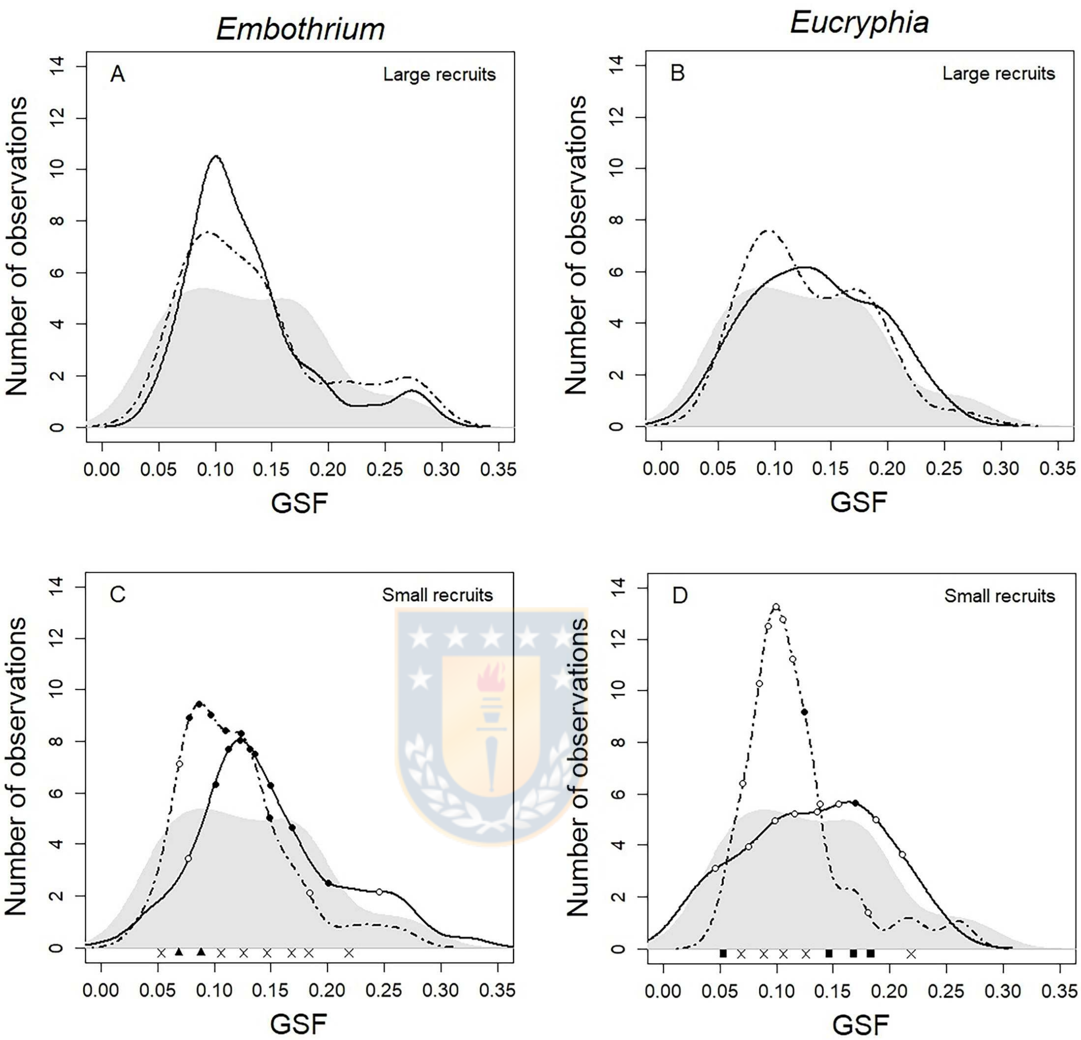
Figure 2.2. Global site factor (GSF) decile differences (± bootstrapping confidence interval) between the understory and small recruits (panels A-B) and between small suckers and small saplings (panels C-D) for the two studied species. Black symbols represent significant differences (i.e. bootstrapping confidence interval does not include zero; type I error was controlled with Hochberg's method); white symbols were used otherwise.

Figure 2.3. Comparisons of the global site factor (GSF) frequency distribution between large and small suckers of *Embothrium*. Panel A shows the GSF frequency distribution for the understory (grey area) and the *Embothrium* root suckers (lines). The continuous line corresponds to small suckers and the discontinuous line to large suckers (<50 cm and ≥50 cm and in length, respectively). Circles along the frequency distribution lines denote the corresponding GSF deciles, different colors represent the results for the GSF decile comparison as shown in panel B. Panel B shows GSF decile differences (± bootstrapping confidence interval) between large and small suckers of *Embothrium*. Black symbols were used for significant differences (i.e. bootstrapping confidence interval does not include zero; type I error was controlled with Hochberg's method); white symbols were used otherwise.

Figure 2.4. Relationship between the initial stem length and relative growth rate (RGR; expressed in terms of stem volume) for each recruit-type and species. Symbols' sizes are proportional to the global site factor (GSF) value above the recruit: the larger the symbol, the higher the GSF. Lines represent the significant estimated relationship of the two variables for low (continuous line) and high (dashed line) light (see alpha FDR values in Table 4). Low and high light levels correspond to the first and third quantile of the GSF frequency distribution, respectively (specifically, GSF=0.09 and GSF=0.16). Shaded areas correspond to 95% confidence intervals. The R^2 and P values for the full model, which tested changes in the RGR in relation

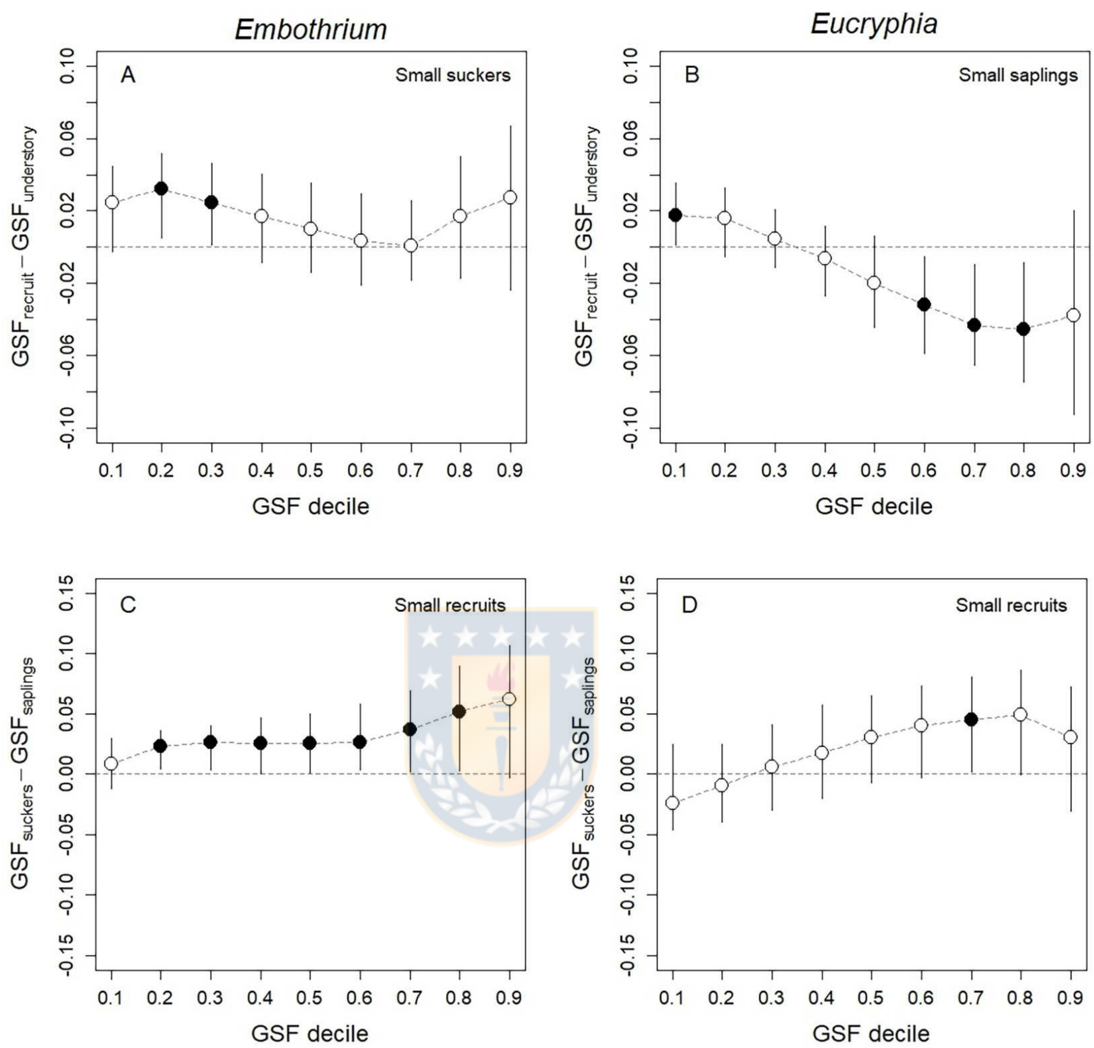
to the initial length and the interaction with GSF, are shown. See full results in Table 4.

Figure 2.5. Boxplot of functional traits for each species (*Embothrium coccineum* and *Eucryphia cordifolia*) and recruit-type (suckers and saplings; grey and white, respectively), shown separately according to the size class: small and large (<50 cm and ≥50 cm in length). Different lowercase letters at the top of each panel indicate significant differences ($P < 0.05$) in the Tukey *posthoc* test conducted for the full factor interaction. The significance level (α) was established by means of the step-up false discovery rate (FDR) procedure. Panels show: A) SLA: specific leaf area; B) [chl]: chlorophyll relative content; C) leaf size; D) SSD: specific stem density; E) Slenderness: slenderness index; F) A_LA_S: leaf area to stem cross-section area; G) aLAR: aboveground leaf area ratio; H) aLMF: aboveground leaf mass fraction; I) Leafing density: number of leaves per stem length. See full results of ANOVA in Table 5.



Embothrium
Eucryphia
A
Large recruits
B
Large recruits
C
Small recruits
D
Small recruits
Number of observations
GSF
0 2 4 6 8 10 12 14
0.00 0.05 0.10 0.15 0.20 0.25 0.30 0.35

Figure 2.1.



2357

2358 Figure 2.2.

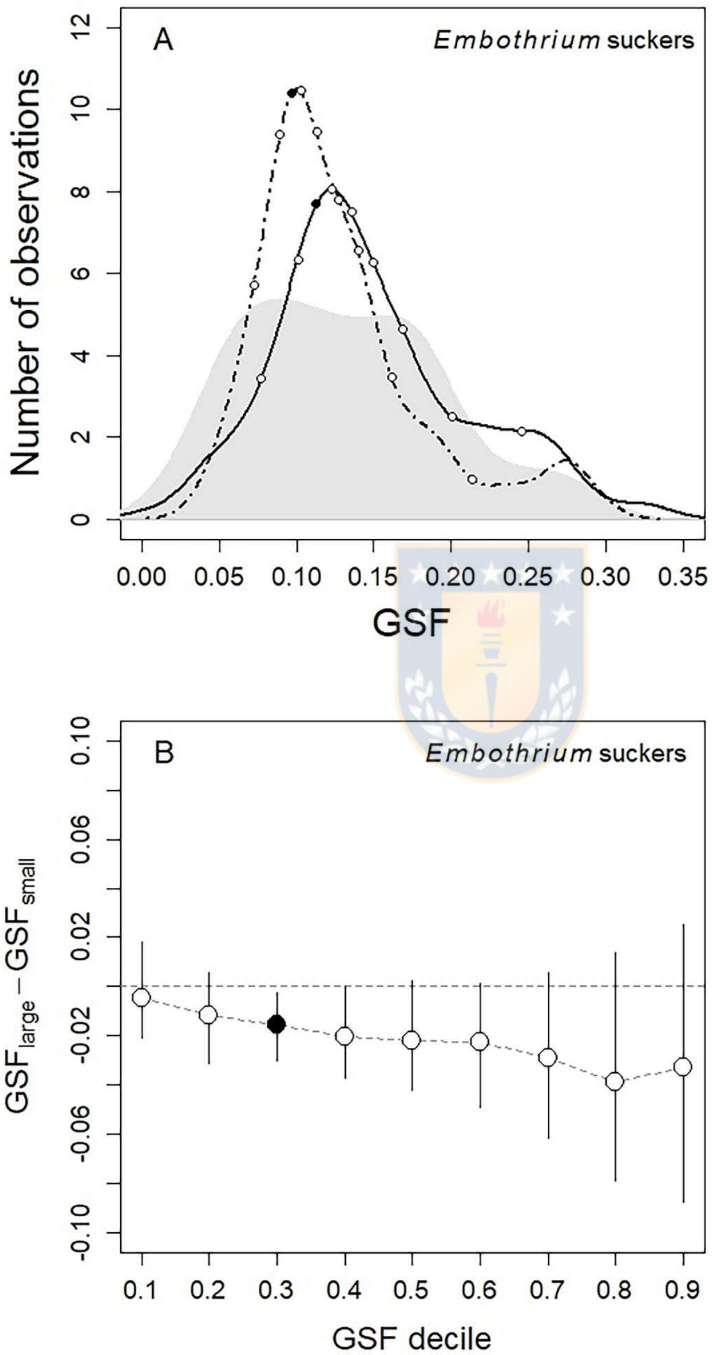

A
Embothrium suckers
Number of observations
0
2
4
6
8
10
12
0.00
0.05
0.10
0.15
0.20
0.25
0.30
0.35
GSF
B
Embothrium suckers
GSF_large − GSF_small
0.10
0.06
0.02
-0.02
-0.06
-0.10
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
GSF decile

Figure 2.3.

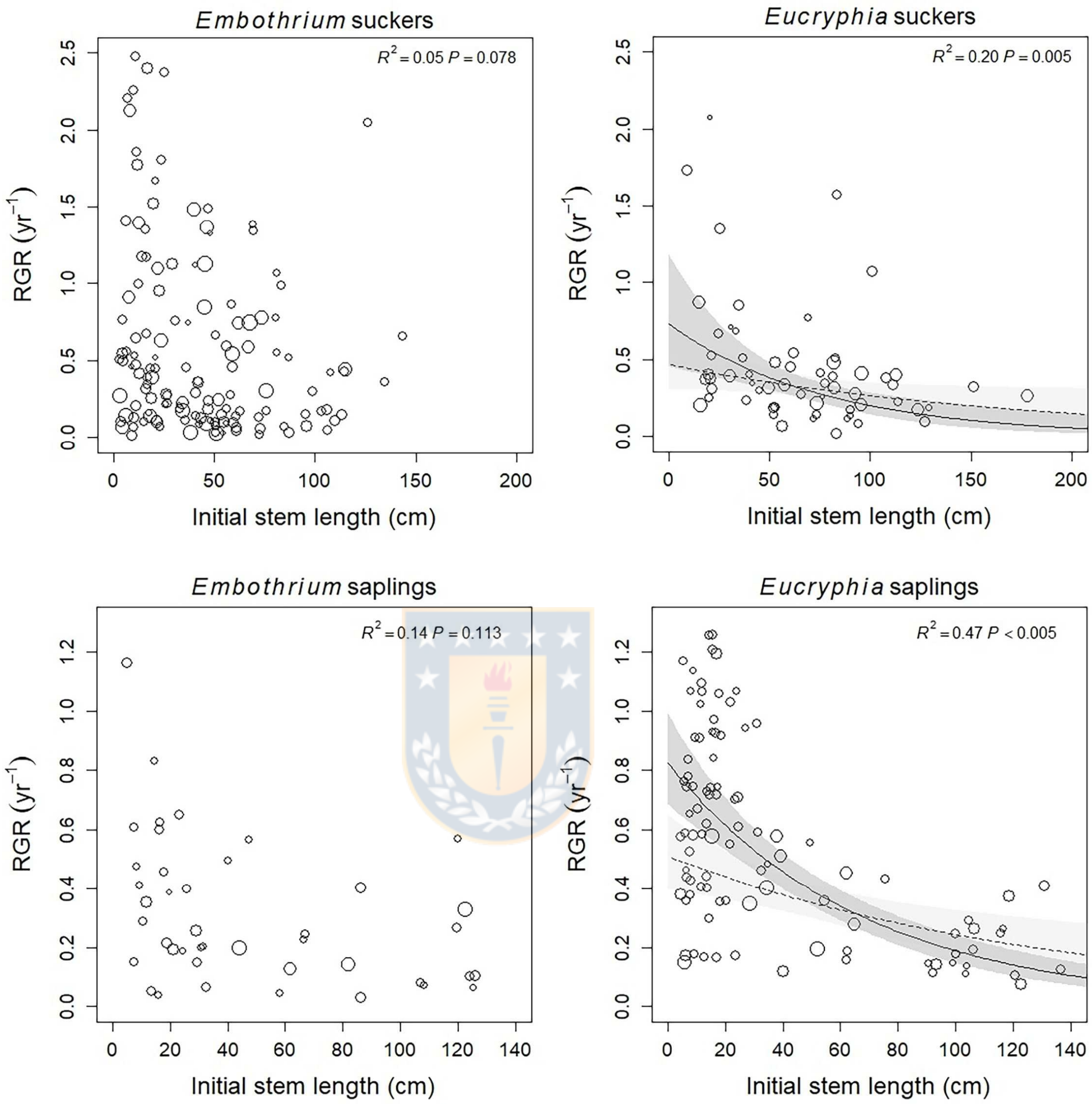
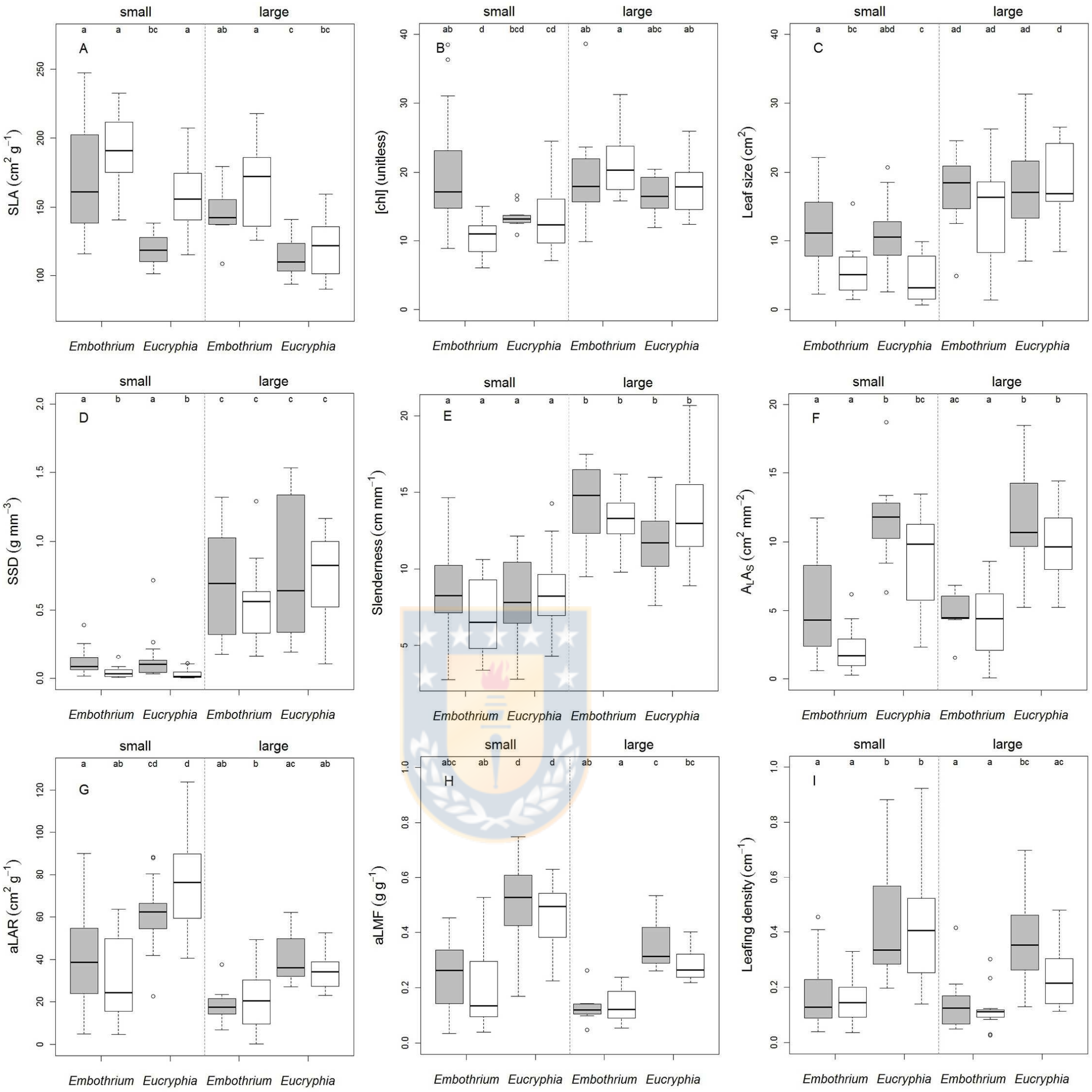

Embothrium suckers
R² = 0.05 P = 0.078
Eucryphia suckers
R² = 0.20 P = 0.005
Embothrium saplings
R² = 0.14 P = 0.113
Eucryphia saplings
R² = 0.47 P < 0.005
RGR (yr⁻¹)
Initial stem length (cm)

Figure 2.4.



small
large
A
SLA (cm^2 g^{-1})
B
[chl] (unitless)
C
Leaf size (cm^2)
D
SSD (g mm^{-3})
E
Slenderness (cm mm^{-1})
F
$A_L A_S$ (cm^2 mm^{-2})
G
aLAR (cm^2 g^{-1})
H
aLMF (g g^{-1})
I
Leafing density (cm^{-1})
Embothrium
Eucryphia
Embothrium
Eucryphia

Figure 2.5.

Supplementary material

Root suckering promotes recruitment in temperate rainforest trees species regardless shade-tolerance

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Supplementary material

Appendix A

Table A.1. Description of the study plots, including the global site factor (GSF) range in the understory and, for the two studied species, the number of adult trees, total basal area of trees, diameter at the breast height (DBH; mean ±SD), number of root suckers and number of saplings.

		Adults			Recruits	
	Species	*n*	Basal area ($m^2 ha^{-1}$)	DBH ± SD (mm)	Root suckers	Saplings
Plot 1	*Embothrium*	23	1.6	102.8 ± 52.7	116	44
GSF: [0.02 – 0.17]	*Eucryphia*	158	12.5	110 ± 55.1	38	93
Plot 2	*Embothrium*	9	1	133.2 ± 59	90	21
GSF: [0.08 – 0.30]	*Eucryphia*	90	5.6	86.3 ± 66.5	38	24
Total		280	20.7		282	182

Table A.2. Results of ANOVA comparing theglobal site factor (GSF) between recruit-types and species, considering only the individuals sampled to measure functional traits. Analysis of variance was conducted using type I of sum of squares. Residuals of the model were normally distributed (Shapiro-Wilk test: W = 0.99, P = 0.44) and homoscedastic (NCV test: χ^2 = 0.63, df = 1, P = 0.43) after Box-Cox transformation of GSF.

	df	SS	MS	*F*-value	P
Recruit-type (RT)	1	0.0001	0.0001	1.25	0.265
Species (SP)	1	0.0002	0.0002	1.94	0.166
RT × SP	1	0.00001	0.00001	0.13	0.722
Residuals	131	0.01	0.000080		

Table A.3. Results of the decile comparison of the global site factor (GSF) distribution between the understory and those recruits for which the Kolmogorov-Smirnov test detected light niche selection (see Table 1): small suckers of *Embothrium* and small saplings of *Eucryphia*. Small recruits are those with less than 50 cm length. The deciles were estimated by bootstrapping and the critical P-value (P-critical) established with Hochberg's method. P-values (P) lower than P-critical (in bold) indicate that the corresponding GSF decile are significantly different between the understory and the recruit.

Decile	Recruit *n*	Forest *n*	Recruit GSF estimate	Forest GSF estimate	Difference GSF estimate	Confidence interval GSF estimate difference	P-critical	P
Small suckers of *Embothrium*								
0.1	139	111	0.0774	0.0531	0.0243	[-0.003 - 0.045]	0.0071	0.021
0.2	139	111	0.1009	0.0689	0.0321	[0.005 - 0.052]	0.0056	**0.001**
0.3	139	111	0.1129	0.0885	0.0244	[0.001 - 0.046]	0.0062	**0.005**
0.4	139	111	0.1228	0.1058	0.0171	[-0.008 - 0.04]	0.0083	0.064
0.5	139	111	0.1354	0.1255	0.0098	[-0.014 - 0.04]	0.0167	0.36
0.6	139	111	0.1496	0.1465	0.0032	[-0.021 - 0.029]	0.025	0.76
0.7	139	111	0.1689	0.168	0.0009	[-0.018 - 0.026]	0.05	0.91
0.8	139	111	0.2004	0.1833	0.0171	[-0.017 - 0.05]	0.0125	0.27
0.9	139	111	0.2458	0.2186	0.0272	[-0.024 - 0.067]	0.01	0.2
Small saplings of *Eucryphia*								
0.1	86	111	0.0705	0.0531	0.0174	[0.002 - 0.036]	0.0083	**0.004**
0.2	86	111	0.0849	0.0689	0.0161	[-0.005 - 0.033]	0.0125	0.063

0.3	86	111	0.093	0.0885	0.0045	[-0.011 - 0.021]	0.05	0.58
0.4	86	111	0.0992	0.1058	-0.0065	[-0.027 - 0.012]	0.025	0.41
0.5	86	111	0.1058	0.1255	-0.0198	[-0.044 - 0.006]	0.01	0.048
0.6	86	111	0.1146	0.1465	-0.0319	[-0.059 - -0.005]	0.0071	**0.002**
0.7	86	111	0.1247	0.168	-0.0433	[-0.065 - -0.01]	0.0056	**0.001**
0.8	86	111	0.138	0.1833	-0.0452	[-0.075 - -0.009]	0.0062	**0.001**
0.9	86	111	0.1809	0.2186	-0.0378	[-0.093 - 0.021]	0.0167	0.13

Table A.4. Results of the decile comparison of the global site factor (GSF) distribution between suckers and saplings for which the Kolmogorov-Smirnov test detected light niche differentiation (see Table 2): small recruits of *Embothrium* and small recruits of *Eucryphia*. Small recruits are those with less than 50 cm length. The deciles were estimated by bootstrapping and the critical P-value (P-critical) established with Hochberg's method. P-values (P) lower than P-critical (in bold) indicate that the corresponding GSF decile are significantly different between the recruit-types.

Decile	Suckers *n*	Saplings *n*	Sucker GSF estimate	Saplings GSF estimate	Difference GSF estimate	Confidence interval GSF estimate difference	P-critical	P
Small recruits of *Embothrium*								
0.1	139	46	0.0774	0.0693	0.0081	[-0.01 - 0.03]	0.05	0.39
0.2	139	46	0.1009	0.0782	0.0228	[0.004 - 0.036]	0.0167	**0.001**
0.3	139	46	0.1129	0.0866	0.0263	[0.004 - 0.04]	0.0071	**0.001**
0.4	139	46	0.1228	0.0973	0.0256	[0.0004 - 0.05]	0.0125	**0.01**
0.5	139	46	0.1354	0.1102	0.0252	[0.001 - 0.05]	0.01	**0.007**
0.6	139	46	0.1496	0.1234	0.0262	[0.004 - 0.06]	0.0056	**0.002**
0.7	139	46	0.1689	0.1318	0.0371	[0.002 - 0.07]	0.0062	**0.005**
0.8	139	46	0.2004	0.149	0.0514	[0.003 - 0.089]	0.0083	**0.006**
0.9	139	46	0.2458	0.1838	0.062	[-0.003 - 0.11]	0.025	0.032
Small recruits of *Eucryphia*								
0.1	28	86	0.0462	0.0705	-0.0243	[-0.05 - 0.025]	0.01	0.2

0.2	28	86	0.0753	0.0849	-0.0096	[-0.04 - 0.024]	0.05	0.67
0.3	28	86	0.0989	0.093	0.0059	[-0.03 - 0.041]	0.025	0.65
0.4	28	86	0.1163	0.0992	0.0171	[-0.02 - 0.057]	0.0125	0.19
0.5	28	86	0.136	0.1058	0.0302	[-0.01 - 0.066]	0.0083	0.06
0.6	28	86	0.1549	0.1146	0.0403	[-0.003 - 0.074]	0.0071	0.014
0.7	28	86	0.1698	0.1247	0.0451	[0.002 - 0.081]	0.0056	**0.004**
0.8	28	86	0.1873	0.138	0.0492	[-0.001 - 0.087]	0.0062	0.007
0.9	28	86	0.2109	0.1809	0.0301	[-0.03 - 0.073]	0.0167	0.27

Table A.5. Results of the decile comparison of the global site factor (GSF) distribution between large and small recruits for which the Kolmogorov-Smirnov test detected differences in the survival of recruits (see Table 3): Large vs small suckers of *Embothrium*. Large and small recruits are those with more and less than 50 cm length, respectively. The deciles were estimated by bootstrapping and the critical P-value (P-critical) established with Hochberg's method. P-values (P) lower than P-critical (in bold) indicate that the corresponding GSF decile are significantly different between the size classes.

Decile	Large *n*	Small *n*	Large GSF estimate	Small GSF estimate	Difference GSF estimate	Confidence interval GSF estimate difference	P-critical	P
				Suckers of *Embothrium*				
0.1	67	139	0.0726	0.0774	-0.0049	[-0.021 - 0.018]	0.05	0.71
0.2	67	139	0.0894	0.1009	-0.0115	[-0.031 - 0.006]	0.0167	0.15
0.3	67	139	0.0972	0.1129	-0.0157	[-0.03 - -0.003]	0.0056	**0.002**
0.4	67	139	0.1026	0.1228	-0.0203	[-0.037 - 0.0003]	0.0062	0.008
0.5	67	139	0.1132	0.1354	-0.0221	[-0.042 - 0.0022]	0.0071	0.015
0.6	67	139	0.127	0.1496	-0.0226	[-0.049 - 0.0011]	0.01	0.017
0.7	67	139	0.1398	0.1689	-0.0292	[-0.062 - 0.0054]	0.0083	0.021
0.8	67	139	0.1614	0.2004	-0.0389	[-0.079 - 0.014]	0.0125	0.07
0.9	67	139	0.2131	0.2458	-0.0327	[-0.087 - 0.025]	0.025	0.29

CHAPTER III

Stable isotope signals in root suckers suggests facilitation of coexistence mediated by parent subsidy in two species differing in shade-tolerance

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Abstract

The high physiological performance of clonal plants has been mainly linked with resource translocation among ramets while remain interconnected, especially for herbaceous species, influencing the species persistence. However, it is not known empirically whether root suckers of tree species with different shade-tolerance could work as strategy for resources capture in light-limiting understory. We explore this in *Embothrium coccineum* (light-demanding) and *Eucryphia cordifolia* (shade-tolerant), two evergreen tree species in a temperate rainforest of Southern Chile. We measured light availability above the apex of both recruits root suckers and saplings (i.e. seed origin plants) of each species, and compared leaf chemical traits (carbon (C) and nitrogen (N) content, $\delta^{13}C$ and $\delta^{15}N$ natural abundance) between recruit-types and species. A pulse labeling experiment with carbon ($^{13}CO_2$) was also performed in the field, labeling a set of *Embothrium* root suckers (donors) to quantify C transfer from high-light (donor) to mid-light (receiver) suckers. For both donor and receiver, young fully expanded leaves were harvested seven days after the pulse labeling to determine chemical traits and excess ^{12}C equivalent from $\delta^{13}C$. In terms of natural abundance, all the quantified parameters differed among species, but only leaf $\delta^{13}C$ were higher in suckers compared to saplings, under similar light environment. In labeling experiment, the excess ^{12}C equivalent was higher in donor than receiver suckers of *Embothrium* reflecting non-carbon transfer, in spite of the strength of the sinks. Natural stable isotope abundance of suckers and saplings together with the labeling experiment on

suckers suggests that root suckering is more important for regeneration and persistence rather than resource acquisition, facilitating species coexistence at an intermediate ecological succession. However, increasing the pool of possible sources and sinksof resources as water and nutrients, and to integrate more environmental factors becomes necessary to reach a better understanding of resource translocation through interconnected root suckers.

Introduction

Clonal growth in plants, i.e. the generation of genetically identical and potentially independent ramets by vegetative growth (Harper 1977, van Groenendael et al. 1996), increases the residence time of a plant on a site (i.e. persistence; Bond and Midgley 2001), thus impacting both populations and forest succession dynamics (Peterson and Jones 1997 and references therein, González et al. 2002, Mateo et al. 2004, Beaudet et al. 2007, Beaudet and Messier 2008, Muñoz and González 2009). Resource translocation among ramets explains the high physiological performance of clonal plants while remain interconnected (Pitelka and Ashmun 1985, Klimeš et al. 1997, Adonsou et al. 2016). Ramets physiologically interconnected to their parents and other ramets can develop a division of labor in which connected ramets specialize to acquire different, locally abundant available resources, being able to share them (Stuefer 1998). For instance, herbaceous ramets growing under high light may have higher leaf area (allowing higher light interception) and higher photosynthetic capacity than the ramets growing under low light (Alpert and Moony 1986, Magda et al. 1988, Stuefer et al. 1996, Roiloa et al. 2014). Similarly, root development of herbaceous ramets may be more profuse when growing under wet conditions than in those rooted in dry patches (Stuefer et al. 1996, Lambers et al. 2012b). Therefore, ramets can show intra-specifically different characteristics when compared to individuals of seed origin under similar environmental conditions (Lambers et al. 2012b, Escandón et al. 2018).

Translocation of water, nutrients and photosynthates between interconnected ramets of herbaceous rhizomatous and stoloniferous plants has been demonstrated in numerous experimental studies (Noble and Marshall 1983, Chapman et al. 1992, de Kroon et al. 1996), but studies of root suckers (i.e. ramets of woody plants mainly) have been less representative under both field and controlled conditions (Pinno and 2014, Adonsou et al. 2016). Besides, in the root-suckering tree *Populus tremuloides*, N translocation is not totally involving a division of labor, suggesting other different functional role of clonal integration in trees compared to herbaceous plants (Pinno and Wilson, 2014). In this sense, resources translocation between ramets could be explained not by the resource availability but by both the size and the source-sink relationship hypotheses. In terms of resource availability, the translocation occurs toward the larger ramets with greater nutrient demand; whereas in terms of source-sink relationship, increasing the sink strength of ramets by shading, clipping, defoliation or natural senescence, has resulted in increased carbon transfer from unstressed ramets (Magda et al. 1988, Marshall 1990, Zhang et al. 2002; see Teste et al. 2009for mycorrhizal networks). However, we are not aware of any field study on carbon flux for clonal growth in trees, so this will be explored here.

The natural abundance of carbon isotope composition ($\delta^{13}C$) reflect different metabolic and transport processes, and hence might be used to test clonal integration. $\delta^{13}C$ is strongly modified by diffusive and biochemical processes during photosynthesis (Farquhar et al. 1982, Farquhar et al.

1989). Under water stress, stomatal closure forces the fixation of the heavier C isotope (^{13}C), leading to less negative $\delta^{13}C$ (O' Leary 1981, Farquhar et al. 1982). Similarly, higher carboxylation rates diminish the discrimination against ^{13}C, resulting in higher $\delta^{13}C$ (O' Leary 1981, O' Leary 1995). Nevertheless, carbohydrates are also enriched during their translocation across plant organs, mainly due to the discrimination against ^{13}C of the invertases involved in the sucrose synthesis (Tcherkez et al. 2011, Rolland et al. 2002). Therefore, $\delta^{13}C$ of the ramet's leaves could be used as a proxy for carbon sharing, reflecting their status as either carbon sources or sinks. On top of that, pulse-chase labeling experiments can be used to trace the fate of carbon assimilates, from source to sink tissues (Ruehr et al. 2009; Brüggeman et al. 2011; Epron et al. 2012). In this context, this technique could be applied to assess the eventual carbon transfer between interconnected root suckers.

Similar to $\delta^{13}C$, nitrogen isotope composition ($\delta^{15}N$) could also be used to trace nitrogen sharing among ramets. One source of variation in $\delta^{15}N$ for plants is related to the soil available nitrogen and his form (nitrate >> ammonium > amino acids). After plant uptake, nitrate and ammonium are assimilated by nitrate reductase and glutamine synthetase enzymes, respectively, which fractionates against ^{15}N (Evans 2001). This enzyme fractionation effect is lower under limited nitrogen availability, which results in a net increment of $\delta^{15}N$. In other words, when nitrate reduction preferentially consumes most of the $^{14}NO_3$ available, no $^{14}N/^{15}N$ net discrimination is possible during reduction (Tcherkez and Hodges 2008). In such a framework, the effect of the parent subsidy, together with the minimal production of an own root system in root suckers would result in a less negative $\delta^{15}N$, as compared to stand-alone saplings.

Embothrium coccineum J.R. et. G. Forster (Proteaceae) and *Eucryphia cordifolia* Cav. (Cunoniaceae) are two evergreen tree species able to recruit both sexual and vegetatively through root suckering (Lusk 2002, González et al. 2002). The two species coexist in the temperate rainforest of southern South America (Lusk 2002, González et al. 2002, Muñoz and González 2009, Escandón et al. 2013). At early ontogenetic stages of development, these two species show contrasting strategies of shade-tolerance, being *Embothrium* a light-demanding and *Eucryphia* a shade-tolerant (Lusk 2002, Lusk and Del Pozo 2002). The role of root suckering in species persistence has been based in its relative abundance (to seedlings) and performance at the regeneration stage of a plant population (e.g. Beaudet et al. 2007), overlooking its role in species coexistence. Here, we evaluated the resource translocation between interconnected root suckers by means of isotopic signal in species with contrasting ecological strategies to explain the coexistence of these two species at an intermediate ecological succession. We hypothesize that, if promoting persistence is the main ecological role of suckering, then suckers would act as net sinks for carbon and nitrogen. If this is the case, they would show higher $\delta^{13}C$ and $\delta^{15}N$ than saplings, as a consequence of ^{13}C and ^{15}N enriched during translocation across the interconnected parent and ramets. As a secondary hypothesis, we propose that root suckers of the light-demanding species would rely on the carbon transfer from interconnected suckers to colonize low-light environments. To assess this, we performed a pulse-labeling experiment in the field to assess the transfer of carbon from high-light to mid-light suckers. For this experiment, high-light and low-light suckers are those suckers living in sites more illuminated relative to low-light suckers, and *vice-versa*.

Methods

Study site

This study was carried out in the Puyehue National Park (40º39' S, 72º11' W, 350 m a.s.l.), located in the western foothill of the Andes in south-central Chile. Although the most area of the park is covered by old-growth forest, this study was conducted in a ca. 50 years-old second-growth forest fragments dominated by *Nothofagus dombeyi*, *Eucryphia cordifolia*, *Caldcluvia paniculata* and *Embothrium coccineum.* In this forest it is possible to find adult trees of *E. coccineum* and *E. cordifolia* together, being representative of the overlapping of the altitudinal and longitudinal species distribution (Smith-Ramírez et al. 2007), thus permitting to sample co-occurring saplings and root suckers for both species. The study plots were established at the sector of Anticura, in the surroundings of the weather climatic station managed by the Forestry National Corporation (CONAF). Following the records from 1980 to 2016, the study area experiments a temperate maritime climate, with 2725 mm of rainfall in average. The rainfall sharply decreases during summer but is greater than 100 mm on average. The warmer month is January, with an average temperature of 14.4º C and the colder is July, with 5.4º C on average, being the mean annual temperature of 9.8º C.

Recruits sampling

Two permanent plots (25 × 60 m each) were established in two different fragments of secondary forest embedded in a matrix of old-growth forest. The plots were separated each other by 400 m. Each plot included >5 adult individuals of the studied species and, altogether, the two plots covered the light availability gradient described for this part of the national park (Gianoli et al. 2010). In a previous study (Escandón et al., under review), we established a subplot of 5 × 50 m in the center of each plot, where all of the recruits of the studied species between 2 and 150 cm in height were identified as either root suckers (from vegetative reproduction) or saplings (from seed origin). To determine whether a recruit was a sapling or a sucker, the root collar was carefully revealed, and the superficial soil was temporarily removed. Recruits were identified as root sucker when their root collars were still connected to their parental roots, and as saplings when they did not show any subsidiary root connections or root scars indicating past connections (Escandón et al. 2018). The light availability for each recruit was determined according to the global site factor (GSF). Briefly, hemispherical photographs were recorded above each recruit's apex, under homogeneous overcast conditions, using a Coolpix 4500 digital camera equipped with a FC-E8 fisheye lens (Nikon, Tokyo, Japan). The GSF was obtained after analyzing individual photographs using canopy analysis software HemiView version 2.1 (1999, Delta-T Devices Ltd, UK). For more details, please see Escandón et al. (under review).

Sampling for the study of natural stable isotope abundance

From the total pool of recruits (i.e. regardless of the plot), 40 individuals per each combination of species and recruit-type were randomly selected to determine the C and N natural stable isotope abundance. Recruit selection was conducted using the "randbetween" function of Microsoft Office Excel software (Microsoft Office Enterprise 2007; Microsoft Corporation, Redmond, WA, USA). After discarding damaged recruits and residual outliers (through *plotresid* function of RVAideMemoire package in RStudio), the sample sizes were as follows: 34 suckers and 28 saplings of *Embothrium*, and 24 suckers and 38 saplings of *Eucryphia*. Selected recruits were carefully excavated, the entire shoot sampled and kept under wet conditions until be rapidly processed in the field laboratory. Leaves and roots were separated from the stems, stored in paper bags and dried in a forced-air oven for 72 h at 60 ºC and weighted. Existence of a root system in root suckers was imperceptible after harvesting. Proportion of root biomass calculated as root biomass divided by total plant biomass was 0.027 and 0.024 for suckers and 0.25 and 0.32 for saplings of *Embothrium* and *Eucryphia*, respectively (see Escandón et al. under review for more details of recruit and species functional traits). The GSF of the sampled plants did not differ between recruit-types ($P = 0.27$), between species ($P = 0.17$), or for the interaction of these factors ($P = 0.72$) (see Table S2). Therefore, differences in leaf chemical traits among recruit-types and/or species could not be attributed to differences in the light environment.

$^{13}CO_2$ isotope labeling

A pulse-labeling experiment was performed on December 2017 in suckers of *Embothrium*, the light-demanding species of our study system. It was replicated in four groups of interconnected root suckers, each consisting of a sucker to be isotopically labeled (hereafter, the donor) and a close sucker (considered as the potential receiver). In each group, the potential receiver was the closest, smallest, and shadiest sucker relative to the donor (Table S2). This design was intended to strengthen the sink of carbon in receivers, and thus increase the resolution of the ^{13}C signal. The donors were covered with an air-tight plexiglass chamber of 45x10x10 cm^3 equipped with a high fluidity box fan (VN-2350, DC 12V, 130mA; Techman Electronics USA), with three independent silicon tubes including three ways stopcocks added for inner gas lectures (Fig. 1S, Supplementary Material for graphical details). The forest floor and the root collar were covered with a four-layer of plastic film to isolate them from aerial CO_2. The chambers were joined to the plastic film using neutral liquid silicon to avoid leaks. The chambers were previously tested to assess their gas-tight: no leaks were detected after four simulations (data not shown). Before the pulse labeling, the [CO_2] inside the chamber was monitored using an Infra-Red Gas Analyzer (CI-340 handheld photosynthesis system, CID-Bio-Sciences, Inc., 4845NW Camas Meadows Drive, Camas, WA, 98607, USA) with airflow set at 0.2 L min^{-1}. Before the pulse labeling, the [CO_2] inside the chamber was scrubbed down by passing the chamber air through soda-lime (see Fig. 1S for graphical details). The chamber emptying started after a diminishing of 30 µmol of CO_2relative to the initial measurements of [CO_2] inside the chamber or after a maximum of 8 minutes. The pulse labeling started when the [CO_2] inside the chamber reached 250 ppm. Chamber was filled up with CO_2enriched with the heavy stable carbon isotope (99.9% ^{13}C; Cambridge Isotope Laboratories, Andover, Massachusetts, USA) until the [CO_2] inside the chamber reached 700 ppm. The [CO_2] was monitored during the subsequent three minutes (Fig. 2S, Supplementary Material). The [CO_2] thresholds used for emptying and filling up the chamber were those avoiding alterations of the RuBisCO activity and activation, based on the global response patterns of RuBisCO to [CO_2] gradients (Galmés et al. 2013). To stimulate gas exchange, the donor was illuminated from the top-outside of the chamber with a red/blue light source adjusted to 1500 µmol of photons $m^{-2}s^{-1}$, delivering ca. 320-800 µmol of photons $m^{-2}s^{-1}$ at the upper third inside the chamber. This amount of light does not represent any potential risk of photoinhibition, because of the photosynthetic performance of the species (e.g. Lusk 2002). During the pulse labeling, neighbor plants were carefully covered with thick plastic bags to prevent accidental aerial enrichment. The temperature and the relative humidity inside the chamber were measured during the pulse labeling with iButton Hygrochron temperature/humidity logger (DS1923; Maxim Integrated Products, Inc.) with 0.0625° C and 0.04% resolution; the averaged (±SD) values recorded were 12.5±1.3° C and 90.9±4.6%, respectively. The chamber was removed 3h after the beginning of the pulse labeling. Donor and receiver shoots were harvested after seven days, according to the carbon transport velocity from leaves to belowground organs and soil reported for 1.5-year-old beech tress (Ruehr et al. 2009). Harvested plants were quickly carried out to the field laboratory, where fully mature leaves of the current year were placed in small paper bags and heated for 7.5 to 10 minutes in a Thomas TH-34DGM microwave at the highest power to stop any enzymatic activity; a beaker with water was placed inside the microwave to maintain air humidity. This procedure preserved plant material for the determination of organic compounds, without quantifiable effects on N levels (Schuman and Rauzi 1981, Popp et al. 1996).

Chemical analysis

Foliar samples were ground in an agate mortar until to pass a 1 mm mesh and afterwards ground in a ball-mill to pass to 0.425 mm mesh (Spex Sample Prep 8000M Mixer/Mill, USA). To avoid contamination, labeled leaves were ground in a separate mortar. The carbon and nitrogen content and isotopic analysis was performed using 2µg of the fine powder encapsulated in a tin capsule, which were burned and the realized gas analyzed to determine C and N content and the $^{13}C/^{12}C$ and $^{15}N/^{14}N$ ratios with an Isotope Ratio Mass Spectrometer CHNS-IRMS autoanalyzer (20-22 IRMS, SERCON, UK) at the Soil, Water and Forest Research Lab (LISAB) at the University of Concepción. The element isotope composition ($\delta^{xx}E$) was calculated as:

$$\delta^{xx}E = \left(\frac{R_{sample}}{R_{standard}} - 1\right) \times 1000,$$

where, R is the ratio between the heaviest and lightest isotope of the element. Carbon and nitrogen isotopes ratios are expressed in parts per mil relative to wheat flour SC0464 (SERCON, UK) standard previously calibrated using a series of primary standards (IAEA-600, IAEA-CH-3, IAEA V9, IAEA-C3, USGS-40, and USGS-41).

The $\delta^{13}C$ of donor and receiver suckers was converted into mg of ^{12}C equivalent excess following a modified version of the procedure and calculations simplified in Teste et al. (2009), which permits to determine whether interconnected suckers had an excess of ^{13}C above natural stable isotope abundance levels. The excess ^{12}C equivalent has been used to measure the enrichment level of a labeled sample, relative to the ^{13}C background level prior to administration of the tracer (Boutton 1991). Background samples consisted in current year leaves from suckers inhabiting under GSF of 0.124 and 0.087 on average, which is a GSF range similar to that of the donors (ANOVA test: $F_{1,5} = 0.005$; $P = 0.95$) and receivers (ANOVA test: $F_{1,5} = 0.009$; $P = 0.93$). As we did not explore the whole genet, this is all the interconnected suckers through the parent root system, we only used root suckers ^{13}C background for the pulse experiment for excess ^{12}C equivalent estimation.

Data analysis

The leaf chemical traits were compared between recruit-types and species by means of two-ways ANOVAs, using a Type-I sum of squares (SS). The evaluated traits were leaf carbon and nitrogen content, the C:N ratio, as well as the isotopic composition of ^{13}C ($\delta^{13}C$) and ^{15}N ($\delta^{15}N$).The significance level (α) was established by means of the step-up false discovery rate (FDR) procedure to control for the probability of Type-I error under repeated testing (Benjamini and Hochberg 1995). The *post-hoc* differences were evaluated estimating the marginal means (least-squares means) through the functions “emmeans” and “cld” of the R package *emmeans* (Lenth 2018) and *multcompView* (Graves et al. 2015), adjusting *P*-values with Tukey method at $\alpha = 0.05$.

To evaluate the diffusion of photo-assimilates between the *Embothrium* root suckers used in the $^{13}CO_2$ isotope labeling experiment, we compared the excess ^{12}C equivalent between the donor and receiver by means of linear mixed model, including the group of root suckers as random effect. The random effect was included in order to reduce the probability of Type-I (false positives) and Type-II (false negatives) error rates (Harrison et al. 2018). The models were subjected to Type-III sum of squares (SS) ANOVA

(“anova” function). The *post-hoc* differences were evaluated as mentioned above. Leaf C, N, $\delta^{13}C$ and $\delta^{15}N$ were compared between donor and receiver root suckers using the same statistical approach.

For all the analyses, the Shapiro-Wilk test and Non-constant Variance Score (NCV) test were used to verify respectively the normality and homoscedasticity of the residuals of the models. The NCV test was applied through the “ncvTest” function available at the *car* library of the R software (Fox and Weisberg 2018). The box-cox transformation was applied when necessary to meet the assumptions of normality and homoscedasticity. Even though the residuals of the model developed for ^{12}C equivalent were not normal after Box-Cox transformation (Table 2), the results have statistical support considering that (1) the P-values for the corresponding ANOVA had values far from the margin of significance, (2) the residuals were homoscedastic, and (3) the absence of normality only had significant effects when it implied heterocedasticity (Quinn and Keough 2002).

Results

Leaf carbon and nitrogen content and natural stable isotope abundance

We found significant effects of species for all the leaf chemical variables. Leaf C content was 2% higher in *Eucryphia* compared to *Embothrium* (Fig 1A). Leaf N content was 0.43% higher in *Embothrium* than in *Eucryphia*. The leaf C:N ratio was significantly higher in *Eucryphia* compared to

Embothrium. Leaf isotopic composition of C and N ($\delta^{13}C$ and $\delta^{15}N$, respectively) was higher in *Embothrium* than *Eucryphia*.

We found significant differences between recruit-type (i.e. saplings and suckers) only for isotopic composition of C and N. Both leaf $\delta^{13}C$ and $\delta^{15}N$ were significantly higher in suckers than in saplings (Table 1, Fig. 1D, 1E).

We found a significant recruit-type × species interaction for C:N, showing significantly higher C:N in saplings than in suckers, but only for *Embothrium* (Table 1, Fig. 1C).

No significant differences were found in leaf C between recruit-types, and there was no significant interaction between recruit-type and species (Table 1). Although suckers tended to have higher leaf N content than saplings (P significant; Table 1), the *posthoc* only detected differences in leaf N content between species (Fig. 1B).

Carbon transfer between interconnected root suckers of Embothrium

No significant differences were found between donors and receivers roots suckers of *Embothrium* for any of the leaf chemical traits evaluated in the $^{13}CO_2$ labeling experiment (leaf carbon and nitrogen content and isotopic composition; Table 2; Fig. 2A-D). Despite the similarities in ^{13}C between interconnected root suckers (Fig. 2C), the excess in^{12}C equivalent was 11% higher in donor than in receivers suckers of *Embothrium* (Table 2; Fig. 2E).

Discussion

Differences between suckers and saplings

As expected for natural abundance sampling, leaf chemical characteristics differed among suckers and saplings, being such differences very consistent for the two studied species, regardless their degree of shade-tolerance. Specifically, suckers showed less negative $\delta^{13}C$ in both *Embothrium* and *Eucryphia*. Leaf enrichment with the heaviest C isotope is usually related to higher water use efficiency, through either higher carboxylation rates or lower stomatal conductance (Farquhar et al. 1989). However, a previous study reported lower instantaneous water use efficiency of *Eucryphia* suckers compared to saplings, due to their lower carbon assimilation rate for a given stomatal conductance (Escandón et al. 2018). An alternative explanation to the low $\delta^{13}C$ values of suckers could be related to differences in the CO_2 diffusion through the mesophyll. Suckers of the two species have higher LMA compared to coexisting saplings (Escandón et al., under review). In this regard, it is known that the mesophyll conductance decreases with LMA, and thus diminishes the isotopic discrimination (Vitousek et al. 1990, Flexas et al. 2008, Chen et al. 2015). However, ^{13}C-discrimination by the RuBisCO is more determinant in $\delta^{13}C$ values than differences in the diffusion rates between the heavy and the light C isotope (Farquhar and Richards 1984, Ubierna and Farquhar 2014). Therefore, the isotopic signal recorded in the suckers likely is also reflecting other processes besides the discrimination against the ^{13}C occurring during the photosynthesis. Autotrophic and heterotrophic plant organs differ in the C isotope composition, with the heterotrophic organs being ^{13}C-enriched (Tcherkez et al. 2011). In this sense, although sucrose synthesis is based on the ^{13}C-depleted triose-phosphates exported form de the chloroplast, invertases are believed to cause progressive ^{13}C enrichment in the sucrose, the form of carbon that is translocated from source to sink organs/tissues (Rolland et al. 2002). Additionally, as the triose-phosphates are ^{13}C-depleted, aldolase reaction within the Calvin cycle favours ^{13}C during production of fructose 1,6-biphosphate, which leads to a relative enrichment of starch (Rossmann et al. 1991, Gleixner and Schmidt 1997).Thus, all together, enriching remote sink organs can occur and, therefore, the isotopic signal of the suckers' leaves could be reflecting carbon sharing between root suckers.

For the two species, suckers tended to have higher leaf N content and less negative $\delta^{15}N$ than saplings. If nitrate reductase and glutamine synthetase enzymes fractionate against ^{15}N (Evans 2001) in adult parent trees with a big root systems biomass developed, more ^{15}N can be available for translocation, thus enriching the root-lacking suckers of studied species. Therefore, our results suggest that sucker's nitrogen is transferred from parent tree. If so, competition for soil N of recruit-types within and between species may be neglected, which would favour plant growth and species persistence, thus facilitating the coexistence of these two species at intermediate stages of the forest succession.

Differences between interconnected suckers of the light-demanding species

Unfortunately, our pulse labeling experiment did not allow us to demonstrate C translocation between *Embothrium* root suckers. The excess ^{12}C equivalent was significantly lower in receiver suckers, suggesting that (i) the supplied $^{13}CO_2$ was uptaken by the donor suckers, and (ii) there is no significant carbon sharing between the sampled suckers of *Embothrium* (e.g. Saitoh et al. 2006, Roiloa et al. 2014). Likely, although receiver suckers were under lower light availability than the donors (Table S2), this relative difference was not enough to provoke the expected strenghten of the sinks. The suckers used in the isotope labeling experiment (both potential donors and receivers) were at the lower end of the light availability range of the *Embothrium* suckers at the study site (Escandon et al., under review). In fact, donors and receivers did not differ in terms of leaf C and N content, either in the isotope composition (Table 2), being this leaf chemical traits usually modulated by the light environment (e.g. Givnish 1988, Niinemets and Kull 1998). The acquisition of ^{13}C supplied to the potential donors was stimulated by the light they received during the experiment. But this easily acquired carbon was quickly incorporated by donor ramets, but not translocated to neighboring root suckers. In addition, the strength of the labeling could have been diluted among multiple interconnected individuals, acting as competing sinks with respect to the sampled receivers, since other interconnected individuals inhabiting under low light could have acted as stronger sinks. So, future pulse labeling experiments may consider sampling all the interconnected suckers along the light gradient (hopefully also the parent tree) in order to certainly detect physiological integration and carbon translocation among them.

Interspecific differences

Our results are also consistent with the degree of shade-tolerance of the two studied species, thereby reinforced its ecological strategies. *Eucryphia* (shade-tolerant) showed higher values of leaf C content compared to *Embothrium* (light-demanding), which likely reflects its higher LMA (Escandón et al., under review). Such differences reflect the different allocation patterns of shade-tolerant and intolerant species, the former with longer leaf lifespan and lower C assimilation rates (Niinemets and Kull 1998, Lusk 2002). Consistently, *Eucryphia* recruits showed lower leaf N content (and thus, higher C:N ratio) than *Embothrium*, reflecting the lower relative inversion in the photosynthetic machinery of shade-tolerant species (Field and Mooney 1986, Niinemets et al. 1998, Lusk 2002). Besides, as colonizer, light-demanding species, *Embothrium* showed higher $\delta^{13}C$, in agreement with its high carbon assimilation rate, water stress resistance, and low transpiration rates (Huber et al. 1986, Lusk 2002). At the more open sites, vapor pressure deficit also increases with canopy openness, and thus plants might to face-up to lower water availability (Lusk and Laughlin 2017).

Differences in leaf N between *Embothrium* and *Eucryphia* would not only be related to shade-tolerance, but also to the sources of and the strategies for nitrogen uptake among the studied species. *Embothrium* forms cluster roots and does not interact with mycorrhyzic fungi, whereas *Eucryphia* does not form cluster roots, and interacts with arbuscular mycorrhiza (Castillo et al. 2006). Even though formation, functioning, and physiological and biochemical processes of cluster roots are mainly induced by soil phosphorus deficiency in volcanic-ash soils (Borie and Rubio 2003; Delgado et al. 2013; Lambers et al. 2012a), there is evidence that the Proteaceae *Hackea actities* can access to soil N from complex nitrogenous compounds mediated by the cluster roots activity (Schmidt et al. 2003). In contrast, plants that interact symbiotically with arbuscular mycorrhiza typically present lower $\delta^{15}N$ values when compared to the original soil nitrogen source (as observed in *Eucryphia*), which is influenced by the depleted amino acids transferred from the fungi to the plant and as a consequence of the fungus N enrichment (Evans 2001).

Conclusions

The carbon and nitrogen supply strategy has been subtly evidenced for understory young suckers by using natural stable isotope abundance compared to saplings, regardless the level of shade tolerance of the studied species. This confirms that formation of root suckers is the more important way for regeneration and persistence (Escandón et al. under review) rather than resource acquisition at an intermediate ecological succession. Thus, resource translocation between parent and suckers facilitate species coexistence through the successional dynamic of this forest. However, given the wide range of light proportion where suckers do occur, future works evaluating the parent subsidy of clonal growth in plant communities deserve to take into account in the design i) to include the parent tree, soil, and even the entire genet; and ii) to consider the succession state of the species, in special when the parent tree could become in a large sink as consequence of its depressed vigor. In this regard, to increase the pool of possible sources and sinks and to integrate more environmental factors becomes necessary to reach a better understanding of subsidy within interconnected root suckers.

Author's contributions

A.B.E., F.A.A. and J.P.F-D. planned and designed the natural stable isotope abundance sampling and field pulse labeling. A.B.E. conducted the field work and data analyses. A.B.E. and S.P. wrote the manuscript with contributions from A.S., F.A.A. and J.P.F-D.

Declarations of conflicts interest

None.

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Supplementary information

Supplementary material related to this article can be found in the online version.

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Table 3.1. Summary of ANOVAs comparing leaf chemical traits between recruit types (RT), species (SP) and interaction term (RT × SP) used for natural abundance sampling. The significance level was established by means of the step-up false discovery rate (α FDR) procedure (significant differences in bold). The results of the tests conducted to evaluate the ANOVA assumptions are also included (Shapiro-Wilk and NCV tests). *Box-Cox transformed variable. $\delta^{13}C$ and $\delta^{15}N$ are isotopic composition of carbon and nitrogen, respectively.

Variable	Source	Df	SS	MS	*F*-value	P	α FDR
Leaf C content (%)	Recruit (RT)	1	0.30	0.30	0.17	0.68	0.043
	Species (SP)	1	132.98	132.98	72.53	**<0.0001**	0.007
	RT × SP	1	0.71	0.71	0.39	0.53	0.04
	Residuals	122	223.69	1.83			
		Shapiro: W = 0.99, P = 0.26; NVC: χ^2 = 1.61, P =0.2					
Leaf N content (%)*	Recruit (RT)	1	2.52	2.52	20.98	**<0.0001**	0.023
	Species (SP)	1	7.47	7.47	62.28	**<0.0001**	0.013
	RT × SP	1	0.47	0.47	3.89	0.052	0.037
	Residuals	88	10.56	0.12			
		Shapiro: W = 0.99, P = 0.46; NVC: χ^2 = 0.51, P = 0.47					
Leaf C:N ratio*	Recruit (RT)	1	0.006	0.006	19.69	**<0.0001**	0.027
	Species (SP)	1	0.021	0.021	69.78	**<0.0001**	0.01
	RT × SP	1	0.002	0.002	5.25	**0.025**	0.033
	Residuals	83	0.025	0.000			
		Shapiro: W = 0.99, P = 0.52; NVC: χ^2 = 1.1, P = 0.3					

$\delta^{13}C$ (‰)	Recruit (RT)	1	14.66	14.66	12.57	**0.0006**	0.03
	Species (SP)	1	58.09	58.09	49.82	**<0.0001**	0.017
	RT × SP	1	0.12	0.12	0.10	0.75	0.047
	Residuals	120	139.90	1.17			
		Shapiro: W = 0.98, P = 0.022; NVC: χ^2 = 0.08, P = 0.77					
$\delta^{15}N$ (‰)	Recruit (RT)	1	201.97	201.97	29.54	**<0.0001**	0.02
	Species (SP)	1	625.84	625.84	91.52	**<0.0001**	0.003
	RT × SP	1	0.53	0.53	0.08	0.78	0.05
	Residuals	88	601.76	6.84			
		Shapiro: W = 0.98, P = 0.37; NVC: χ^2 = 3.1, P = 0.078					

Table 3.2. Results of the linear mixed models comparing leaf chemical traits for the *Embothrium* root suckers used in the $^{13}CO_2$ labeling experiment. Mean values (±SD) for each variable are also shown. Significant differences are in bold. Residual tests of normality (Shapiro-Wilk) and homoscedasticity (NCV test) are showed for each variable.* Box-Cox transformed variable. $\delta^{13}C$ and $\delta^{15}N$ are isotopic composition of carbon and nitrogen, respectively.

Variable	SS	MS	Num DF	Den DF	*F*-value	P	Root suckers	
							Receiver	Donor
Leaf C content (%)	2.71	2.71	1	3	1.66	0.29	45.87 ± 0.55	44.71 ± 2.18
	Shapiro: W = 0.86, P = 0.13; NCV test: χ^2 = 3.11, df = 1, P = 0.078							
$\delta^{13}C$	5.8	5.8	1	6	1.14	0.33	-33.12 ± 1.67	-31.42 ± 2.72
	Shapiro: W = 0.92, P = 0.43; NCV test: χ^2 = 0.81, df = 1, P = 0.37							
Leaf N content (%)	0.06	0.06	1	3	0.78	0.44	2.16 ± 0.47	2.34 ± 0.64
	Shapiro: W = 0.88, P = 0.21; NCV test: χ^2 = 0.35, df = 1, P = 0.55							
$\delta^{15}N$	0.023	0.023	1	3	0.12	0.75	0.81 ± 3.07	0.7 ± 2.64
	Shapiro: W = 0.94, P = 0.63; NCV test: χ^2 = 0.09, df = 1, P = 0.76							
^{12}C eq*	1.28	1.28	1	3	20.76	**0.02**	0.49 ± 0.01	0.54 ± 0.033
	Shapiro: W = 0.82, P = 0.045; NCV test: χ^2 = 2.25, df = 1, P = 0.13							

Figure legends

Figure 3.1. Boxplot of leaf chemical composition and natural stable isotope abundance of root suckers (gray box) and saplings (white box) of *Embothrium* and *Eucryphia* in a second growth forest. Different lowercase and uppercase letters at the top of each panel indicate significant differences ($P < 0.05$) in the Tukey *posthoc* test conducted for recruit and species, respectively. The results of the analysis comparing each of these variables between recruit-types (RT), species (SP) and the interaction of these two factors (RT × SP) are shown in Table 1. $\delta^{13}C$ and $\delta^{15}N$ are isotopic composition of carbon and nitrogen, respectively.

Figure 3.2. Mean±SD of leaf chemical traits and ^{12}C equivalent for the *Embothrium* root suckers used in the $^{13}CO_2$ labeling experiment. See full results of ANOVA in Table 2.

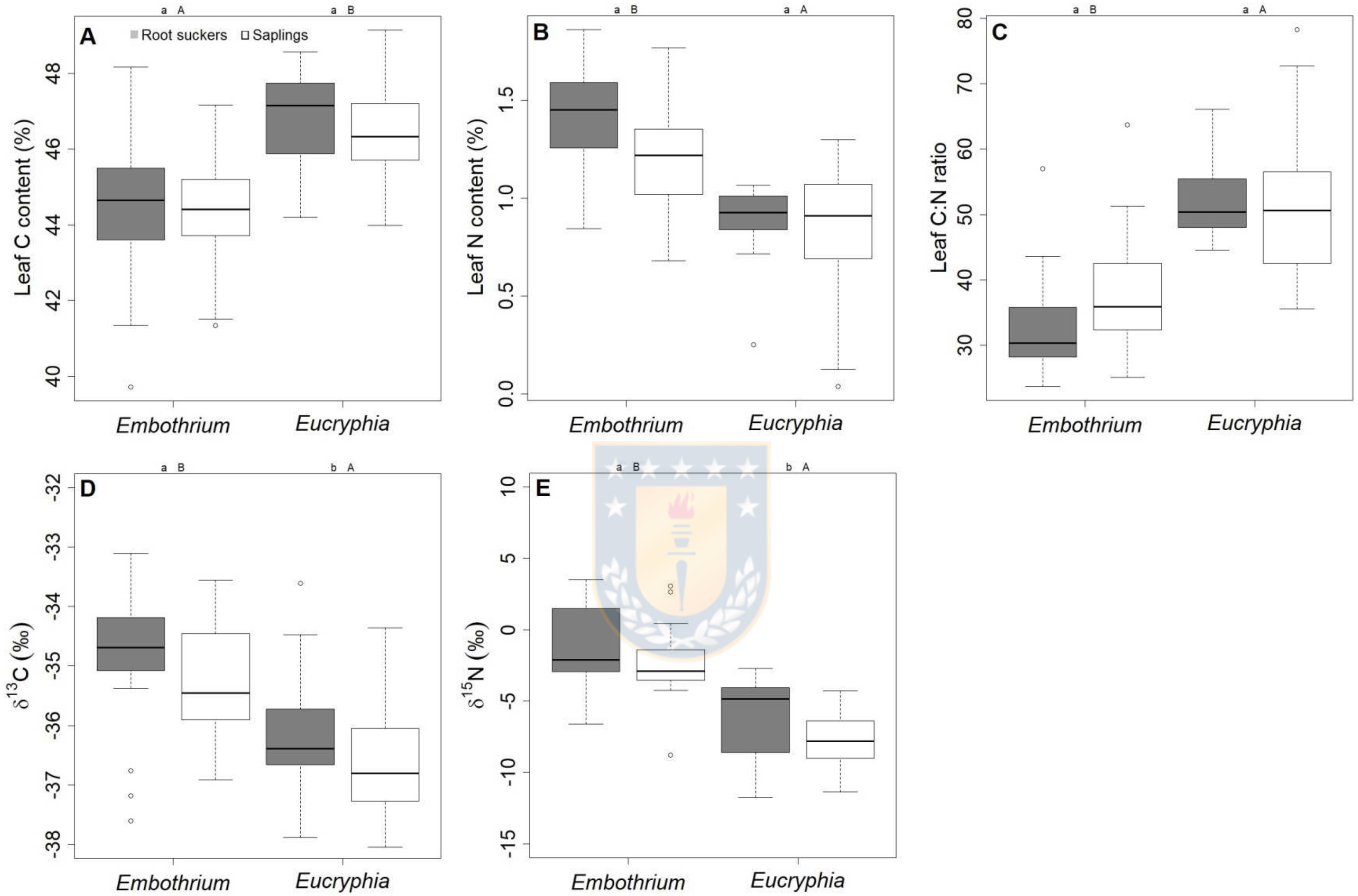

A
Root suckers
Saplings
Leaf C content (%)
B
Leaf N content (%)
C
Leaf C:N ratio
D
δ13C (‰)
E
δ15N (‰)
Embothrium
Eucryphia
a A
a B
b A

Figure 3.1.

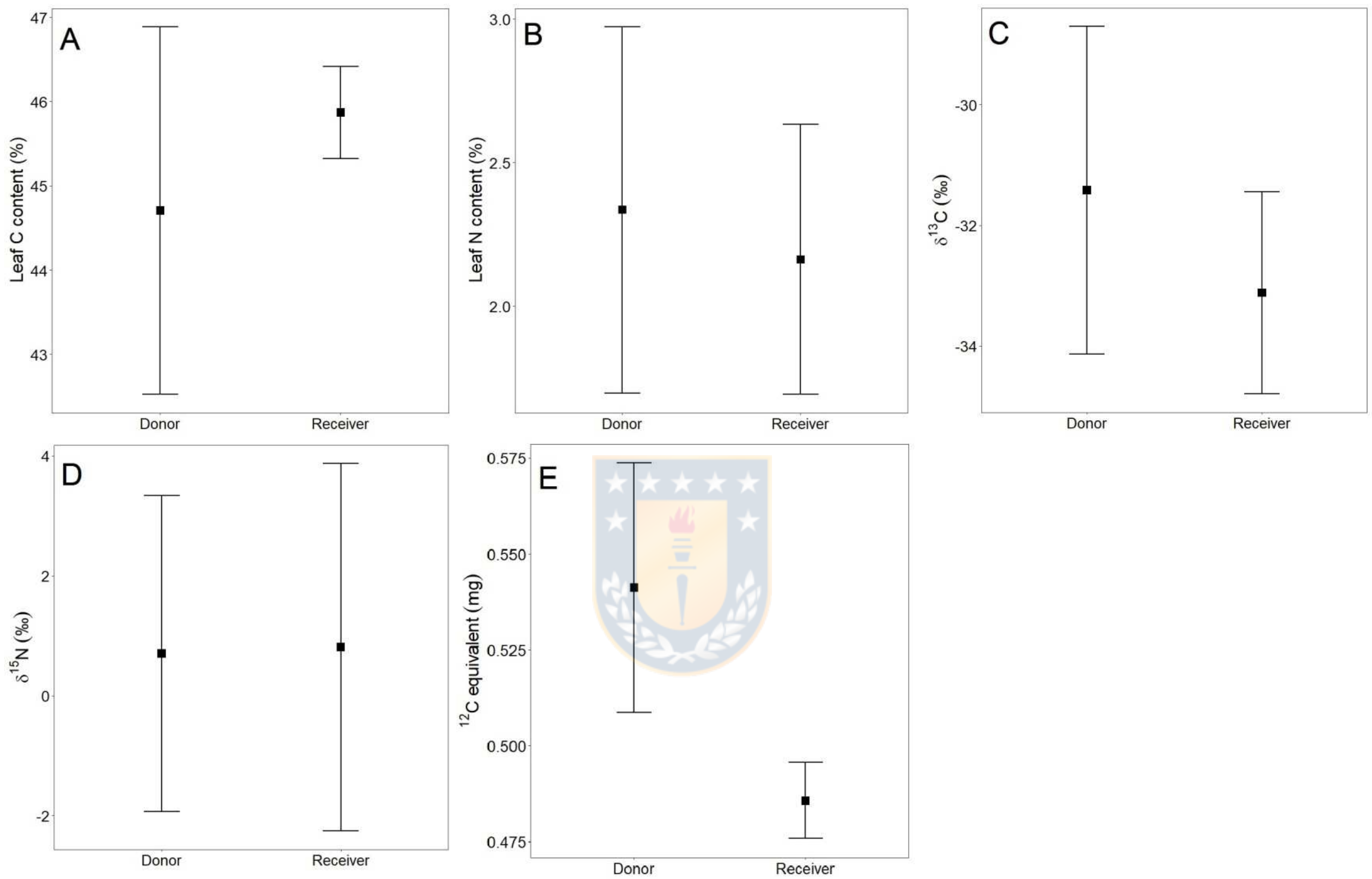

A
Leaf C content (%)
47
46
45
44
43
Donor
Receiver
B
Leaf N content (%)
3.0
2.5
2.0
Donor
Receiver
C
δ13C (‰)
-30
-32
-34
Donor
Receiver
D
δ15N (‰)
4
2
0
-2
Donor
Receiver
E
12C equivalent (mg)
0.575
0.550
0.525
0.500
0.475
Donor
Receiver

Figure 3.2.

Supplementary material

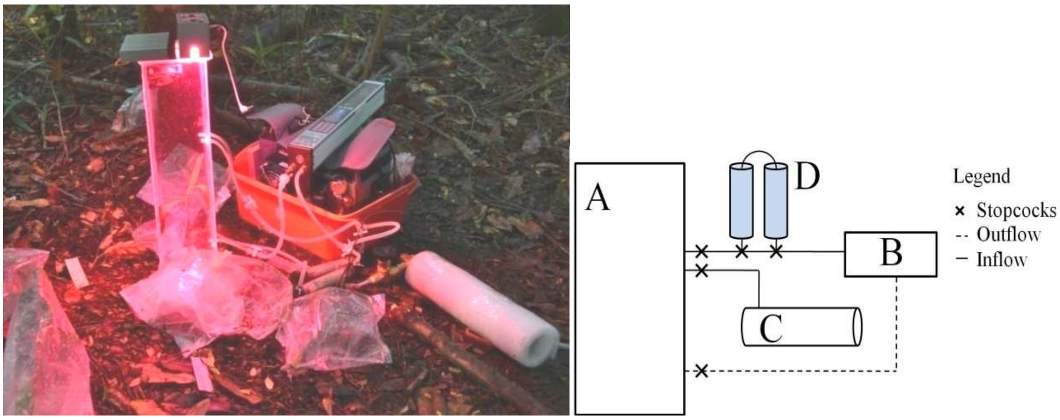


Figure S1. Picture (left) and diagram (right) of the pulse-labeling system. The treated plant was inside the chamber "A" and its neighboring plants were covered with thick plastic bags to avoid accidental aerial enrichment. Inside the chamber, a DS1923 hygrochron iButton (Maxim Integrated, San Jose, CA, USA) was located in one of the wall, in front of the gas exchange holes, and a fan (energized with an external battery) was placed at the top. On the chamber, a light source coupled to the IRGA "B" was placed. The insertions of the silicon tubes for gas exchange measurements were sealed with neutral silicon. Two silicon tubes were inserted at the mid of total height (upper) and the third one was located in the first quarter from the bottom (under). Each silicon tube had a three way stopcock to close/open the chamber once the steps for labeling started/ended. One of the upper tubes was used to diminish the CO_2 concentration inside the chamber, whereas the other was used to fill the chamber with $^{13}CO_2$ (i.e. this one was directly connected to the $^{13}CO_2$ cylinder "C"). To reduce the CO_2 concentration inside the chamber, the air coming from the chamber was forced to pass through two soda lime interconnected-columns "D". For "steady-state" lectures, the soda lime columns were by-passed using the three way stopcocks. To reach the CO_2 ppm values proposed, we controlled manually the three ways stopcocks correspondingly. The IRGA was used for logging lectures of CO_2 concentration inside the chamber (see Fig. S2).

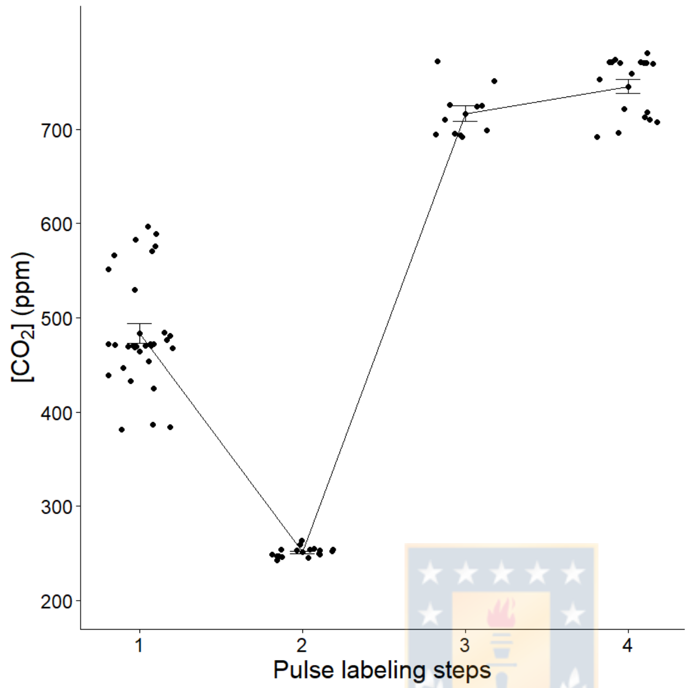


Figure S2. Mean±SE of CO_2 concentration inside the chamber during the four-step pulse labeling procedure. Steps as follow: 1: initial monitoring prior to the CO_2 extraction; 2: reduction of the CO_2 concentration inside the chamber; 3: $^{13}CO_2$filled up; 4: final monitoring of CO_2 concentration. For more details see Methods section.

Table S1. Summary of ANOVA comparing the global site factor (GSF) between recruit-types (i.e. saplings and suckers) and species (*Embothrium* and *Eucryphia*) (also the interaction of these two factors) used for natural abundance sampling. Significance level was established by means of the step-up false discovery rate (α FDR) procedure. Residual tests of normality (Shapiro-Wilk) and homoscedasticity (NCV test) are showed.

Variable	df	SS	MS	*F*-value	P	α FDR
Recruit-type (RT)	1	0.0001	0.0001	1.62	0.21	0.017
Species (SP)	1	0.0001	0.0001	1.36	0.25	0.033
RT × SP	1	0.000007	0.000007	0.086	0.77	0.05
Shapiro: W = 0.99, P = 0.3; NCV test: χ^2 = 0.56, df = 1, P = 0.46						

Table S2. Results of the linear mixed model comparing light availability, size, and basal diameter for the *Embothrium* root suckers used in the $^{13}CO_2$ labeling experiment. Mean values (±SD) for each variable are also shown. Differences between receiver and donor suckers are marked with different lowercase letters (significant at $\alpha = 0.05$). Residual tests of normality (Shapiro-Wilk) and homoscedasticity (NCV test) are showed for each variable. *Box-Cox transformed.

Variable	SS	MS	Numdf	Den df	*F*-value	P	Root sucker	
							Receiver	Donor
GSF*	0.00019	0.00019	1	3	13.42	0.035	0.084[a] ± 0.05	0.125[b] ± 0.02
	Shapiro: W = 0.91, P = 0.37; NCV test: χ^2 = 0.84, df = 1, P = 0.36							
Length (cm)	824.18	824.18	1	6	32.93	0.0012	42.9[b] ± 3.7	22.6[a] ± 6
	Shapiro: W = 0.92, P = 0.44; NCV test: χ^2 = 0.82, df = 1, P = 0.37							
Basal diameter (mm)	0.086	0.086	1	3	0.06	0.82	3.78 ± 1.5	3.99 ± 1
	Shapiro: W = 0.92, P = 0.39; NCV test: χ^2 = 0.6, df = 1, P = 0.44							

General Discussion

Persistence niche in the light gradient and forest succession

The importance of persistence has been largely neglected in favor of recruitment, despite sprouts can grow much faster than seedlings, filling their gaps after a tree is blown over (Bond and Midgley 2001). As clonal growth constitutes an advantage for regeneration when regeneration by seed is not successful, to study clonal growth is essential to understand tree species persistence along forest succession. This is the case of the evergreen short and long-lived species *Embothrium coccineum* and *Eucryphia cordifolia*, which showed characteristics explaining their differentiated tolerance to shade and a not-randomly distribution in relation to the available light in the secondary forest (Lusk and Del Pozo 2001, Escandón et al. 2013, this work). Although only small recruits of both species showed differences in frequency distribution and functional traits in response to the environment, these were consistent with the resulting survival probability and growth, surely contributing to shape species light niche (Chapter II). In spite that *Embothrium* suckers and *Eucryphia* saplings showed light niche selection, there was light niche differentiation between recruit-types in both species. In this regard, here *Eucryphia* showed the same trend found for recruit-type distribution than another second-growth forest (Escandón et al. 2013), meeting with our expectations of suckers distributed towards the more luminous microsites than saplings (Chapter II), where saplings displayed traits that enhance the crown carbon capture (Chapter I): lower leaf biomass, higher water use, light interception and capture efficiencyin saplings than suckers. Contrarily to our expectations and to the evidence found by Kowarik (1995), suckers of *Embothrium* were also distributed towards the more luminous sites than saplings (Chapter II). However, Homma et al. (2003) studiying the shade-intolerant species *Populus tremula* reported a similar pattern: its suckers were distributed mainly inside gaps and in the edge of the canopy, were it is expected a successful establishment of seedlings. Summarizing, our first and second hypotheses were totally and partially meted, respectively. Therefore, our results suggest, by one hand, that root suckers of evergreen trees tend to inhabit more luminous sites independently of the shade-tolerance level (but see Hosaka et al. 2008 for more information on shade-tolerant species), although very early suckering regeneration was observed under closed canopy for *Embothrium* (Lusk 2002). We aware of the scarcity of studies including several levels of shade-tolerance in the same forest type, so this later deserves to be tested including even more than two species. On the other hand, the significant amount of *Embothirum* suckers compared to saplings helps to understand species persistence, which converges to occupy the same light niche than saplings through the ontogenetic trajectory. Persistence of the mid shade-tolerant *Eucryphia* could be less pronounced than was for *Embothrium*, basically due to the lower amount of suckers compared to saplings explained by its early successional stage (Chapter II). Though *Eucryphia* suckers allow this species to be present in sites were saplings are less successful at the early development stage. However, the distribution of *Eucryphia* recruit-types converged when they have grown. Therefore, suckers permit to extend the regeneration to the luminous sites at early ontogenetic stages, but root suckers contribute only to shape the light niche when became taller throughout the species regeneration dynamics.

The effect of parental subsidy can be an explanatory variable to comprehend the regeneration light niche extensions as well as the persistence of the tree species (Chapters I and II). Our results of functional responses compared between recruit-types under similar light availability (Escandón et al. 2018) support that idea. Taking persistence in its temporal scale and understanding that species persistence can be mediated by clonal growth, then tree species coexistence can occur temporally under similar resources and environmental conditions, regarless resource requeriments of the coexisting species. In this sense, we highlight the scarce recruitment by sexual reproduction in the pioneer *Embothrium*, which notice that the closer successional fate of this species is to disappear from this second-growth forest (*sensu* Connel and Slatyer 1977). However, its high production of suckers contribute to delay its desappearance, especially because i) *Embothrium* suckers had a high probability of survival in its light regeneration niche through the early ontogenetic trajectory (Chapter II), and ii) sapling mortality rate is higher than any other species under poor-lit microsites (Lusk 2002). A contrary fate can be predicted for the mid shade-tolerant *Eucryphia*, since its recruitment was mainly by seed origin plants. Thus, the relative abundance of saplings and suckers of a given species can offer a tool to analize how the reproductive strategies are proportionally represented in each species, depending on species dominance with the forest successional stage and the light niche occupied within the light gradient of the studied forest fragment.

Functional traits and recruits'performance

Intraspecifically, the ecological and physiological functional responses of suckers differed from that of saplings suggesting an important effect from a potential subsidiary-parent tree, reflected by a faster ontogenetic trajectory in suckers (Chapters I and II). This is well supported by the results related to biomass allocation to photosynthetic tissue (higher in suckers), water transport (higher in suckers), crown architecture (less efficient in suckers), and those related to carbon gain (lower in suckers). Similarly, leaf carbon (C) and nitrogen (N) content and isotopic composition (natural abundance) differed mainly among recruit-types and species, but not between recruit-type of each species (Chapter III). In summary, these results showed that *Eucryphia* suckers had thick bigger long-lived leaves than saplings due to the higher C content, which explains the early differences in leaf surface per gram of leaf biomass (SLA). This pattern disappears when the whole plant biomass is considered (aLAR), due to the higher specific stem density of suckers (Chapter I and II), which has been reported previously for this species (Escandón et al. 2018). Overall, in early ontogenetic stages, although root suckers tended to show leaf functional traits that would help to increase leaf carbon gain, the leaf gas exchange resulted higher in saplings, whereas the isotopic composition (natural abundance of ^{13}C and

^{15}N) suggests parental subsidy for suckers, although pulse labeling under low suggests non-labor division among ramets. The higher carbon isotopic composition ($\delta^{13}C$) averaged of suckers over saplings suggests that the parents translocate resources to suckers. In this sence, as it was expectable found enriched remote organs (i.e. suckers more enriched than saplings), because sucrose is the form that carbon is translocated from source to sink organs/tissues and fractionation of invertases cause progressive sucrose enrichment (Rolland et al. 2002). However, we hipothesize that, if carbon translocation from parent tree to ramets is essential for sucker survival, then the stem of suckers could be a stronger sink tissue than leaves, because i) suckers had a higher stem density and (slightly) vessel density than saplings; ii) no evidence from leaves for carbon sharing between ramets were found; and iii) by descarding: where else is carbon being aboveground allocated? In general, these reasons also help to explain the higher carbon gain of saplings and understand why parent root diameter is more significant for aboveground biomass gain in suckers (Chapter I). In addition, results of nitrogen isotopic composition ($\delta^{15}N$) evidenced N translocation from parents to suckers, regardless of species strategy for nitrogen acquisition (by clustered roots in *Embothrium* and arbuscular mycorrhizal symbiotic interaction in *Eucryphia*).

The meaning and future work

Recently, the information and understanding of the ecological role of clonal growth in herbs had significantly increased, but much less is known about woody plants. The aim of this thesis was to compare the ecophysiological responses between interconnected-ramets (i.e. suckers connected to the parent tree) and saplings in order to elucidate its functional role into the regeneration and persistence niche in the forest understory. This comparative approach by using two contrasting shade tolerant trees of small (<50 cm length) and large size (≥50 cm length; i.e. effective recruitment) into a second growth evergreen rainforest also conseidered the shade-tolerance and the early ontogenetic trajectory, as well as resource translocation among ramets, on the evaluation of light niche shaping. So, beyond the importance of the presence of clonal growth in tree species of the evergreen temperate rainforest (see Table GA1), this thesis compiles several results deepening in the persistence-ecological role of clonal growth in trees. All the results are filling the lack of information at local (i.e. specifically for the studied species) and global scale (i.e. for the clonal growth in root suckering trees) in an undisturbed forest ecosystem. Most functional variability associated with the type of recruit (sucker and sapling) was only detected in small plants, and also similar for the two studied species, suggesting faster ontogenetic trajectory in suckers. This faster ontogenetic trajectory means that smaller suckers had similar functional responses than those taller ones, which, unexpectedly, did not improve survival probability in *Embothrium* suckers. Thus, these results suggest the importance of parental support for suckers maintenance, growth and survival, independently of the species and their shade tolerance. Here, we are contributing to understanding that the species coexistence can be mediated by the persistence of tree species with contrasting shade-tolerance during the early successional phases of the forest dynamics. We highlight the necessity of a better understanding of clonal growth and its ecological meaning in the context of forest succession. In this vein, by using stable isotopes, information of the origin of resources acquired by parent plants, the processes governing resource uptake and transformation, and the physiological performance of suckers along resource and condition gradients will permit us to elucidate and comprehend in deep the flux of resource within the genet.

General Conclusions

The main conclusions of this thesis work are:

1. Functional traits related gas exchange are more consistent in saplings than in suckers in order to increase CO_2 uptake at low water losses, in accordance with leaf physiological response to light and explained by the parent supply in suckers. In this sense, root suckers are apparently water, carbon and nitrogen subsidized in both light-demanding and shade-tolerant species. Further studies are needed to quantitatively and proportionally estimate the resource contributions from the parent tree to the root sucker for statement an empirical parental subsidy model.

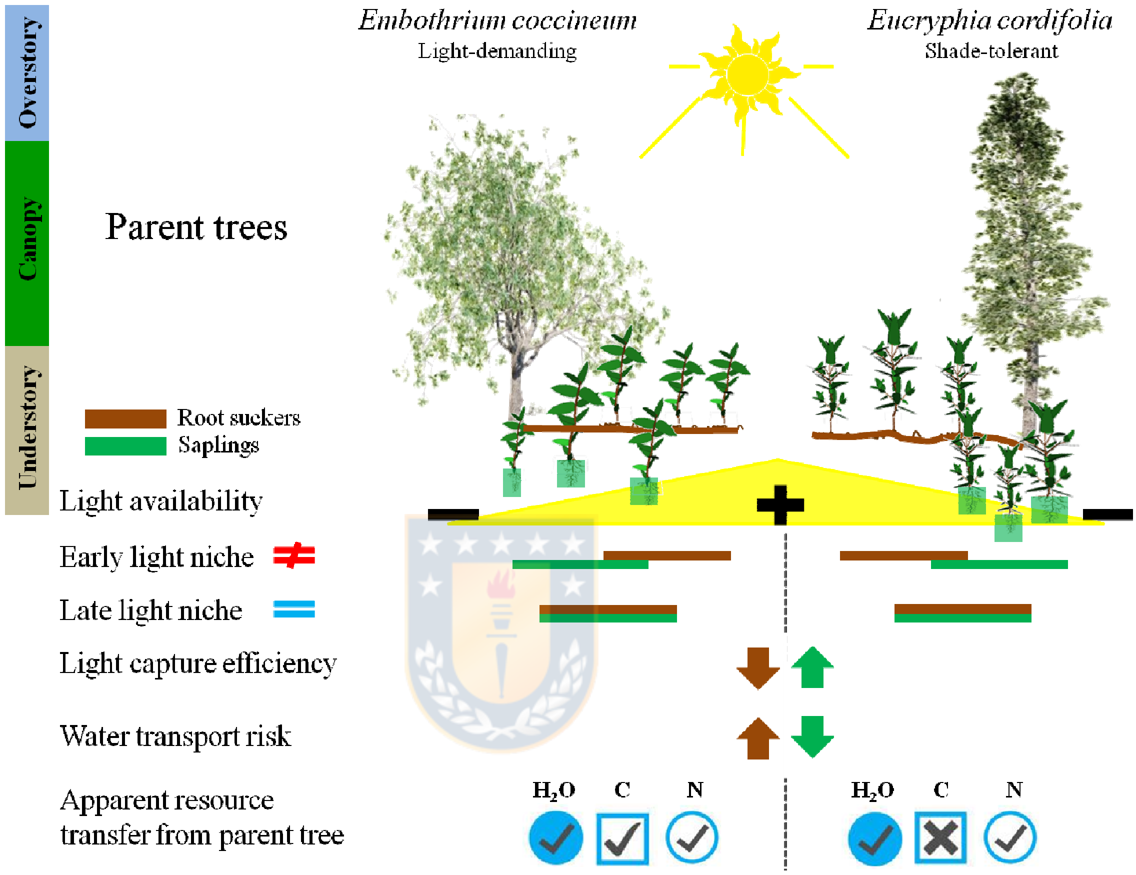
2. Root suckering extends the regeneration niche towards open and illuminated microenvironments during the early stages of ontogeny for both species, regardless of their shade-tolerance. However, the functional responses that underlie that pattern differ with species shade-tolerance. The increment of the light niche disappears when the mortality probability decay through the ontogeny, promoting the coexistence of this two species contrasting in its succession dynamics in a secondary temperate rainforest.

3. Although that carbon transfer between ramets in the mid-to-less illuminated sites in the forest understory may be neglected due to that parental subsidy favouring persistence and recruitment, root suckers are nitrogen subsidized in its regeneration light niche, regardless the

differing species nitrogen uptake strategy in the advanced forest succession. To what extent suckers are subsidized by high-illuminated ramets remains unclear, so more efforts are needed to disentangle its functional role within regeneration and reproductive (vegetatively and sexually) phases.

4. Root suckering promotes the regeneration and persistence of the species by means of the parental subsidy independently of species shade tolerance, rather than resource translocation among ramets. Ontogenetically early, suckers incline the regeneration light niche towards more illuminated sites, promoting the coexistence by persistence as its main role in temperate rainforest understory.

Thesis Model



Model description: Regardless the early differing distribution of recruit-types (root suckers –in brown- and saplings –in green), with suckers occupying more illuminated sites than saplings, and species shade tolerance, the ontogenetic shift (“Late light niche”) of recruit distribution was overlapped, which indicates that suckering promotes the coexistence of contrasting shade-tolerant species that combine sexual and vegetative reproduction. Traits related to light capture and water transport responded contrastingly when recruit-types were compared, albeit not necessarily intraspecific differences were found. Light-demanding *Embothrium* suckers posses chemical traits that suggest them as strength sinks in terms of water, carbon and nitrogen than its counterpart shade-tolerant *Eucryphia*.

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General Appendix

Table GA1. Tree species of the temperate rainforest that regenerate by both sexual and clonal growth (root suckering).

Family	Species	Clonal growth?	Reference*
Aextoxicaceae	*Aextoxicon punctatum*	Yes	Muñoz & González 2009
Myrtaceae	*Amomyrtus luma*	Yes	González et al. 2002, Muñoz & González 2009
Myrtaceae	*Amomyrtus meli*	No	González et al. 2002
Araucariaceae	*Araucaria araucana*	Yes	Decombeix et al. 2011
Elaeocarpaceae	*Aristotelia chilensis*	No	Muñoz & González 2009
Cunoniaceae	*Caldcluvia paniculata*	Yes	Observación de campo
Asteraceae	*Dasyphyllum diacanthoides*	No	Saldaña 2013
Winteraceae	*Drimys winteri*	No	Saldaña 2013
Proteaceae	*Embothrium coccineum*	Yes	Lusk 2002
Cunoniaceae	*Eucryphia cordifolia*	Yes	González et al. 2002, Muñoz & González 2009, Escandón et al. 2013
Cupressaceae	*Fitzroya cupressoides*	Yes	Donoso et al. 1993
Proteaceae	*Gevuina avellana*	Yes	González et al. 2002, Muñoz & González 2009
Monimiaceae	*Laurelia sempervirens*	No	Muñoz & González 2009
Monimiaceae	*Laureliopsis philippiana*	Yes	Veblen et al. 1980
Proteaceae	*Lomatia dentata*	Yes	González et al. 2002
Proteaceae	*Lomatia ferruginea*	Yes	Observación de campo
Myrtaceae	*Luma apiculata*	No	Saldaña 2013
Myrtaceae	*Myrceugenia planipes*	Yes	Observación de campo
Fagaceae	*Nothofagus dombeyi*	No	Saldaña 2013
Fagaceae	*Nothofagus nitida*	No	Saldaña 2013
Lauraceae	*Persea lingue*	No	Muñoz & González 2009
Podocarpaceae	*Podocarpus nubigena*	Yes	Lusk 1996
Podocarpaceae	*Podocarpus saligna*	No	Muñoz & González 2009
Podocarpaceae	*Saxegothaea conspicua*	Yes	Veblen et al. 1980, Lusk 1996
Cunoniaceae	*Weinmannia trichosperma*	Yes	A. Saldaña M. (Com. Pers.)

* References that reported root suckering of the species.

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