Carbon dynamics and woody growth in *Fitzroya cupressoides* forests of southern Chile and their environmental correlates, from seasonal to decadal timescales

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Thesis submitted to the University of Oxford in
fulfilment of the requirements for the degree of
Doctor of Philosophy.

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Abstract

Among the most compelling and least well-understood tree species growing in the temperate forests of southern South America is *Fitzroya cupressoides,* a high biomass species and the second longest-lived tree species in the world. This thesis quantified the main components of the carbon cycle in *Fitzroya* forests (i.e. net primary productivity (NPP) and soil respiration) and evaluated the environmental variables that are most related to them. The study was focused on medium-age and old-growth forests growing in the Coastal Range (Alerce Costero National Park, AC) and the Andean Cordillera (Alerce Andino National Park, AA) of southern Chile, respectively. The specific objectives of this thesis were to: 1) assess the forest structure, species composition and characterise the environmental conditions of these forests; 2) assess biomass, aboveground NPP, carbon allocation and mean wood residence time in these forests; 3) assess soil respiration and relate it to soil environmental conditions. Additionally, to use a mass balance approach to estimate fine root productivity; 4) estimate total NPP using biometric and indirect estimates of productivity; 5) evaluate the climatic factors mainly related to *Fitzroya* stem radial change on an intra-annual basis; and 6) evaluate changes in *Fitzroya*'s tree growth and carbon isotopes during recent decades, and determine which environmental factors are more related to them. The last two objectives focus on *Fitzroya* as the dominant species and the subject of this study. Two 0.6 ha plots were installed within each national park; NPP was estimated for a year and soil respiration and high resolution stem growth measurements were monitored over almost two years.

Aboveground biomass estimates for the Andean site are among the most massive reported in the world and carbon fluxes in *Fitzroya* forests are among the lowest reported for temperate wet forests worldwide. The longevity as well as the particularly rainy and nutrient poor soil conditions where these ecosystems grow may influence their exceptionally slow carbon dynamics.

Differences in carbon fluxes between sites seem most probably driven by different environmental conditions rather than by developmental stage. Moreover, carbon fluxes were more sensitive to interannual climate variability in AC than AA. Warmer and drier summer conditions, likely to become more common under future climate change, more significantly affected stem growth and soil respiration in the Coastal Range than in the Andes. Regarding long-term changes, tree growth has been decreasing in the coastal site in the last 40 years and increasing in the Andes since the 1900s. These trends have been accompanied by an increase in intrinsic water use efficiency which is likely caused by rises in CO_2 and changes in climate conditions in both sites.

Although *Fitzroya* grows in particularly wet and cool areas, projected drier and warmer conditions may have a negative effect on *Fitzroya* stem growth and carbon sequestration in both study sites. This effect would be more critical in the Coastal Range though, because of its more Mediterranean climate influence and more restrictive soil conditions in this area. Adequate resources are needed for the monitoring and conservation of these slow growth and massive forests especially in the Coastal Range, in order to avoid ongoing illegal cuttings and threatening forest fires.

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Dedicated to my husband Aldo Farías and to my beloved Alerce forests

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List of Acronyms, notations and abbreviations

AGB	Aboveground Biomass
NPP_{ACW}	Aboveground Coarse Wood Productivity
$Mort_{AG}$	Aboveground Mortality
NPP_{AG}	Aboveground Net Primary Productivity
AA	Alerce Andino National Park
AA1	Alerce Andino Plot 1
AA2	Alerce Andino Plot 2
AC	Alerce Costero National Park
AC1	Alerce Costero Plot 1
AC2	Alerce Costero Plot 2
c_a	Ambient or Atmospheric Carbon
F_{DOC}	Aqueous Carbon Leakage
R_a	Autotrophic Respiration
BAI	Basal Area Increment
$Mort_{BG}$	Belowground Mortality
NPP_{BG}	Belowground Net Primary Productivity
BEF	Biomass Expansion Factor
$NPP_{branch\ turnover}$	Branch Turnover Productivity
$NPP_{litterfall}$	Canopy Productivity
$\delta^{13}C$	Carbon Isotopic Ratio in plant material, if talking about tree rings similar to $\delta^{13}C_{tree}$
$\delta^{13}C_{atm}$	Carbon Isotopic Ratio of Atmospheric CO_2
$\delta^{13}C_{tree}$	Carbon Isotopic Ratio in tree rings
C/N	Carbon/ Nitrogen Ratio

R_{sample}	Carbon Ratio ($^{13}C/^{12}C$) of the sample
$R_{standard}$	Carbon Ratio ($^{13}C/^{12}C$) of the Standard
ΔC	Change in Carbon Stocks
$NPP_{coarse\ root}$	Coarse Root Productivity
CWD	Coarse Woody Debris
$R^2_{conditional}$	Coefficient of Determination associated to fixed and random factors in linear mixed models
$R^2_{marginal}$	Coefficient of Determination associated to fixed factors in linear mixed models
CSIC	Consejo Superior de Investigaciones Científicas (Spain)
CITES	Convention on International Trade in Endangered Species
CONAF	Corporación Nacional Forestal
$\delta^{13}C_c$	Corrected Carbon Isotope Ratio
DBH	Diameter at Breast Height
DGA	Dirección General de Aguas
DMC	Dirección Meteorológica de Chile
DOC	Dissolved Organic Carbon
DWS	Durbin Watson Statistic
EGM	Environmental Gas Monitor
EPS	Expressed Population Signal
$NPP_{fine\ root}$	Fine Root Productivity
F_{CWD}	Fraction of coarse woody debris entering the soil
FACE	Free-Air CO_2 Enrichment
GPP	Gross Primary Productivity
R_h	Heterotrophic respiration

IRGA	Infrared Gas Analyser
c_i	Intercellular Carbon
IPCC	Intergovernmental Panel on Climate Change
IUCN	International Union for Conservation of Nature
iWUE	Intrinsic Water Use Efficiency
$\Delta^{13}C$	Isotopic Discrimination against ^{13}C
T	Kendall Tau-b Correlation Coefficient
A	Net Carbon Assimilation Rate /Photosynthetic Rate
NEP	Net Ecosystem Productivity
NPP	Net Primary Productivity
NA	Not Available
PAR	Photosynthetically Active Radiation
PVC	Polyvinyl Chloride
PIN correction	Pre-industrial Correction
PCA	Principal Component Analysis
Q_{10}	Q_{10} factor by which soil respiration increases for an increase of 10° C in temperature
RAINFOR-GEM	Red Amazónica de Inventarios Forestales-Global Ecosystems Monitoring Network
REDD	Reducing Emissions from Deforestation and Forest Degradation
SOM	Soil Organic Matter
R_S	Soil Respiration
SWC	Soil Water Content
SAM	Southern Annular Mode
SD	Standard Deviation

SE	Standard Error
SR	Stem Radius
ΔR	Stem Radius Increment
g_c	Stomatal Conductance to CO_2
g_w	Stomatal Conductance to Water Vapour
TBCA	Total belowground Carbon Allocation
VPD	Vapour Pressure Deficit
V	Tree Volume
VPDB	*Vienna Pee Dee Belemnite*
VOC	Volatile Organic Compounds

Chapter 1: Introduction

1.1.Background

The temperate forests of southern South America are mainly distributed on the western margin of the continent between 35° and 55° S in Chile and adjacent portions of Argentina (Armesto et al. 1996). A very important ecological feature of these forests is their biogeographical isolation (Villagrán & Hinojosa 1997), which has led to unusually high endemism at species and higher taxonomic levels (Armesto et al. 1996; Kalyn Arroyo et al. 1996; Villagrán & Hinojosa 1997). This high endemism concentration in a relatively small territory and the threaths that it faces due to habitat loss, have given this region a global conservation priority (Olson & Dinerstein 1998; Myers et al. 2000; Armesto et al. 2009). Other important features of these ecosystems are that they can contain large amounts of biomass (Keith et al. 2009) and that they hold some of the most pristine temperate forests in the world (Mittermeier et al. 2003). Old-growth forests from southern Chile have been considered as a baseline for biogeochemical cycle studies and characterised as having an “unpolluted nitrogen cycle”, due to the low atmospheric nitrogen (N) inputs, compared with northern hemisphere temperate forests (Perakis & Hedin 2002; Armesto et al. 2009).

The temperate forests of southern South America are a mixture of endemic, tropical and Austral-Antarctic phytogeographic elements, where the ancestors of the current flora arrived to this area in successive Neotropical, warm Australasian and Austral-Antarctic waves during the early Tertiary. The structural features and distribution patterns that these forests present nowadays, are closely related and particularly shaped by the repeated glaciations occurring during the Pleistocene (Villagrán & Hinojosa 1997).

Within this temperate zone, there is an area characterized by abundant precipitation and cool temperatures (38°-55° S), which has been categorized as one of the main rainforest zones worldwide, together with the Pacific coast of North America (Alaback 1991). These temperate rainforests constitute the largest area of these ecosystems in the southern hemisphere (Armesto et al. 2009).

One of the most compelling species of these temperate rainforests, because of its beauty, scientific, cultural and historical relevance, is *Fitzroya cupressoides* ((Molina) Johnston), the only extant species of the genus *Fitzroya* (Wolodarsky-Franke & Lara 2005). *Fitzroya* or alerce is an endangered species, endemic to these rainforests and one of the longest-lived trees in the world (with a lifespan of one individual reported as more than 3600 years, Lara & Villalba 1993). *Fitzroya* is a giant conifer that can reach heights of 50 m with trunk diameters of 5 m (Veblen et al. 1976; Donoso et al. 2006), thus carbon sequestration and long-term carbon (C) storage are important features of these long-lived ecosystems.

The total area covered by *Fitzroya* forests in Chile reaches 258,371 ha (CONAF 2011). *Fitzroya* only occurs in a disjunct distribution along the Andes of Chile and adjacent Argentina and along the Coastal Range of Chile from 39° 50’ to 43° S; small forest remnants are also present in the Chilean Central Depression at ~41° S (Lara et al. 2002; Donoso et al. 2006, Figure 1.1). There are no fossil records that date the early existence of *Fitzroya* forests in South America. Subfossil stumps found in southern Chile were dated to approximately 50,000 ^{14}C years before present (Roig et al. 2001); however a related species (*Fitzroya acutifolia*) was found to grow during the Early Oligocene-Early Miocene period in mixed forests in Tasmania (Paull & Hill 2010). Genetic studies found that the current species populations originated from at least two glacial refugia located in Coastal Chile and the Andes of Argentina and that the populations that were more differentiated were the ones from the eastern slope of the Andes and the northern distribution in the Coastal Range (Allnutt et al. 1999; Premoli et al. 2000).

Due to the beauty and quality of its wood, *Fitzroya* has suffered a long history of exploitation since the European colonization in the sixteenth century (Donoso et al. 1993; Veblen et al. 1995; Lara et al. 1999). In 1976, this species was declared a Natural Monument (Decree 490) and its exploitation was prohibited in Chile. However, an exemption in this Decree permits the cutting and use of "dead *Fitzroya*", if the trees were killed before 1976 (Lara et al. 2003). This has stimulated illegal cutting and intentional burning to obtain "dead wood" (Wolodarsky-Franke & Lara 2005).This species was listed on Appendix I of the CITES Convention in 1973 and is currently listed as endangered in the IUCN Red list of threatened species (IUCN 2013).

The structural features of *Fitzroya* forests vary throughout their range, from old-growth forests with giant trees in the Andes, to young forests, commonly affected by fires, in the highlands of the Coastal Cordillera (Donoso et al. 1993). *Fitzroya* populations in both the Andes and Coastal Ranges are different in terms of disturbance regimes, soil conditions and, to a certain degree, climate (Veblen & Ashton 1982; Donoso et al. 1990; Lara 1991; Lara et al. 2002; Donoso et al. 2006). Given these differences, it can be expected that biomass, the main components of the carbon cycle, as well as the sensitivity of carbon processes to climate variables might be different too.

Despite the intrinsic significance of *Fitzroya* as one of the oldest and more carbon massive forests in the world (Keith et al. 2009), there is a lack of knowledge on essential ecological aspects related to the carbon cycle in this species. Estimates of biomass and net primary productivity (NPP), the net rate of organic compounds construction by the plants, are only available for *Fitzroya* populations from Chiloé Island, the southern distribution of these forests in the Coastal Range. Measurements of soil respiration, the major source of CO_2 to the atmosphere from terrestrial ecosystems, are completely lacking. Additionally, there is no information on what are the main climate and environmental variables that are related with productivity and respiration in these two Ranges. Therefore, it is not clear how much carbon these forests are accumulating per year, how much CO_2 they are releasing to the atmosphere, and how both estimates compare between *Fitzroya* forests and with other temperate forest ecosystems. This ecological information, as well as the environmental correlates of productivity and respiration, are key for improving the general understanding of the carbon cycle in this long-lived species and for making accurate scientific predictions on the fate of growth and carbon sequestration in *Fitzroya* forests.

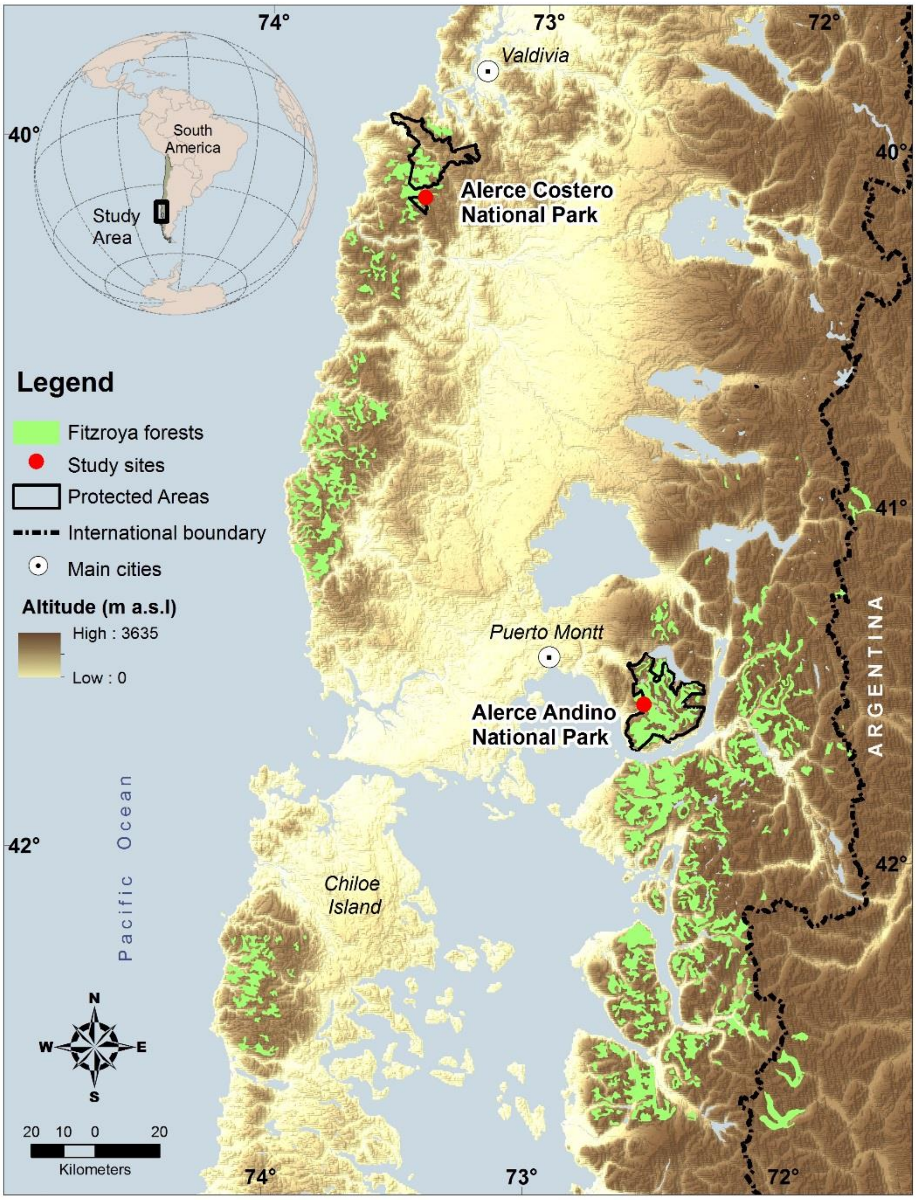

Figure 1.1. Distribution of *Fitzroya* forests in Chile and Argentina and location of the study sites. The small size populations in the Central Depression close to Puerto Montt are not shown.

It is very labour intensive and expensive to carry out long-term NPP studies, so an appropriate understanding of the relationships between climate and total productivity is challenging. Moreover, *Fitzroya* forests do not develop along a definite altitudinal gradient that easily permits an evaluation of changes in productivity with changing climate conditions. Due to these facts, the only feasible way of assessing these relationships at an intra-annual, annual and decadal basis is using woody productivity, the most visible aspect of forest productivity (Malhi et al. 2009). It has been suggested that stem growth is a sensitive indicator of total tree carbon balance, because it is a low priority for carbon allocation (Waring & Pitman 1985); therefore, the decrease in annual tree-growth associated to a specific cause could be pointing to a similar response in the whole forest productivity (Clark 2004). It is important to understand though, the relationship between the interannual variation in woody growth and variation in total NPP, to determine the extent to which changes in growth reflect changes in total NPP or just the allocation of NPP. Allocation trade-offs, rather than changes in total productivity, have been found to govern the response of a tropical forest to seasonal and interannual drought events (Doughty et al. 2014). Despite this, partitioning to wood, together with belowground carbon fluxes, are considered the most responsive to resources and environment (Ryan et al. 1996; Litton et al. 2007; Epron et al. 2012) and likely provide a good proxy to assess the sensitivity of this species to environmental change. A clear advantage of using stem growth is that it allows evaluation of long-term changes in woody production and carbon sequestration that can be directly linked with long-term changes in climate.

Fitzroya tree-ring width chronologies and their relationship with climate have been studied in both Ranges during the past decades (Villalba 1990; Villalba et al. 1990; Lara & Villalba 1993; Neira & Lara 2000; Barichivich 2005). These studies have made clear the strong and negative (positive) effect that especially previous and current summer temperature (precipitation) have on *Fitzroya* tree-radial growth. However, these dendrochronological relationships usually do not indicate a direct growth mechanism and tree-ring width chronologies have shown a different trend over time in some areas of the Andes (positive) and Coastal Range (no-trend or negative) during recent decades (Lara and Villalba, unpublished data, Figure 1.2). It is important to understand this phenomenon and the potential drivers behind these different growth patterns, since approximately the same trends in climate are affecting both study areas: a decreasing trend in precipitation and a slightly positive or no trend in temperature (Falvey & Garreaud 2009; Hartmann et al. 2013).

Due to climate change, warming and drying are expected in south-central Chile (Collins et al. 2013); summer temperatures in the region are projected to increase by up to 4° C and precipitation to decrease by up to 50% by 2100 (Fuenzalida et al. 2007). Precipitation has already decreased in south central Chile, in combination with an increase in the frequency of droughts, especially during the last 50 years (Christie et al. 2011; González-Reyes & Muñoz 2013; Hartmann et al. 2013). Therefore, evaluating the response of *Fitzroya*'s growth to recent environmental changes is important to understanding the effects that future climate change may have on these ecosystems' productivity and growth and their associated carbon sequestration. Furthermore, it has been suggested that negative growth trends and enhanced variability and/or sensitivity to climate are key features of declining and dying trees (Linares & Camarero 2012), so studying growth patterns can provide information on features that lead to potential forest decline.

A better understanding of how different species will be affected by climate change is needed to inform land management and policy decisions (Law 2014). This scientific information is critical for the formulation of adequate conservation policies particularly applied to regulations and adequate resources to better protect *Fitzroya* from illegal cuttings and intentional fires. These threats continue to occur mainly in the Coastal Range of southern Chile.

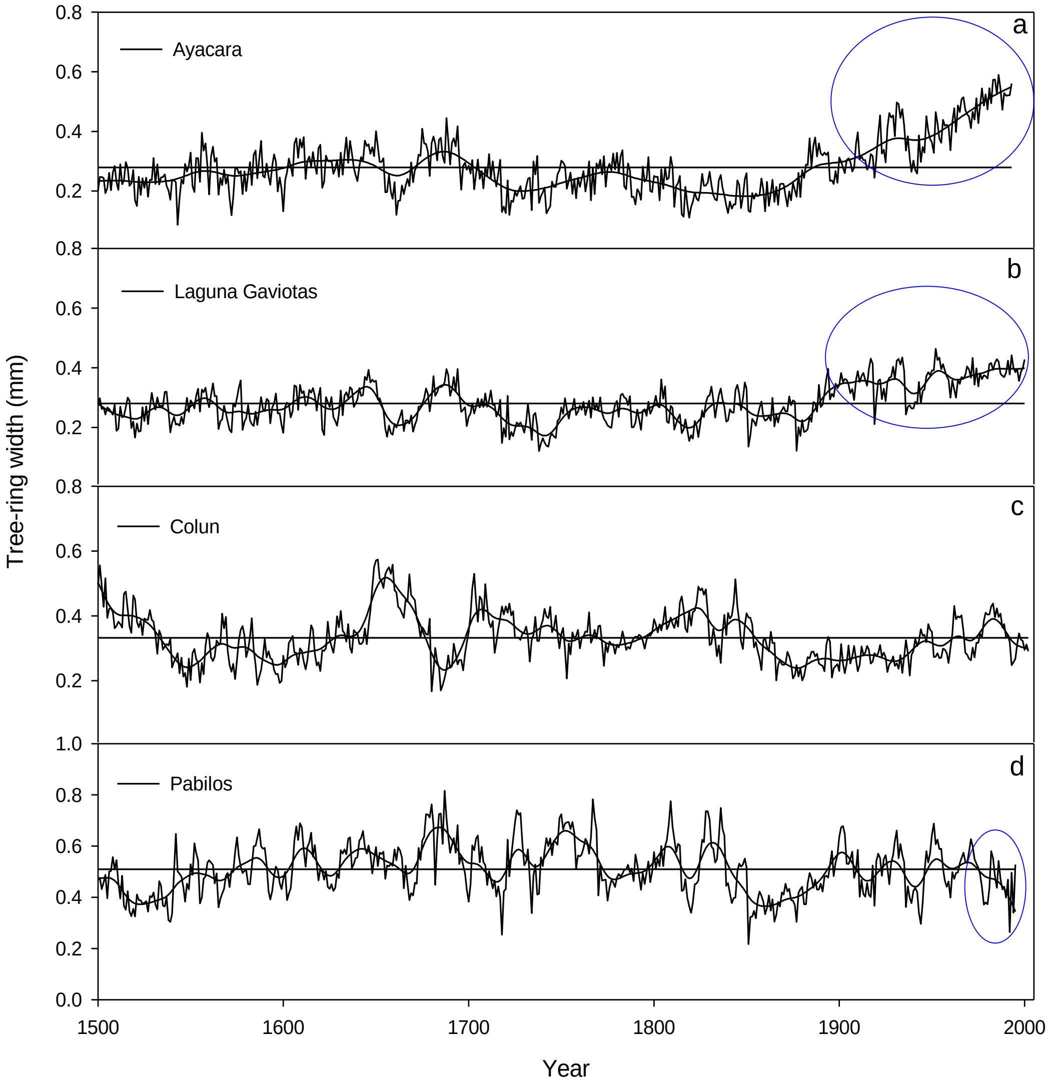

Figure 1.2. a) and b) Raw tree-ring width chronologies from two sites in the Andes Cordillera since 1500 showing a pronounced trend of increasing tree-ring growth in the last 100 years. c) and d) Tree-ring width chronologies from two sites in the Coastal Range showing no trend and a slight decreasing trend in the last decades (Lara and Villalba, unpublished data). Blue circles highlight the positive and negative trends. To emphasize the long-term variations, a cubic spline version designed to reduce 50% of the variance in a sine wave with a periodicity of 25 years is also shown (Cook & Peters 1981).

1.2. Aim and objectives

The aims of this research are to assess the main components of the carbon cycle, represented by net primary productivity and soil respiration, in *Fitzroya cupressoides* forests growing in the Coastal Range and the Andean Cordillera of southern Chile, and determine the environmental factors that are most related to their variability. The studied stands are representative of the widespread condition of *Fitzroya* forests in these two main areas of its distribution (i.e. medium-age forests in the Coastal Range and old-growth forests in the Andean Cordillera).

These two aims can be further divided in six specific objectives:

1. To assess the forest structure, species composition and characterise the environmental conditions associated with *Fitzroya* forests growing in the Coastal Range (Alerce Costero National Park) and the Andean Cordillera (Alerce Andino National Park) of southern Chile.
2. To assess biomass and aboveground net primary productivity (NPP_{AG}) and estimate carbon allocation and mean wood residence time in these two sites.
3. To assess soil respiration and its partitioning between autotrophic and heterotrophic components on a seasonal and annual basis in each of the study areas, and relate total respiration to soil environmental conditions. In addition, to utilise a mass balance approach that uses heterotrophic respiration and belowground carbon inputs to estimate fine root productivity in each site.
4. To provide the first estimate of total NPP for *Fitzroya* forests in southern Chile using biometric estimates of aboveground components and indirect estimates of belowground productivity.

5. To evaluate which climatic factors are mainly related to *Fitzroya* stem radial change on an intra-annual basis using high precision point dendrometers in trees from these two areas.
6. To evaluate changes in *Fitzroya*'s annual radial development in both Ranges during recent decades using tree ring width chronologies in combination with tree-ring stable carbon isotopes data, and determine which climatic and environmental factors are more related to these changes.

The first four objectives provide the framework to comprehend the ecological status and carbon dynamics of these forests and the main differences between stands growing in both Ranges. Objective 3 specifically looks at the understanding of the soil environmental drivers of soil respiration. Objectives 1 to 4 focus on the whole forest in each site, while objectives 5 and 6 only focus on *Fitzroya* as the dominant species in these forests and the subject of this thesis. Objective 5 allows a better definition of the environmental correlates of tree radial growth on short temporal scales and Objective 6 allows an assessment of any physiological or any radial growth (woody productivity) change in *Fitzroya* during recent decades and their probable environmental causes. Thus, Objectives 5 and 6 together provide an assessment of the environmental correlates of radial growth and carbon sequestration at a short and long-term basis in these contrasting *Fitzroya* ecosystems.

1.3. Outline of the thesis

The core of this thesis is composed of four independent and interconnected empirical chapters that have been submitted as articles to peer-reviewed scientific journals. Around these chapters are an introduction (Chapter 1), a literature review (Chapter 2), a site description and methodology section (Chapter 3) and a discussion section (Chapter 8) which provides the concluding remarks of this thesis. Details about the methods used in this thesis are provided in the respective empirical chapters.

The four empirical chapters are:

Chapter 4*: The oldest, slowest rainforests in the world? Massive biomass and slow carbon dynamics of Fitzroya cupressoides temperate forests in southern Chile*. This chapter describes aboveground biomass, productivity, carbon allocation and wood residence times in *Fitzroya* forests in both sites. Results are placed within the context of other studies in temperate forests in the US, New Zealand and South America.

Chapter 5: *Soil respiration patterns and mass balance estimation of fine root production in Fitzroya cupressoides forests of southern Chile.* This chapter examines belowground dynamics in these forests focusing on total, as well as on autotrophic and heterotrophic soil respiration. The soil environmental drivers of total respiration interannual variation are evaluated. This chapter also provides a mass balance assessment of fine root productivity that adds to the understanding of total productivity in these forests.

Chapters 6 and 7 examine the main environmental variables that are related to *Fitzroya* tree radial development on an intra-annual and interannual-decadal basis, respectively.

Chapter 6: *Environmental correlates of stem radius change in the endangered Fitzroya cupressoides forests of southern Chile*. This chapter examines the environmental variables mostly related to tree radial growth at a high temporal resolution using automatic point dendrometers.

Chapter 7: *Increased water use efficiency but contrasting tree-growth patterns in Fitzroya cupressoides forests of southern Chile.* This chapter focuses on the changes that tree radial development (expressed as tree radial growth and carbon isotopic composition) have experienced in recent decades and their potential environmental drivers.

Finally, Chapter 8 highlights and links the key findings of each chapter, discusses the broader ecological and policy implications of these findings and lays out future research needs that arise from this study.

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Chapter 2: Literature review

2.1. The terrestrial ecosystem carbon cycle in forests: net primary productivity and ecosystem respiration

Forests are major reserves for terrestrial carbon and the largest component of global terrestrial primary productivity (Malhi et al. 1999). They cover approximately 30% of the terrestrial surface and play a crucial role regulating environmental and climatic conditions (Bonan 2008).

The carbon cycle involves the movement and exchange of carbon (C) between organisms and the biosphere, with photosynthesis and respiration being the fundamental processes of this cycle. Photosynthesis is a metabolic process by which plants use solar energy to convert CO_2 and water into carbohydrates and oxygen. Part of the oxygen is used in respiration by the mitochondria, the rest being emitted into the atmosphere; while protons and electrons, released during the split of water molecules, combine with CO_2 to produce sugars (carbohydrates). These are essential for plant metabolism and are stored as energy and biomass.

The gross uptake of CO_2 that is employed for photosynthesis in an ecosystem corresponds to the Gross Primary Productivity (GPP). Due to the costs associated with growth and maintenance of different components (foliage, wood and roots), a large fraction of photosynthates are utilised for vegetation metabolism resulting in release of CO_2 through autotrophic respiration (R_a). The amount of photo-assimilated compounds that remains available for the construction of organic material is known as Net Primary Productivity (NPP) and connects to the previous components through the following relationship (Luyssaert et al. 2007).

$$GPP = NPP + R_a \tag{2.1}$$

NPP can be defined as the total new organic matter produced during a determined period of time (Clark et al. 2001) or as the rate of carbon accretion in plants after losses from plant respiration and other metabolic processes are considered (IPCC 2003). NPP is a fundamental ecological variable that indicates the condition of an ecosystem (e.g. if a forest is intrinsically productive or not compared with others) and the status of a variety of ecological processes (ORNL DAAC 2013). Most of NPP is allocated to the production of biomass in foliage, flowers and fruits ($NPP_{litterfall}$), aboveground wood composed by stem and branches (NPP_{stem} and NPP_{branch}) and fine and coarse roots ($NPP_{fine\ root}$ and $NPP_{coarse\ root}$, Luyssaert et al. 2007). In addition, NPP also includes other components that are more difficult to measure such as carbon lost through herbivory, carbon emitted as volatile organic compounds and methane, losses of root material to belowground consumers, root exudates and carbohydrate inputs to mycorrhizae (Clark et al. 2001; Luyssaert et al. 2007). Estimates of losses to volatile organic compounds and root secretions (root exudates and transfers to mycorrhizae) have been estimated in 0-5% and 10-30% of total NPP, respectively; while losses to herbivory and mortality can reach up to 40% in some ecosystems (Chapin III et al. 2011). NPP is frequently estimated by measuring the major components mentioned above, but due to the difficulty in measuring belowground components, aboveground NPP (NPP_{AG}) is more commonly estimated. NPP_{AG} has been found to represent between 37 and 78% of total NPP in different temperate forests, with most of the stands having more than 60% of total NPP allocated aboveground (Waring et al. 1998).

Carbon allocation is the proportion of productivity assigned to different tree components and is commonly assessed through woody, canopy and fine root productivity rates measured in the field (Doughty et al. 2014). Plants seek to maximize growth and distribute it among their parts in response to the balance between above- and belowground resource supply rates. Generally, plants allocate productivity to reduce limitation by any particular resource. Thus, they allocate more new biomass to roots when there is growth limitation by water or nutrients and they allocate more to shoots when there is light limitation (Chapin III et al. 2011).

Biomass corresponds to the mass of the different components of a plant that is accumulated over the years and is frequently expressed as the amount of carbon (ca. 50% of biomass) that can potentially be sequestered or emitted to the atmosphere (Brown et al. 1999). The residence time of carbon corresponds to the time between construction of biomass through NPP and loss of biomass through litterfall, herbivores consumption, root shedding or mortality. This residence time varies between different NPP components, from years to centuries in the case of carbon in wood and from months to years in the case of foliage or fine roots (Luyssaert et al. 2007).

Every year a portion of the standing biomass is lost to litter and/or soil layer carbon pools and these pools are decomposed by animals and microbes, through a process referred as heterotrophic respiration (R_h). The net gain of carbon to the ecosystem is the Net Ecosystem Production (NEP) and corresponds to the difference between NPP and R_h.

$$NEP = GPP - R_a - R_h \quad (NPP + R_a - R_a - R_h) \qquad (2.2)$$

The net carbon balance of a forest is a subtle balance between carbon gain processes (photosynthesis, tree growth, carbon accruing in soils) and carbon release processes (plant respiration, litter decomposition by microbes, soil carbon oxidation, forest degradation and disturbances, Malhi et al. 1999). In near-equilibrium systems (e.g. forests not affected by major disturbances in decades, in which changes in carbon stocks are subtle), total R_h

roughly equals NPP, because in these systems inputs (e.g. litterfall, root inputs) equal decomposition fluxes.

The sum of R_a and R_h corresponds to total ecosystem respiration (R_e) and soil respiration (R_s) to the sum of the belowground fractions of R_a and R_h. Soil respiration is the largest source of CO_2 from terrestrial ecosystems, normally contributing 30 to 80% of total respiration in forests (Davidson et al. 2006a).

Biomass estimations are especially useful to assess the magnitude of forests carbon storage and are relevant to negotiations involving reducing emissions from deforestation and forest degradation (REDD, Keith et al. 2009). Accurate NPP estimates are required to increase the understanding of environmental and ecological controls on carbon cycling, and therefore to better predict ecosystem responses to climate change (Bernier et al. 2007). Assessing the allocation of NPP to different tissues is crucial to understand an ecosystem's functioning and is an important parameter to represent in ecosystem models (Malhi et al. 2011). Finally, soil CO_2 efflux is a determining factor of atmospheric CO_2 levels and carbon sequestration, and a better understanding of this efflux is key to improve the prediction of net ecosystem exchange responses to climate change (Lavigne et al. 2003; Metcalfe et al. 2011).

2.2. Carbon cycling components in temperate forests

The temperate rainforests of southern South America are located along the western margin of the continent with a latitudinal range of ~17 degrees and embracing a large physiographic diversity (Kalyn Arroyo et al. 1996). This region resembles the western coast of North America which encompasses a similar latitudinal climatic gradient, and both areas constitute the largest areas of temperate rainforests in the world (Alaback 1991). Despite this resemblance, large differences remain: floristic biodiversity at the species and genus levels is higher in the south; there is a dominance of angiosperms rather than gymnosperms in southern forests and, with their isolation at the edge of a continent dominated by either arid lands or tropical ecosystems, the level of endemism is much higher in these forests compared to the ones in the northern hemisphere (Armesto et al. 1996).

Taking into account these differences, but acknowledging that *Fitzroya* is a conifer, that somewhat similar climatic conditions characterise both regions, that there is abundant literature on North American forests and that biomass storage is high in coniferous forests from both areas, this review will largely be focused on carbon studies in coniferous forests from western North America. Information on other forests from the southern hemisphere will be also provided when available.

This review will concentrate on components of the carbon cycle that are mainly related to my research: aboveground biomass, net primary productivity (mostly focused on aboveground components) and soil respiration in temperate forests.

2.2.1. Aboveground biomass and productivity in temperate forests

Aboveground biomass estimates in mature and old-growth temperate forests were reported to range globally between 43 and 1650 Mg C ha^{-1} (discarding low biomass dry sites, Keeling & Phillips 2007). The highest biomass values reported were for *Sequoia sempervirens* forests in California and for *Tsuga heterophylla* forests in the western Coast Range in Oregon (Westman & Whittaker 1975; Gholz 1982, Table 2.1). More recently even higher biomass values were reported for these *Sequoia* forests (Busing & Fujimori 2005, Table 2.1). A study at a tree level reported that the largest known trees of this species and of *Sequoiadendron giganteum* are storing 212 and 291 Mg C, respectively; amounts even higher than carbon stocks per hectare reported for some temperate ecosystems (Sillet et al. 2015). Particularly high stand values have also been reported for *Pseudotsuga menziessi* and *Pseudotsuga-Tsuga* forests from the Oregon Cascades, old-growth forests from the Oregon Coast and *Agathis australis* forests from New Zealand (Fujimori et al. 1976; Silvester & Orchard 1999; Smithwick et al. 2002; Keith et al. 2009, Table 2.1). A high amount of biomass was also reported for a *Eucalyptus regnans* stand in Australia, and the highest biomass carbon stock worldwide was attributed to cool temperate moist forests as a biome (Keith et al. 2009, Table 2.1). Higher biomass accumulation in forests from the Pacific Northwest compared with other temperate ecosystems, have been reported to be due to their sustained growth in height and diameter and their species longevity (Waring & Franklin 1979).

There have been only a few studies that have biometrically assessed biomass in temperate forests in southern South America (e.g. Ferrando et al. 2001; Battles et al. 2002; Laclau 2003; González et al. 2005; Joshi et al. 2006; Schlegel & Donoso 2008; Peri et al. 2010). Among these studies, the high aboveground biomass values reported for an evergreen *Nothofagus*-dominated forest in southern Chile (Schlegel & Donoso 2008) and for dense *Fitzroya* forests from Chiloé (Battles et al. 2002; Joshi et al. 2006, Table 2.1) stand out.

NPP_{AG} estimates in mature coniferous forests from the Pacific Northwest ranged between 1.1 and 7.5 Mg C ha^{-1} yr^{-1} in an East (dry) to West (wet) transect in Oregon, respectively (Gholz 1982). Values in particularly rainy sites in the western Coast Range and western Cascades ranged between 5.25 and 8.75 Mg C ha^{-1} yr^{-1} (Runyon et al. 1994; Waring et al. 1998, Table 2.1). NPP_{AG} estimates in *Sequoia sempervirens* forests in northern California, were estimated to range from 2.65 to 9.40 Mg C ha^{-1} yr^{-1} (Westman & Whittaker 1975), but later estimates by Busing & Fujimori (2005) were considered more accurate and around the average for old coniferous forests of the Pacific Northwest (3.5-5 Mg C ha^{-1} yr^{-1}, Table 2.1). Westman & Whittaker (1975) pointed out that the high biomass in

Sequoia forests would not be a consequence of high productivity, but of the great longevity and size of *Sequoia* trees. A few other temperate rainforests sites have been reported to have large aboveground biomass compared to their productivity, with the longevity of the dominant species contributing to their massive/high biomass (Pan et al. 2013).

There have been even fewer studies that have assessed productivity in temperate forests of the Southern Hemisphere and southern South America. Relatively low NPP_{AG} values were reported for *Agathis australis* and *Nothofagus solandri* forests in New Zealand (Silvester & Orchard 1999; Clinton et al. 2002) and for *Austrocedrus chilensis* forests in the Argentine Patagonia (Ferrando et al. 2001, Table 2.1). Vann et al. (2002) and Joshi et al. (2006) studied NPP_{AG} in *Fitzroya* and broadleaf dominated stands in the Coastal Range of the Chiloé Island (42.5° S, 74° W), where a significant variation was found between similar forests (Table 2.1). Differences were mainly due to much lower woody productivity in the conifer forest and much higher litterfall in the montane broadleaf forest in the study of Joshi et al. (2006) compared to Vann et al. (2002). Broadleaf Chiloé forests appeared to have higher productivity than *Fitzroya* forests, and overall productivity values were lower or within the range of values reported for coniferous forests of North America (Vann et al. 2002). It was particularly stated that forests in the Coastal Range of Chiloé had low N mineralization rates and a high rate of NPP_{AG} per unit of N mineralized, compared to northern temperate forests (Vann et al. 2002). Lower rates of net N mineralization and high nitrogen use efficiency have been reported in primary montane forests compared with northern hemisphere forests. This indicates a tighter internal N cycle, with tree species largely independent of external N inputs and able to retain nutrients with minor losses to downstream ecosystems (Vann et al. 2002; Joshi et al. 2006; Pérez et al. 2003a; Pérez et al. 2003b).

Total productivity estimates are scarcer due to the difficulty in assessing belowground components. Total NPP has been estimated to range between 6.81 and 11.22 Mg C $ha^{-1}yr^{-1}$ in coniferous forests from the Pacific western Coast Range and western Cascades (Runyon et al. 1994; Waring et al. 1998, Table 2.1). A mean estimate of 7.9 Mg C ha^{-1} $year^{-1}$ was reported for evergreen needleleaf forests from the US Pacific Northwest (Hessl et al. 2004), and a similar mean estimate was reported for evergreen temperate humid forests in a worldwide database (Luyssaert et al. 2007, Table 2.1).

Belowground production in the same sites where total NPP was reported above ranged between 1.56 and 2.5 Mg C ha^{-1} yr^{-1}, values much lower than the aboveground production (Runyon et al. 1994; Waring et al. 1998; Hessl et al. 2004). However, in drier *Pinus ponderosa* forests in Oregon, total root production surpassed NPP_{AG} (2.99 vs 1.73 Mg C ha^{-1} yr^{-1}, Runyon et al. 1994;Waring et al. 1998). Mean fine root productivity in temperate forests was estimated to be ~ 2.14 Mg C ha^{-1} yr^{-1}, a value higher than boreal, but lower than tropical forests productivity (Finér et al. 2011).

In terms of allocation, aboveground carbon in mature temperate forests worldwide appears to be more allocated to woody productivity than canopy production (Keeling & Phillips 2007; Litton et al. 2007). Evergreen temperate humid forests as a biome appear to allocate carbon primarily to woody productivity (mean of 2.80 Mg C ha^{-1} yr^{-1}), then to root productivity (2.35 Mg C ha^{-1} yr^{-1}) and lastly to foliage productivity (1.59 Mg C ha^{-1} yr^{-1}, Luyssaert et al. 2007). Allocation values in the temperate forests of the Pacific Northwest fluctuated between 46-61% for woody 16-26% for foliage and 23-28% for belowground productivity (Runyon et al. 1994).

It has been generally thought that forest stands decline growth rates and productivity with age. Two underlying explanatory mechanisms have been progressive increases in respiratory demands (respiration hypothesis) and declining carbon assimilation rates (assimilation hypothesis, Sala et al. 2011). However, these hypotheses have been questioned and the main underlying mechanisms are still unresolved and under debate (Sala et al. 2011). In recent years, it has even been suggested that old-growth forests continue to accumulate carbon with increasing age and therefore constitute carbon sinks (Luyssaert et al. 2008). A review of carbon datasets for old-growth temperate forests also concluded that these forests may accumulate woody biomass at an almost stable rate for centuries (Schulze et al. 2009). The mechanisms that allow long-term accumulation of carbon in forests are still unclear, but a sustained wood production by old and large trees can be a major contributor to their sink capacity (Sillett et al. 2010; Hinckley et al. 2011; Stephenson et al. 2014). The low mortality rates that characterize long-lived trees would also contribute to this sink capacity. It is uncertain how long these forests will remain as carbon sinks under current and projected climate warming; mortality being a key issue to pay attention to. Regional mortality events have been reported in different forests globally due to severe water deficits and tall trees of old-growth forests have been recently placed at a greater risk of loss due to higher vapour pressure deficits (McDowell & Allen 2015).

Since old-growth forests progressively accumulate carbon, they are able to store considerable quantities of it. Long tree life spans and slow growth rates, such as the ones that characterize *Fitzroya*, as well as the sequestration of a large proportion of carbon in decay-resistant heartwood in this species and other long-lived species such as redwoods (Sillett et al. 2015), largely contribute to carbon storage properties of temperate forests.

Table 2.1. Aboveground biomass (AGB), productivity (NPP_{AG}) and total net primary productivity (NPP) from different studies in temperate forests worldwide (studies in forests from the US Pacific Northwest and the Southern hemisphere are mostly shown).

Forest type	**Study area**	**AGB (Mg C ha^{-1})**	**NPP_{AG} (Mg C ha^{-1} yr^{-1})**	**NPP (Mg C ha^{-1} yr^{-1})**	**Reference**
Sequoia sempervirens	California	1650	2.65-9.40	-	(Westman & Whittaker 1975)
Sequoia sempervirens	California	2320	3.5-5.0	-	(Busing & Fujimori 2005)
Tsuga heterophylla	Western Coast Range, Oregon	746	6.5	-	(Gholz 1982)
Pseudotsuga Menziessi	Oregon Cascades	587	-	-	(Keith et al. 2009)
P.Menziessi-T. heterophylla	Oregon Cascades	781	-	-	(Fujimori et al. 1976)
Old-growth temperate forests	Oregon Coast	464.7	-	-	(Smithwick et al. 2002)
Picea sitchensis, T. heterophylla	Western Coast Range, Oregon	355.4	5.25	6.81	(Runyon et al. 1994)
P. menziessi	Interior valley, Oregon	235.6	5.80	7.70	(Runyon et al. 1994)
T. heterophylla, P. menziessi	West Cascades, Oregon	204.2	8.75	11.22	(Runyon et al. 1994)
Evergreen needleleaf forests	Pacific Northwest	228.4	7.35	7.9	(Hessl et al. 2004)
Evergreen temperate humid forests	Worldwide	149.3	-	7.83	(Luyssaert et al. 2007)
Mature-old-growth temperate forests	Worldwide	43-1650	1.67-11.34	-	(Keeling & Phillips 2007)
Mature-old-growth cool temperate moist forests	Worldwide	377	-	-	(Keith et al. 2009)
Eucalyptus regnans	Victoria, Australia	1819	-	-	(Keith et al. 2009)
Agathis australis	North Auckland, New Zealand	729	3.25	-	(Silvester & Orchard 1999)
Nothofagus solandri	Craigieburn Range, New Zealand	122.6	2.62	-	(Clinton et al. 2002)
Nothofagus dombeyi dominated forest	Andes Cordillera, Southern Chile	435	-	-	(Schlegel & Donoso 2008)
Fitzroya cupressoides	Chiloé Island, southern Chile	268.4-285.9	2.28-3.42	-	(Battles et al. 2002; Vann et al. 2002; Joshi et al. 2006)
Mixed broadleaf evergreen forest	Chiloé Island, southern Chile	116.4	4.42-6.06	-	(Vann et al. 2002; Joshi et al. 2006)
Austrocedrus chilensis	El Bolsón, Argentina	78.4-99.9	2.95-3.6	-	(Ferrando et al. 2001)

2.2.2. Soil respiration in temperate ecosystems

Soil respiration is the largest source of CO_2 from terrestrial ecosystems and is commonly measured as the soil CO_2 efflux, which means the rate at which CO_2 leaves the soil and enters the atmosphere (Raich et al. 2014).

Soil respiration is the combination of autotrophic (R_a) and heterotrophic (R_h) respiration. R_a is mainly contributed by living plant roots and their associated mycorrhizae, while R_h mainly comes from microbes responsible of the detritus and soil organic matter decomposition (Butler et al. 2012). The proportion of these two components varies with seasons and among forests, but an approximate 50% - 50% proportion at an annual basis is within the range reported in other temperate forests (Epron et al. 2001; Irvine & Law 2002; Lavigne et al. 2003; Ruehr & Buchmann 2010). In addition, a global metaanalysis of partitioning studies found that heterotrophic respiration averaged 52% of total soil respiration among temperate coniferous forests (Subke et al. 2006).

Mean soil respiration in northern hemisphere forests during the growing season was the highest in mixed deciduous/evergreen forests (4.9 μmol m^{-2} s^{-1}), followed by evergreen needleleaf (3.6 μmol m^{-2} s^{-1}) and by deciduous broadleaf forests (3.2 μmol m^{-2} s^{-1}, Hibbard et al. 2005). Annual soil respiration in evergreen coniferous forests ranged between 4.27 and 18.05 Mg C ha^{-1} yr^{-1} (Hibbard et al. 2005), and values were well within this range in an old- growth *Pseudotsuga menziessi-Tsuga heterophylla* forest in Oregon (Sulzman et al. 2005, Table 2.2). Annual soil respiration measurements along a chronosequence in Oregon, demonstrated that soil CO_2 effluxes in mature and old-growth forests were the highest in mountain *P.menziessi* forests (West Cascades), a little lower in mesic *T.heterophylla-Picea sitchensis* forests (Coast Range) and the lowest in semi-arid *Pinus Ponderosa* forests (East Cascades, Campbell & Law 2005, Table 2.2). Trends with age were not consistent among forests, and rates were high in old-growth, but also in initiation stages. Edaphoclimatic conditions had a higher effect on soil respiration variation than disturbance regime in these ecosystems (Campbell & Law 2005).

Annual estimates were a little lower in another old-growth *P. ponderosa* stand in the drier East Cascades (Irvine & Law 2002) and values did not surpass 10 Mg C ha^{-1} yr^{-1} in diverse studies performed in the Eastern US, Europe and China (Davidson et al. 1998; Buchmann 2000; Savage & Davidson 2001; Rodeghiero & Cescatti 2006; Wang et al. 2006a; Ruehr et al. 2010, Table 2.2).

At the global scale soil respiration rates are well correlated with temperature and precipitation (Raich & Potter 1995). Seasonal variability in soil CO_2 efflux is often explained by soil temperature changes in mesic sites and by soil moisture content when soils are dry or excessively wet. Conversely, soil water content is the main driver of soil respiration in Mediterranean and arid ecosystems, where small water inputs after dry periods contribute to the largest C-losses contributed by microbial activity (Carbone et al. 2011). Soil efflux is generally largest in warm, moist and productive sites, decreases in seasonally dry or cold climates and is lowest in dry or cold sites. Interannual climate variability is the main factor attributed to affect interannual changes in soil respiration in diverse ecosystems (Raich et al. 2014).

Soil respiration and microbial decomposition have been usually modelled using soil temperature, as there is evidence that it is a generally reliable predictor of soil CO_2 efflux, when there is no severe drought stress (Davidson et al. 1998). Exponential functions, particularly the Q_{10} function, which reflects the temperature sensitivity of soil respiration, have been regularly employed to estimate soil respiration from temperature (Davidson et al. 2006b). However, this function estimated from annual datasets not only includes temperature, but also seasonal changes in other variables such as soil water content, root biomass and litter inputs, among others (Davidson et al. 1998).

Q_{10} values are defined as:

$$Q_{10}= \exp^{10b} \tag{2.3}$$

Where b is the regression coefficient obtained from the relationship between respiration and temperature:

$$R= \exp^{b(T)} \tag{2.4}$$

Where R is soil respiration and T soil temperature

Q_{10} values might be particularly high when temperature and the production of photosynthetic substrates positively covary naturally during the growing season (Davidson et al. 2006b; Ruehr & Buchmann 2010). In addition, particularly drier conditions in wet sites can lead to higher Q_{10} values, mainly due to a large efflux of CO_2 and an enhancement of microbial and root respiration at intermediate water contents (Davidson et al. 1998; Davidson et al. 2006b).

The Q_{10} function strongly depends on the ecosystem studied, being important to evaluate the environmental controls on soil respiration at a site level, so potential responses of ecosystems to ongoing changes can be identified (Curiel Yuste et al. 2004; Fenn et al. 2010).

The effects of soil moisture availability on soil metabolic activity vary depending on the water content of the soil: in dry soils metabolic activity and hence soil respiration increases with increasing soil humidity, thereafter soil moisture does not have much effect (biological activity is near its potential) until soils get too wet and the lack of oxygen constraint aerobic respiration (Raich & Potter 1995). Soil water content affects the diffusion of soluble substrates and the diffusion of oxygen at low and high water contents, respectively; and both mechanisms limit soil respiration (Davidson et al. 2006b).

Table 2.2. Annual soil respiration values from different studies in temperate forests worldwide.

Forest type	**Study area**	**Annual soil respiration ($Mg\ C\ ha^{-1}\ yr^{-1}$)**	**Reference**
Temperate coniferous forests	Northern hemisphere	4.27-18.05	(Hibbard et al. 2005)
Old-growth *P. menziessi* *T. heterophylla*	Central Cascades, Oregon	7.27-8.41	(Sulzman et al. 2005)
Mature and old-growth *P. menziessi*	West Cascades, Oregon	16.7-20.7	(Campbell & Law 2005)
Mature and old-growth *T. heterophylla, P.sitchensis*	Coast Range, Oregon	10.8-15.6	(Campbell & Law 2005)
Mature and old-growth *Pinus ponderosa* forest	East Cascades, Oregon	7.2-8.7	(Campbell & Law 2005)
Old-growth *Pinus ponderosa* forest	East Cascades, Oregon	4.83-5.97	(Irvine & Law 2002)
Tsuga forest	Harvard forest, Massachusetts	6.7	(Davidson et al. 1998)
Mixed boreal transition forest	Howland forest, Maine	4.3-8.6	(Savage & Davidson 2001)
Picea abies forest	Bavaria, Germany	7.10	(Buchmann 2000)
Mixed evergreen forests	Alps, Trento, Italy	7.72	(Rodeghiero & Cescatti 2006)
Mixed mountain forest	Lägeren, Switzerland	8.69-9.07	(Ruehr et al. 2010)
Mixed temperate forests	Heilongjiang, China	4.03-8.13	(Wang et al. 2006a)

2.3. Woody growth as a proxy for evaluating the sensitivity of total productivity to climate: Stem growth-climate relationships and long-term changes in tree radial development

When long-term NPP studies are not possible or natural forests distributions along elevational or rainfall gradients do not exist, probably the only way of figuring out the effects of climate on productivity is studying stem radial growth. Tree rings are unique archives that allow the assessment of interannual variation and trend changes in forests productivity and carbon sequestration at decadal or longer timescales. Tree-ring widths, as well as the isotopic composition of tree rings, have been commonly used to assess the climate variables that mostly influence tree species development, as well as the impacts of environmental changes on tree species during recent decades. Although the isotopic composition in tree rings is not a proxy for productivity, it is probably the best way to investigate important physiological changes (e.g. photosynthetic rate, stomatal conductance) that might be related to long-term trends in growth and productivity (McCarroll & Loader 2004; Silva & Anand 2013).

The coming sections (2.3.1., 2.3.2. and 2.3.3) will review the basis and main research findings for the following topics: tree-ring width - climate relationships on an intra-annual timescale, tree-ring width - climate relationships during recent decades, and tree-ring carbon isotopic studies. These topics will help addressing specific objectives 5 and 6 and the literature review will primarily focus on temperate, as well as on Mediterranean forests.

2.3.1. Stem radial growth-climate relationships on an intra-annual basis

Cambial cell division and cell expansion, the two processes underlying radial growth proceed at timescales of hours to a few days. Dendrochronological studies usually assess growth- climate relationships at longer time periods, leaving a gap in the understanding of the causal chain between cellular and radial growth (Köcher et al. 2012). This means that the variables identified as the ones controlling tree-growth in dendrochronological analysis are not always the same as the ones that determine instantaneous changes in cambial activity. The assessment of tree radial growth on short temporal scales can provide valuable information regarding the environmental triggers of radial growth, differences in climate sensitivity of different species or tree populations, and potential limitations to forest productivity due to climate change (Pérez et al. 2009).

High precision dendrometers have been widely used to continuously monitor stem radial variation throughout the year at a high temporal resolution (e.g. every 30 minutes). This monitoring is fundamental to understanding the reaction of the tree to short-term variations in environmental conditions (Deslauriers et al. 2007). These instruments commonly measure stem radial variation that is comprised of diurnal changes (cycles) in water storage depletion and replenishment (Offenthaler et al. 2001) and seasonal tree growth (Downes et al. 1999; Tardif et al. 2001; Deslauriers et al. 2003; Bouriaud et al. 2005; Deslauriers et al. 2007). Several studies have used high precision dendrometers to assess growth-climate relationships (Tardif et al. 2001; Deslauriers et al. 2003; Bouriaud et al. 2005; Biondi & Hartsough 2010) finding that these instruments can be useful to assess radial increment-climate relationships in different species all over the world.

There are two main approaches to extract stem growth from dendrometer data where the key step consists of removing the daily variations produced by stem shrinking and swelling. The *daily approach* consists of extracting one value per day (average or maximum) from the time series, so it can be quickly reduced from 24 or more daily readings to just one. The stem variation between two subsequent days is successively calculated using first differences. The *stem cycle approach* is based on the stem shrinking and swelling patterns and consists of separating three distinct phases: contraction, expansion and stem radius increment, which is considered an estimate of growth. These phases represent the diurnal sap flow variations and the associated change in stem radius (Deslauriers et al. 2007). This last approach has the advantage of continuously extracting stem contraction and expansion and has been especially recommended when a high frequency of long cycles due to rainfall events occur, since the stem expansion calculated through this method lasts for several days (Deslauriers et al. 2007). Thus, this approach is especially suitable for study sites where precipitation is high and can last for several days.

Dendrometers have been used in boreal species where Tardif et al. (2001) and Deslauriers et al. (2003) found that precipitation had the strongest influence on stem radial growth and temperature and vapour pressure deficit were secondary factors. Drew et al. (2008) found that temperature and precipitation had a primary influence on stem radius change of Eucalyptus in Tasmania. Temperature was the main driver of stem radial growth of a conifer species at the timberline (Gruber et al. 2009) and air humidity and vapour pressure deficit were the main variables influencing stem radius change in temperate broadleaved species in Europe (Köcher et al. 2012). Only one study so far has assessed the relationship between *Fitzroya* tree growth and daily climatic variables at the southern distribution of the species in the Coastal Range (Pérez et al. 2009). The authors used band dendrometers and the *daily cycle* approach to assess daily growth in three endemic species growing in the Chiloé Island. Pérez et al. (2009) found that tree growth of the shade-tolerant species *Podocarpus nubigenus* was positively related to photosynthetically active radiation and that *Fitzroya* stem growth was positively related to precipitation and negatively related to radiation. Radial growth in this species was greatly and negatively affected by warm, rainless periods.

2.3.2. Stem radial growth changes during recent decades and their relationship with environmental conditions

Climate is the main driver of forest growth, so changes in climate conditions are likely to affect forest productivity and carbon sequestration (Boisvenue & Running 2006). Climate change might positively influence tree growth through CO_2 fertilisation and longer growing seasons, as well as negatively affect growth due to increasing water stress (Allen et al. 2010).

The increase in atmospheric CO_2, has been one of the most prominent environmental changes in the last century (increasing from ~ 285 ppm at start of industrial revolution to 396 ppm in 2013, Robertson et al. 2001; Keeling et al. 2014). Increases in CO_2 promote photosynthesis by rising CO_2 uptake (i.e. increase carboxylation compared with oxygenation, Norby et al. 1999; Beedlow et al. 2004). They might also produce a partial closure of stomata, reducing the amount of water lost by transpiration, and therefore increasing the ratio of carbon gain to water loss (i.e. water use efficiency, WUE, Farquhar 1997). This effect can increase plant growth, especially in dry ecosystems and enhance NPP (Amthor 1995; Huang et al. 2007). The enhancement in productivity and WUE due to CO_2 is commonly known as direct CO_2 fertilisation (Beedlow et al. 2004).

An increase in tree-ring growth and productivity mostly during the 20th century has been reported for different forests since the 1980s (LaMarche et al. 1984; Graumlich et al. 1989). LaMarche et al. (1984) attributed the increase in tree ring widths of *Pinus longaeva* growing in subalpine habitats in the western US to CO_2 fertilisation, rather than to direct climatic effects. Meanwhile, Graumlich et al. (1989) attributed the increase in NPP of old-growth mountain forests in western Washington to summer temperatures and solar radiation, rather than to CO_2 fertilisation. Later, Salzer et al. (2009) attributed the increasing trend in tree-ring growth in *Pinus longaeva* at the upper-treeline to a temperature increase. They argued that recent climate datasets do reveal a positive trend in temperature that matched the increase in growth, which was not found by LaMarche et al. (1984). Regarding another long-lived species, tree growth has been reported to increase in old *Sequoia sempervirens* and *Sequoiadendron giganteum* trees during recent decades and attributed causes vary from increases in radiation (for *S. sempervirens*) to probable CO_2 fertilisation and nitrogen deposition (for *S. giganteum*, Sillet et al. 2015).

There have been a number of tree-ring studies that have ascribed the increase in tree-ring growth during recent decades to the rise of CO_2 (Knapp et al. 2001; Bunn et al. 2003; Soulé & Knapp 2006; Wang et al. 2006b; Leal et al. 2008). An analysis of a tree-ring global database, found that approximately 20% of the sites exhibited an increasing trend in growth that could be attributed to CO_2 fertilisation. There were within-species differences which were attributed to various factors, including different soil properties, competition and disturbance regime (Gedalof & Berg 2010).

Despite these findings, there are other studies that suggest that CO_2 fertilisation might be limited or constrained by nutrient deficit, air pollution and temperature and precipitation changes (Beedlow et al. 2004). According to Körner (2013) CO_2 fertilisation is only possible when soil resources match the enhanced demand, being unlikely in a natural system where plants commonly compete for these resources. However, Lloyd & Farquhar (2008) argued that at least tropical trees are able to acquire the resources that they need under higher CO_2. It appears that there is still an open debate about the true effect of CO_2 increase on forests growth.

On the other hand, there have been studies, especially in Mediterranean ecosystems, that have shown decreases in tree growth mainly due to reduced water availability (Andreu-

Hayles et al. 2011). Climate has been reported to become more limiting for radial growth in Iberian forests, causing higher growth synchrony among diverse sites and species, and higher interannual growth variability associated with increasing water stress during the last fifty years (Macias et al. 2006; Andreu et al. 2007). A decline in tree radial growth started in the early 1980s in four conifer species from the western Mediterranean mountains in Spain and Morocco (*Abies alba, Abies pinsapo, Pinus nigra and Cedrus atlantica*), which was partly associated with precipitation decrease and regional warming. Sharp growth declines were observed during drought events, and highest/moister sites were less affected (Linares et al. 2011a). The same growth reduction pattern due to reduced annual precipitation was observed in *Pinus halapensis* in Greece during the last two decades (Sarris et al. 2011).

Although not only related to long-term climate change, decreases in precipitation in southern South America associated with the positive phase of the Antarctic Oscillation or Southern Annular Mode (SAM), have been linked to reduced tree growth in the dry-mesic forests of Chile and Argentina (Villalba et al. 2012). Thus, tree-growth in long-lived species such as *Araucaria araucana* and *Austrocedrus chilensis* has been lower than the long-term average since the 1950s and appears to be unprecedented in the last 600 years (Villalba et al. 2012).

There have been no studies to date that have assessed tree growth changes in *Fitzroya cupressoides* forests during the last few decades. Section 2.3.2.1 reviews the main dendrochronological studies and findings for this species.

Going beyond tree growth decreases, it has been reported that changes in precipitation patterns and increasing temperatures are likely to produce forest decline in areas predicted to undergo more severe and longer lasting droughts (Allen et al. 2010). In addition, a worldwide review on woody species found that 70% of them potentially face long-term decreases in productivity and survival, mainly because they operate with low hydraulic safety margins against detrimental levels of drought stress (Choat et al. 2012). The authors concluded that there is a global convergence in the vulnerability of forests to drought independent of the amount of rainfall that these forests currently receive.

These last conclusions are particularly important to be taken into account in the case of *Fitzroya* forests, since they grow under very rainy conditions, but at the same time the growing season is the driest season of the year, and trees can be affected by summer droughts. Drier conditions could potentially have a higher impact on *Fitzroya* populations from the Coastal Range, where climate has a stronger Mediterranean influence and where soils are generally shallower than in the Andes.

2.3.2.1. Dendrochronological studies in *Fitzroya cupressoides*

The strong negative relationship between *Fitzroya* tree-ring growth and previous summer temperature allowed a 3622-year summer temperature reconstruction for southern South America (Lara & Villalba 1993). An 1120-year summer temperature reconstruction was also developed earlier for northern Argentine Patagonia by Villalba (1990); who also found a weaker positive relationship with precipitation from the previous summer.

Neira & Lara (2000) studying one tree-ring chronology from the Andes (Contao, 41^0 33' S, 72^0 38' W) and one from the Coastal Range (Mirador, 40^0 10' S, 73^0 42' W) found a negative (positive) relationship between both tree-ring chronologies and previous summer temperature (precipitation). Moreover, the Andean chronology also presented a positive correlation with precipitation from the current summer. Lara et al. (2000) reviewed a network of 23 *Fitzroya* tree-ring width chronologies along Chile and Argentina finding that chronologies from the Andes had a stronger climate signal compared to chronologies from the Coastal Range, where there has been a high degree of alteration, mainly by fires, during the last 400 years (Veblen & Ashton 1982).

Finally, Barichivich (2005) studied the relationship between climate and two tree-ring width chronologies from the Coastal Range, finding a negative relationship with temperature and a positive one with precipitation during the months of the previous and current growing season. Maximum temperature had a stronger negative relationship with tree growth and minimum temperature had a weaker and mixed (positive and negative) correlation during the growing season periods.

It can be concluded from these previous studies that *Fitzroya's* growth is positively affected by rainier and cooler conditions during the growing season, and that the previous season's weather predominantly has an effect on stem radial growth.

2.3.3. Stable carbon isotope studies in tree rings

Before going into the literature review about the main findings in these studies (2.3.3.3), a brief review of the isotopic theory and some considerations on isotopic studies will be presented in Sections 2.3.3.1 and 2.3.3.2.

2.3.3.1. Theoretical basis and fundamentals of stable carbon isotope studies

Besides tree-ring widths, stable isotopes of carbon, hydrogen and oxygen (the main components of wood) constitute an additional powerful source to understanding tree-climate interactions. Their advantage over tree-ring width data, is that they provide a clearer mechanistic understanding of the influence that climate has on the processes through which these three elements pass during assimilation and wood development (Loader et al. 2007). Tree-ring isotopes can provide an excellent record of the ecophysiology of the tree, the resources they utilise and the current and past environmental conditions where they have developed (Dawson et al. 2002). In this review, the focus will be on carbon isotopes, which are the ones assessed in this study.

Carbon has two stable isotopes, a light ^{12}C and a heavy ^{13}C. The difference in mass between these isotopes allows different processes (biological, physical or chemical) to discriminate against the heavier ^{13}C isotope, thus revealing an environmental signal. By convention, and because of the small absolute abundance of isotopes in any material, the carbon isotope ratio of ^{13}C to ^{12}C ($^{13}C/^{12}C$) is expressed with reference to a standard material for which the isotopic ratio is known (Dawson & Simonin 2011). This ratio is known as $\delta^{13}C$ and is expressed in per mille (‰) as:

$$\delta^{13}C\ (‰) = (R_{sample}/R_{standard} - 1)1000 \qquad (2.5)$$

Where R_{sample} and $R_{standard}$ are the carbon ratios ($^{13}C/^{12}C$) of the sample and standard, respectively. The standard corresponds to the Vienna Pee Dee Belemnite (VPDB, McCarroll & Loader 2004).

During the diffusion of CO_2 from the atmosphere into the intercellular space within the leaf, this molecule is fractioned against ^{13}C, in favour of the light isotope ^{12}C by ~ 4.4‰, due to the differential mobility of both isotopes (^{12}C being more mobile). During photosynthesis, the intercellular CO_2 (c_i) combines with leaf water to produce sugars through carboxylation, which produces another fractionation against the heavy isotope causing a further isotopic depletion by 27‰. The dominant signal in the tree-ring carbon isotope ratios reflects the balance between the supply and demand of CO_2 at the site of photosynthesis, directly associated with the balance between stomatal conductance and photosynthesis rate (Loader et al. 2007).

The isotope discrimination (Δ) in plants, resulting from the preferential use of ^{12}C over ^{13}C during photosynthesis is expressed as:

$$\Delta^{13}C = \frac{\delta^{13}C_{atm} - \delta^{13}C_{tree}}{1 + \delta^{13}C_{tree}/1000} \approx \delta^{13}C_{atm} - \delta^{13}C_{tree} \qquad (2.6)$$

Where $\delta^{13}C_{atm}$ corresponds to the isotopic value of atmospheric CO_2 and $\delta^{13}C_{tree}$ to the measured isotopic ratio in tree rings. This discrimination represents approximately the difference between both $\delta^{13}C$ values. The steady increase in the fossil fuel emission of ^{13}C-depleted CO_2 during the last two centuries, has caused a progressive lowering of the $\delta^{13}C_{atm}$. This change has been reflected in most trees as a steady decrease in $\delta^{13}C_{tree}$ through time, a trend that needs to be removed when relating isotopic data to climate variables (Saurer et al. 2004; Loader et al. 2007).

The dependence of discrimination on plant physiological properties, specifically on c_i/c_a (c_a being the ambient CO_2 concentration) was described by Farquhar et al. (1982):

$$\Delta^{13}C \cong a + (b-a)\, c_i/c_a \qquad (2.7)$$

Where a is the fractionation related to the diffusion of atmospheric CO_2 through stomata (4.4 ‰) and b the fractionation associated with the enzymatic carbon fixation (27 ‰).

The dominant climatic factors affecting carbon isotopic fractionation are those that dominate stomatal conductance (controlled by air humidity and soil moisture conditions) and those that control photosynthetic rates (dominated by light levels and leaf temperature, McCarroll & Loader 2004).

The $\delta^{13}C$ in tree rings is usually sensitive to humidity conditions prior to and during the ring formation in water-limited systems. This is mainly due to the stomatal regulation of water loss. In particularly warm and dry conditions, the rate of CO_2 diffusion into the leaf decreases due to partial stomatal closure, leading to a reduced discrimination against ^{13}C and higher $\delta^{13}C$ (Figure 2.1, Johnstone et al. 2013). The opposite occurs under high humidity conditions (Figure 2.1).

In environments where water is not limiting, such as in cool, high altitude or latitude sites, solar radiation can be the main driver of δ^{13}C changes. This is mainly because higher radiation during the growing season positively affects photosynthetic rates and carbon assimilation, also leading to a reduced discrimination and higher δ^{13}C (Gagen et al. 2011a; Hafner et al. 2013; Johnstone et al. 2013; Loader et al. 2013).

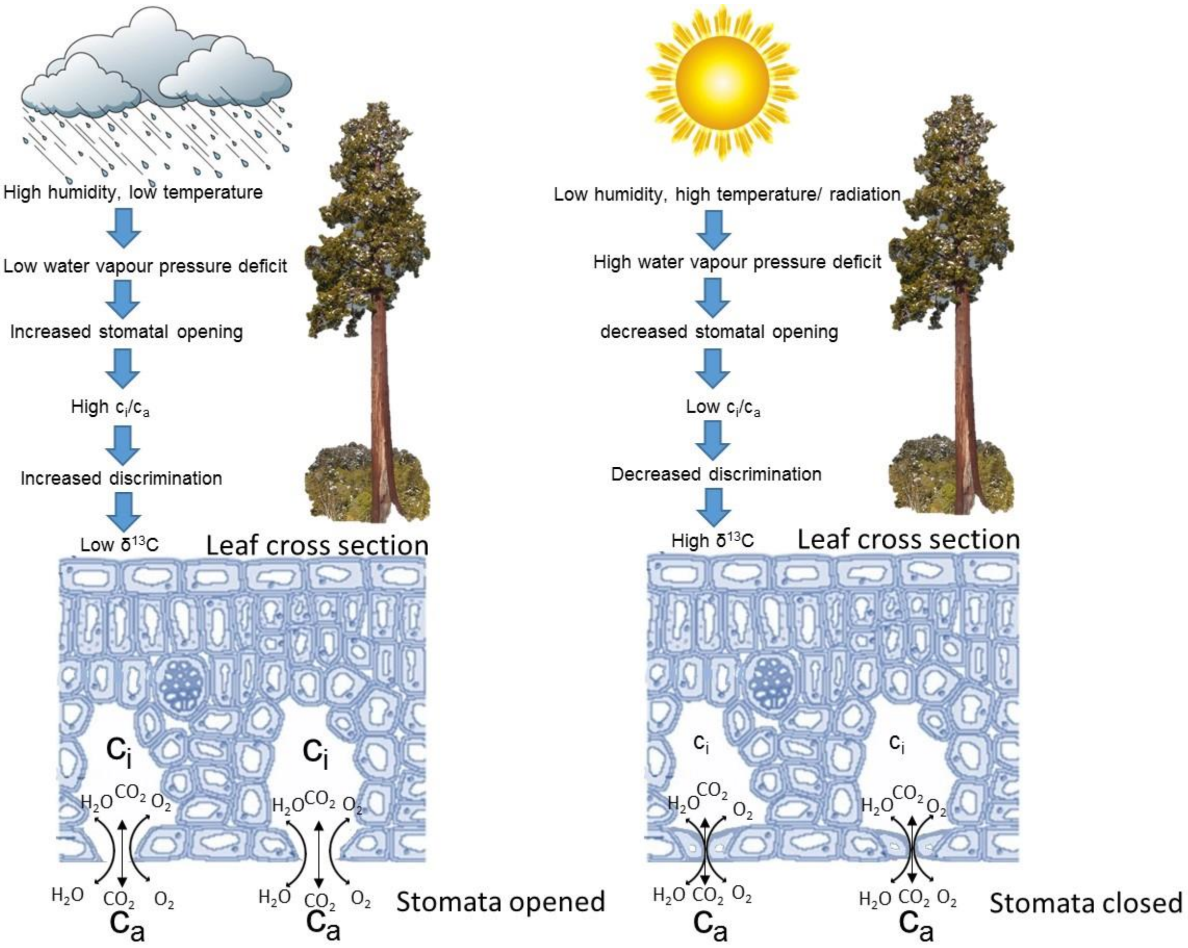

Figure 2.1. Constraints of carbon isotope fractionation given by different climate conditions. The two examples show how climate conditions impact stomatal opening, and how this opening influences changes in δ^{13}C of tree organic matter. Diagram adapted from Helle & Schleser (2004).

Changes in the assimilation rate within the leaf will influence c_i, while changes in stomatal conductance will influence the rate at which this internal CO_2 can be replenished. Where climate variables directly affect either of these controls, it will be integrated by the plant during photosynthesis and expressed as a change in the intrinsic water use efficiency (iWUE) and $\delta^{13}C$ (Loader et al. 2007). iWUE is a proxy of the water used per unit carbon gain and corresponds to the ratio between the net assimilation rate (A) and stomatal conductance to water vapour ($g_{w,}$ Ehleringer 1993). Since the stomatal conductance for CO_2 (g_c) and water vapour (g_w), are related by a constant $g_{w=}$ 1.6 $g_{c,}$ and the net carbon uptake by diffusion follows Fick's law corresponding to $A= g_c(c_a - c_i)$; iWUE can be calculated as follows:

$$iWUE = c_a(1- c_i/c_a)*0.625 \tag{2.8}$$

iWUE values can provide insight into the natural responses of trees to increasing atmospheric CO_2 concentrations (Waterhouse et al. 2004). As it was mentioned above, stomata tend to close under elevated CO_2 concentrations, a mechanism that allows water saving. When there is a high stomatal limitation of photosynthesis, the leaves display a low intercellular CO_2 concentration (c_i), a decreased fractionation and an improved iWUE (Saurer et al. 2004).

Several authors have detected changes in iWUE that may relate to changing atmospheric CO_2 (Saurer et al. 2004; Waterhouse et al. 2004; Linares et al. 2009; Andreu-Hayles et al. 2011). It has been reported that a positive relationship between tree growth changes and iWUE, without the direct influence of climate, would indicate CO_2 stimulation, while a negative relationship would reflect the main influence of stressors (Silva & Anand 2013). Since iWUE is a direct function of c_a, its trend will likely be positive; so variations in tree ring discrimination through time (which captures the variability only coming from physiological reactions to environmental changes), will be always needed to avoid making conclusions just based on iWUE (Silva & Anand 2013).

2.3.3.2. Considerations for isotopic studies

There are some considerations that need to be taken into account regarding the general methodology employed in carbon isotopic studies:

- Annual $\delta^{13}C$ chronologies are recommended to be developed by averaging the carbon isotope composition of different trees for the same year. However, overall restrictions in time and resources only allow pooling samples before the analysis, which has the limitation that the relative contribution of individual rings may be variable. Since this approach also means losing the standard deviation, an error estimate can be obtained if the pooled records are broken into individual trees at certain regular intervals (Loader et al. 2007).
- In tree-ring stable carbon isotope analyses purified α-cellulose is a regularly used material, because it constitutes the framework around which lignin and resins are then deposited (Loader et al. 2007; Taylor et al. 2008). Cellulose has been mainly used to avoid the effect of a variable relative abundance of wood constituents on the stable isotopes, since different components of wood differ isotopically (Borella et al. 1998). However, since it is very time consuming to extract cellulose, different authors have used or recommended to use whole wood or whole wood without extractives, due to strong correlations with the isotopic signal in cellulose (Borella et al. 1998; Warren et al. 2001; Loader et al. 2003; Harlow et al. 2006; Taylor et al. 2008; Granados 2011). A constant offset has been found between cellulose and whole wood (Loader et al. 2003) and between hollocellulose and wood without extractives (Harlow et al. 2006) in several species, giving more confidence to the use of whole wood instead of cellulose in carbon isotope studies. Resins, which are mobile components, are recommended to be removed prior to the isotopic analysis, since they can reduce the temporal integrity of the chronology (Loader et al. 2007).

- Some advantages of tree ring carbon isotope chronologies compared with tree-ring width chronologies is that the first can explain a similar proportion of climatic information using fewer replicates (Robertson et al. 1997). In addition, they do not present an apparent long-term age trend, so they do not need statistical detrending to correct age-related effects (Loader et al. 2007). The only age effect is the "juvenile effect" that is characterized by a steady increase in $\delta^{13}C$ values during the first decades of forest development until they reach more stable values. This can be mainly produced by the recycling of respired air by young trees that grow close to the forest floor, by shading and/or by changes in hydraulic conductance when trees get taller (Francey & Farqhuar 1982; McCarroll & Loader 2004; Loader et al. 2007; Brienen et al. 2011). This juvenile effect can be easily identified, and the period affected removed prior to any analysis.

2.3.3.3. Findings in carbon isotope studies in tree rings

Tree ring carbon isotope studies have been used to assess trends in discrimination and iWUE in different temperate and Mediterranean species. They have also been used to assess the main environmental variables related to the tree isotopic composition.

Studies in Mediterranean ecosystems have found different degrees of increases in iWUE during the last few decades. However, these increases have, in most cases, not translated into higher growth, but in narrower rings and basal area growth rates, as in the case of some *Abies* and *Pinus* species (Andreu-Hayles et al. 2011; Linares et al. 2011b). Granda et al. (2014) found significant differences in growth between coexisting Mediterranean species, driven by physiological responses to drought. Droughts appear to negatively affect species like *Quercus faginea* and *Pinus nigra*, which are experiencing reductions in stomatal conductance to avoid water loss and further growth declines. On the other hand, species such as *Juniperus thurifera* appear to benefit from the CO_2 rise, through increased C assimilation despite dry conditions.

A tree ring study in broadleaved trees and a conifer species from northern Europe (Eastern England and southwest Finland) found an increase in iWUE and constant c_i levels during the last century. However, a reduction in the rate of increase of iWUE was found at higher levels of c_a, demonstrating a decrease in the sensitivity of trees to atmospheric CO_2 (Waterhouse et al. 2004). The same was found later in *Pinus sylvestris* from northern Finland, where iWUE showed a plateau since the 1970s, indicating that there is a limit to the capability of the trees to exert stomatal control over c_i under increasing CO_2 levels (Gagen et al. 2011b).

A significantly higher iWUE was detected for some species in Free Air CO_2 Enrichment (FACE) experiments compared with control plots. This higher iWUE was explained by a significant decrease in c_i/c_a in all species and was related to changes in CO_2 and climate (Battipaglia et al. 2013). The authors concluded that the effect of increased CO_2 on the hydrological balance of forests will highly depend on species composition.

Lévesque et al. (2014) investigated the growth and physiological changes of different conifer species growing in xeric and mesic sites in Switzerland during recent decades. They found that iWUE significantly increased during the last 50 years and varied for different species and site conditions. Radial growth decreased in xeric sites and did not increase in mesic sites, inferring that stomatal closure caused by dry conditions has reduced carbon uptake and growth. This study concluded that temperature-induced drought might negatively affect forest productivity even in temperate mesic sites (Lévesque et al. 2014).

Regarding long-lived species, a millennial $\delta^{13}C$ chronology of the longest-lived tree species *Pinus longaeva*, revealed that there was not a significant trend in this record during the last century. $\delta^{13}C$ ratios were sensitive to moisture availability (negative correlation), allowing a summer precipitation reconstruction for the last millennia (Bale et al. 2011). Meanwhile, a strong positive correlation between $\delta^{13}C$ of middle wood and summer maximum temperature was found in *Sequoia sempervirens*. A negative relationship was also found with summer low cloud frequency. Thus, middle wood $\delta^{13}C$ increased under clear skies, reduced precipitation and high atmospheric vapour pressure deficit (VPD) during summer as expected from theory; while the effect of cloud frequency was mainly explained by the positive effect of solar radiation on CO_2 assimilation rates. The strongest correlation with maximum temperatures was interpreted as a combined effect of solar radiation and high VPD (Johnstone et al. 2013). $\delta^{13}C$ in late wood on the other hand, was negatively associated with precipitation in late summer and early fall, when rainfall helped reducing the drought stress (Johnstone et al. 2013).

In South America, Srur et al. (2008) evaluated changes in radial growth and iWUE in *Nothofagus pumilio* forests growing along an altitudinal gradient in the Argentine Patagonia. The authors found increasing iWUE along the gradient, but diverging tree-growth patterns during the last century, with significant decreases at low elevations, no significant changes at middle elevations and significant increases at high elevations (Srur et al. 2008).

Finally, a 290-year tree-ring $\delta^{13}C$ chronology (1700-1987) was developed for *Fitzroya* growing in the Andes Cordillera (Alerce Andino National Park) at 450 m a.s.l (Leavitt & Lara 1994). The hollocellulose component was analyzed in pentads and ten cores were pooled for most of the period, except every 50 years, when the pentads from each tree were processed separately. The main finding was a decreasing trend in the $\delta^{13}C$

chronology, with a steeper decline of approximately 1.2‰ after 1900. The c_i/c_a ratios did not present any significant trend throughout the period, showing strong evidence that the *Fitzroya* $\delta^{13}C$ chronology was tracking the $\delta^{13}C_{atm,}$ and that the $\delta^{13}C_{tree}$ was not being affected by any shifts in environmental conditions and/or atmospheric CO_2 concentration (Leavitt & Lara 1994).

As it can be inferred from carbon isotope and tree growth analyses, there are different species responses to recent changes in CO_2 and climate. Most of the species show an increase in iWUE during recent decades. However, species growing under restrictive moisture conditions appear to be negatively affected by a warmer and drier climate (decreasing growth). Some species do not show significant trends in tree growth, while some others show an increasing trend probably due to a positive effect of either CO_2 or favourable climate conditions (e.g. increasing precipitation).

Given that forests responses to CO_2 rises and climate change are mostly species-dependent or even site dependent, studies to assess current physiological as well as growth and productivity changes in endangered ecosystems are strongly encouraged for conservation purposes. In the case of *Fitzroya*, this type of study contributes to exploring potential negative changes occurring in stands of this endangered species and their possible explanations. In a broader context, studies on *Fitzroya* also contribute providing insights into the climate change response capacity of long-lived and old-growth ecosystems.

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Chapter 3: Site description and methods

This chapter will cover a general site description and field methodology mostly not included in the four empirical chapters. Pictures will complement some of the field methods description to exemplify them. Details about site characteristics and specific methods and statistical analyses are presented in the respective manuscripts.

Study sites were chosen in accessible areas of the Coastal Range and the Andes Cordillera. These two ranges are the main areas where *Fitzroya* grows and stands that broadly represented the mean structure of adult forests in each Range were selected. *Fitzroya* forests in the Coastal Range are mostly medium-age and have not reached an old-growth stage, mainly due to the occurrence of fires and human cuttings in the last centuries. Fires in this area appear to have been an important disturbance even before the European settlement in southern Chile (Lusk 1996; Lara et al. 1999). On the other hand, old-growth stands are much more common in the Andes, where disturbances are sparse and mainly associated with landslides (due to the rugged topography) and volcanic eruptions (Clement et al. 2001). Accessibility to the study sites was an important factor, since productivity measurements are very intensive and require relatively easy access all year round. This is quite difficult in the case of *Fitzroya* forests since they are always located in remote areas in the Andes or in private areas without adequate protection in the Coastal Range.

In order to develop this study, I conducted prospective field visits to choose my study sites within protected areas. Once they were selected I established two 0.6 ha plots in each study site. To perform fieldwork I had the permanent help of my husband and field assistants from Universidad Austral de Chile. Initially, I also had the help of a Peruvian assistant who helped me to learn and apply the protocol for productivity measurements that had been widely applied in studies of tropical ecosystems in that country (Marthews et al. 2012).

3.1. Study sites

We installed two 0.6 ha plots (AC1 and AC2) on very gentle slopes of the Alerce Costero National Park (AC) at 40° 10’ S- 73° 26’ W in winter 2011 (July-August, Figure 1.1). This Park is located on the Coastal Range and the mean altitude of both plots is 850 m a.s.l. Plots were chosen with the help of the Park Ranger who knew the area and forests characteristics very well. A researcher from Universidad Austral de Chile (Prof. Antonio Lara), with vast experience in *Fitzroya* forests, agreed with the location and stands chosen, since they were representative of adult forests conditions in the Coastal Range. Medium-age stands dominated by *Fitzroya* and with access all year long were selected (Figure 3.1).

We also installed two similar 0.6 ha plots (AA1 and AA2) on a southwest-facing slope (ca. 20%) in the Alerce Andino National Park (AA) at 41° 32’ S- 72° 35’ W (~ 760 m a.s.l). They were installed in winter-spring 2011 (August-October, Figure 1.1). It took longer to install these plots, because it was not possible to go up to the study site between August and early October, due to permanent snow cover and dangerous road conditions. In the Andes, the stand chosen was a pristine old-growth forest contiguous to the site where the oldest *Fitzroya* tree was found in the 1980s (Lara & Villalba 1993, Figure 3.1). This site is the only old-growth *Fitzroya* forest in the Andes with relatively easy accessibility (except in winter due to snow accumulation), due to a private road that ends at a former mountain lodge close to the study site. Most of *Fitzroya* forests in the Andes are reached only after several hours walking, so this stand was particularly suitable for intensive productivity monitoring.

The climate regime where *Fitzroya* grows is characterized by high annual precipitation (ranging from 2000 to over 4000 mm), and is classified as oceanic wet temperate with a reduction in summer precipitation (Lara et al. 2000; Lara et al. 2002). Precipitation in the Coastal site reaches ~ 4180 mm (period 1999-2010, DGA 2011). The closest station to the Andean site, located at 240 m elevation (Lago Chapo, ~18 km northeast of the area), recorded a mean annual precipitation of 4140 mm and a mean annual temperature of 10.3°C (Lara & Villalba 1993). There are no temperature records close to the Coastal site and snowfall is common during winter in both areas, but especially in the Andes where it can last until spring (Donoso et al. 1990).

Soils in the Coastal Range are developed from metamorphic schist, they are generally thin, podzolized and poor in nutrients (Veblen & Ashton 1982; Donoso et al. 1993). Soils in the Andes are volcanic, with a high organic matter content and are in general deeper and more fertile than soils in the Coastal Range. The pH in both ranges is low (< 5, Peralta et al. 1982; Donoso et al. 1993; Veblen et al. 1995).

Figure 3.1. Structure of the studied forests at top: the Alerce Costero National Park and bottom: Alerce Andino National Park (Photos: Yadvinder Malhi).

3.2. Climate characterization and soil sampling

In order to characterize climate in both areas, we installed automatic weather stations (Skye instruments, Powys, UK) at less than 1 km from the study plots in both areas (Figure 3.2). Stations were installed in forest gaps as open as possible, given the available site conditions. These stations recorded precipitation, relative humidity, temperature and total solar radiation every 30 minutes. Soil temperature was recorded with a Decagon sensor EC-T (Pullman, WA, USA) installed at a single point at 10 cm depth in AC1 and AA1.

Figure 3.2. Weather stations at the Alerce Costero (left) and Alerce Andino (right) National Parks (Photos: Aldo Farías).

In terms of soil sampling, soil conditions were first tested across each plot by an expert using a steel soil sampler. Two and three soil pits were subsequently excavated in AC1 and AC2 plots respectively, for physical and chemical characterization. In the Andes two soil pits were excavated in each plot (Figure 3.3). Soils were physically characterized in the field by an expert and chemical analyses were done separately for each surface horizon, where most of the roots develop. Laboratory analyses included the main macro and micro-nutrients, pH and bulk density and were performed at the Laboratorio de Suelos Forestales at Universidad Austral de Chile, Valdivia.

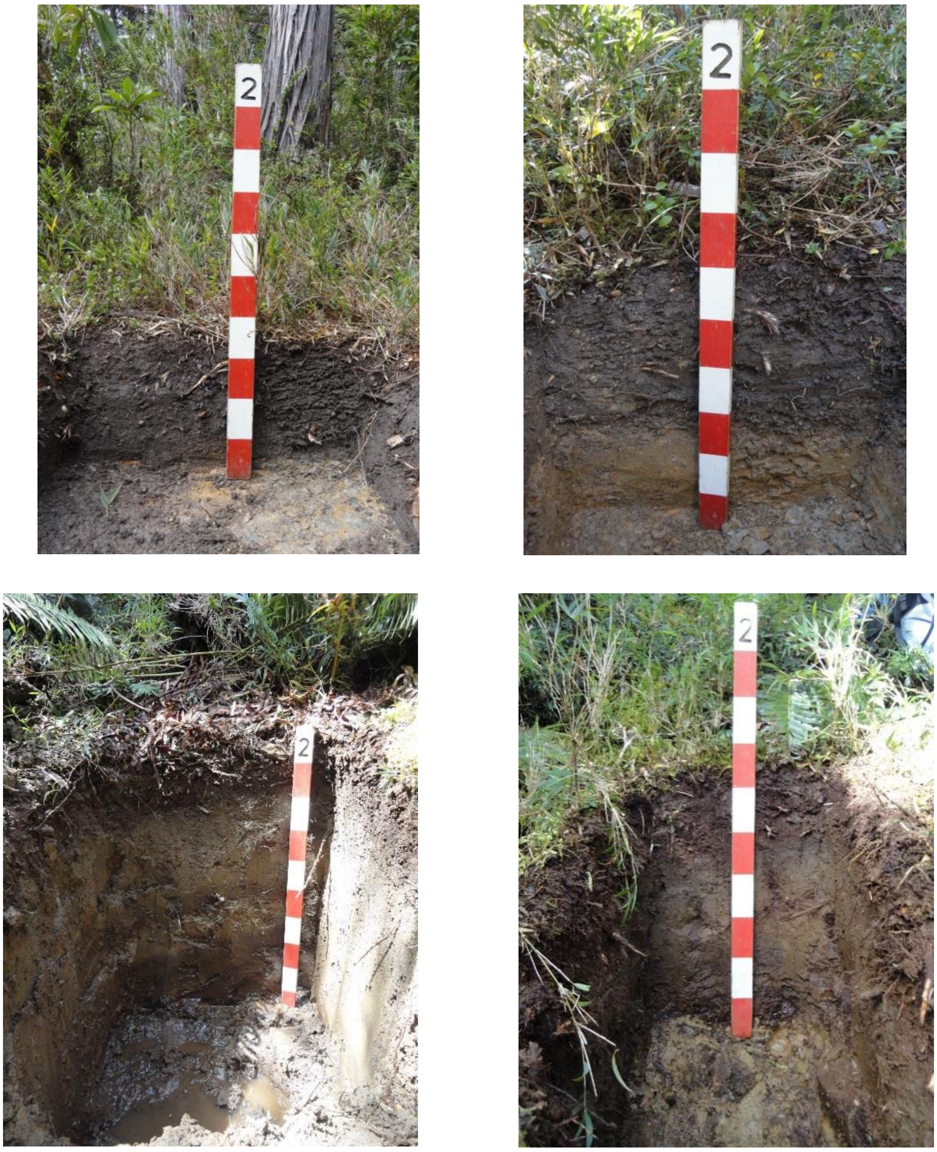

Figure 3.3. Examples of soil profiles in the Alerce Costero (top) and Alerce Andino (bottom) sites (Photos: Oscar Concha).

3.3. Aboveground biomass and net primary productivity (NPP)

3.3.1. Plots and tree census

To measure biomass and aboveground net primary productivity (NPP_{AG}) the two 0.6 ha plots in each study site were divided into 15 20x20 m subplots (Figure 3.4).

Measurements were made mostly following and adapting the RAINFOR-GEM network protocol (Marthews et al. 2012) and were carried out from November 2011 until October 2012 in both study sites. According to this protocol a one-ha plot is commonly used to assess biomass and productivity. However, it was not possible to fit such a plot in the Coastal site, since there were no accessible forest patches of this size in the Park, and 0.6 ha plots were used instead. Similar 0.6 ha paired plots were used in AA, to facilitate comparison and consistency between the two sites.

I was in Chile from July 2011 to May 2012 to establish plots, take most of the biomass and productivity measurements, measure soil respiration and process the collected data. I went back again from December 2012 to February 2013 to collect tree ring samples and continue taking productivity and the associated soil respiration measurements, since some of these measurements were continued for another year until October 2013.

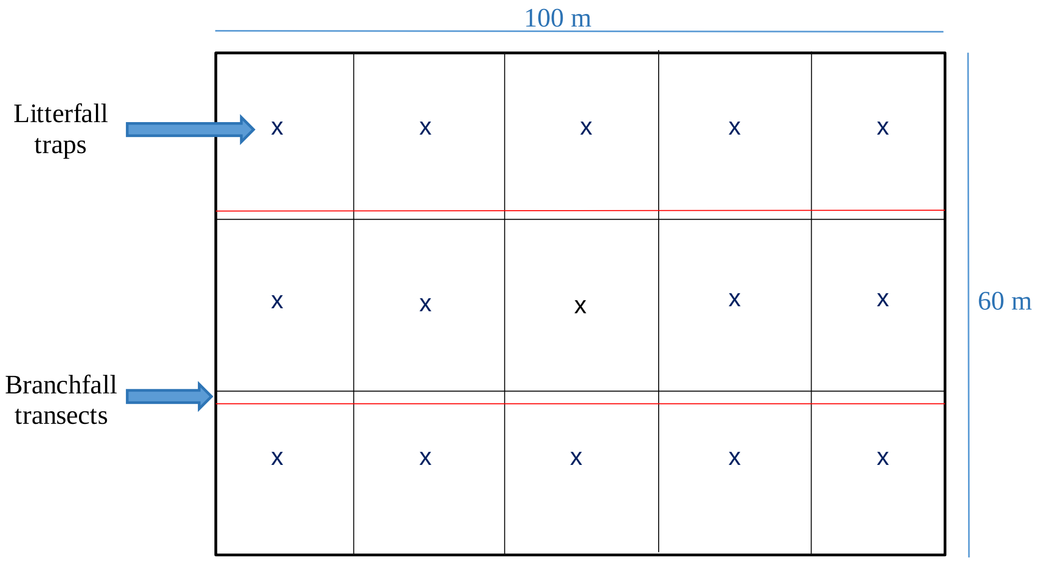

Figure 3.4. Diagram of the 0.6 ha plot with its fifteen 20 m x 20 m subplots. The position of the litterfall traps and branchfall transects is also shown.

We tagged and measured the diameter at breast height (DBH, 1.3 m) of all trees ≥ 10cm diameter in each 0.6 ha plot (Figure 3.5). Special care was taken to lift small epiphytes to measure diameter as precisely as possible in *Fitzroya* trees. We also measured the heights of at least 10% of the individuals per species and diameter class (10 cm classes) using a vertex IV (Haglof, Sweden, Figure 3.5).

To have an estimate of biomass and productivity from trees <10 cm DBH, we established two 10x10 m plots within each 0.6 ha plot and all small trees (2-10 cm DBH) were censused using callipers. Further details on this methodology are described in Chapter 4.

Figure 3.5. Measuring diameter in the Alerce Andino site (left) and measuring height using a vertex IV in the Alerce Costero site (right, Photos: Aldo Farías).

3.3.2. Biomass and aboveground coarse wood productivity (NPP$_{ACW}$)

Biomass and productivity measurements were performed according to the methods described in detail in Chapter 4 (main manuscript and supplementary material).

The woody growth rate per species was a crucial factor employed in the woody productivity calculations and it was estimated by collecting increment cores from trees as shown in Figure 3.6. The last year in the collected cores corresponded to 2011, but to have a more robust estimate of productivity since in some cases the last ring was not clear or complete; I used the mean growth of the last five rings in the calculations of woody productivity. Hence, the woody growth estimates employed in the NPP calculations are based on a five-year average, rather than specifically the year 2011.

Figure 3.6. Sampling tree ring cores of *Fitzroya cupressoides* in the Andean site (Photos:Waldo Iglesias)

3.3.3. Branch turnover (NPP$_{\text{branch turnover}}$) and canopy productivity (NPP$_{\text{litterfall}}$)

We measured branch turnover every three months in two permanently marked 100 m long transects (1 m wide) within each plot. Each transect was subdivided into five 20 m sub transects, corresponding to the sides of each 20x20 m subplot (Figure 3.4).

Canopy litterfall was collected on a monthly basis from 15 0.25 m^2 litter traps installed approximately at the center of every 20x20 m subplot and placed at 1 m height above the ground (Figures 3.4 and 3.7). More details on these methods are provided in Chapter 4.

Figure 3.7. Litterfall traps in the Coastal site (left) and the Andes (right, Photos: Aldo Farías).

3.3.4. Fine and coarse root productivity

Fine root production can be assessed biometrically measuring the amount of fine roots ($\leq$ 2 mm diameter) that develop in a certain period of time using techniques such as ingrowth cores or rhizotrons (Girardin 2010; Marthews et al. 2012). It can also be estimated using a mass balance approach that assumes carbon inputs equal belowground carbon outputs, including any change in carbon stocks (Malhi et al. 2009; Fenn et al. 2015).

Fine root productivity ($NPP_{\text{fine roots}}$) estimation was tested in the study sites using ingrowth cores. We actually installed five ingrowth cores in the four corners and centre of each plot and roots were extracted every three months following the RAINFOR-GEM protocol (Marthews et al. 2012) and the methodology proposed by Metcalfe et al. (2007). However, after performing consecutive measurements, I realised that soils in each area significantly lost their structure after these manipulations and the ingrowth cores were not properly recording real root growth. Due to this reason, ingrowth cores were just used to have an initial estimate of the fine root biomass in each site and $NPP_{\text{fine roots}}$ was rather estimated using a mass balance approach (see section 3.4.4.).

Coarse root (> 2 mm diameter) productivity ($NPP_{\text{coarse roots}}$) is more difficult to measure in the field and is commonly assumed to be a proportion of the stem productivity (root to shoot biomass ratios are usually employed, Malhi et al. 2009). In this study the coarse root/aboveground biomass ratio found in *Fitzroya* forests from Chiloé Island (6.9%, Battles et al. 2002) was used for this purpose.

3.3.5. Total aboveground productivity (NPP_{AG}) and total NPP

Total aboveground productivity (Mg C ha^{-1} yr^{-1}) was calculated by adding the above mentioned components:

$$NPP_{AG} = NPP_{ACW} + NPP_{branch\ turnover} + NPP_{litterfall} \tag{3.1}$$

Total NPP was calculated adding NPP_{AG} plus belowground productivity ($NPP_{\text{fine roots}}$ + $NPP_{\text{coarse roots}}$). Details on this methodology are presented in Chapter 4.

3.4. Soil respiration, its environmental drivers and mass balance estimation of fine root productivity

3.4.1. Soil respiration measurements

In order to measure the belowground carbon fluxes coming from *Fitzroya* forests, total soil respiration (R_s) measurements were carried out in each study area. Furthermore, a partitioning experiment helped in estimating the autotrophic (R_a) and heterotrophic (R_h) components.

We measured total soil respiration at 15 regularly distributed points in each plot (fifteen 10 cm depth collars inserted in the soil) using an infra-red gas analyser (IRGA) EGM-4 system with a closed static chamber technique (PP Systems, MA, USA). The fifteen points corresponded to the centre of each 20x20 m subplot next to each litterfall trap (Figures 3.8 and 3.9). The sampling frequency was once a month mostly from August 2011 to August 2013 in AC and from October 2011 to May 2013 in AA. Soil surface temperature (12 cm temperature probe, Electronic Temperature Instruments, West Sussex, UK) and volumetric water content (12 cm Hydrosense probe, Campbell Scientific Ltd., Loughborough, UK) were also measured at the same time at a distance of less than 50 cm from each collar (Figure 3.9).

The partitioning of autotrophic and heterotrophic respiration was estimated using two tubes that we installed at the four corners of each 0.6 ha plot (Figure 3.8). Heterotrophic respiration was estimated using a long tube (inserted 30 cm into the soil) from which roots were previously removed and total respiration was measured using 10 cm depth collars; the difference in fluxes between both tubes was used as a measure of autotrophic respiration. These measurements were less continuous than those of total respiration from October 2011 to August 2013 in AC and from November 2011 to May 2013 in AA, but with a longer gap during winter 2012. The longer gap was mainly because total soil respiration rather than the partitioning measurements were prioritized, and cold conditions during winter-spring sometimes only allowed total respiration measurements to be taken during each field campaign (i.e. batteries did not last for all measurements).

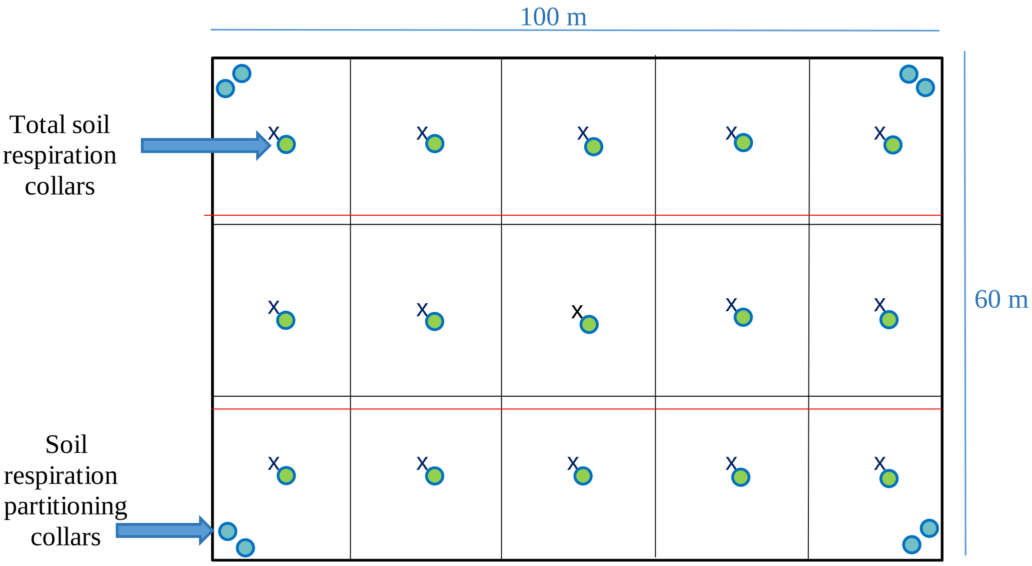

Figure 3.8. Diagram of the 0.6 ha plot with its fifteen 20 m x 20 m subplots. The position of the total soil respiration collars and soil partitioning experiments is also shown.

Figure 3.9. Measuring soil respiration and soil temperature and water content in the Alerce Costero site (Photos: Aldo Farías).

3.4.2. Seasonal and annual soil respiration estimates

For any single soil respiration measurement, soil efflux was calculated from the rate of CO_2 concentration increase within the closed chamber for a certain period of time. All records except the first nine seconds (to avoid the potential initial disturbance caused by chamber placement on the collar) were used to calculate fluxes using a linear fitting. Subsequent concentration data were in occasional cases also removed if they deviated from the linear fit.

I calculated soil CO_2 efflux per collar (µmol CO_2 m^{-2} s^{-1}) following the procedures presented in Marthews et al. (2012), where the main equation to obtain soil respiration is:

$$R = \frac{C_{124} - C_{14}}{T_{124} - T_{14}} * \frac{P*273}{(T_a + 273)} * \frac{V_d}{A} * 1.964*0.0036 * \frac{V_d + V_{collar}}{V_d} * 6.312 \qquad (3.2)$$

Where R is the volume corrected respiration in µmol CO_2 m^{-2} s^{-1}. C_{124} and C_{14} correspond to the CO_2 concentration (ppm) at time 124 and 14 seconds, respectively. T_{124} and T_{14} are the times of the respective CO_2 concentration, P is the ambient pressure at T_{124} and T_a is the air temperature recorded during measurement. V_d is the chamber volume (upper part) that corresponds to 0.0012287 m^3 and A to the area of exposed soil (area of the collar) that corresponds to 0.00882 m^2. 1.964 is 44.01/22.41, where 44.01 g is the molar mass of CO_2 and 22.41 l the volume of one mole of this component. Finally, V_{collar} is the volume of the collar and 6.312 is the factor to convert g CO_2 m^{-2} h^{-1} to µmol m^{-2} s^{-1}.

A diurnal temperature correction was applied to convert spot measurements (in each collar) into estimates of daily mean respiration. Mean daily fluxes for the fifteen collars were used to have an estimate of monthly soil respiration and, integrated over the year, an estimate of annual soil effluxes. Annual soil efflux estimates (in Mg C ha^{-1} yr^{-1}) were calculated by linear interpolation of fluxes between measurement days. The annual periods considered were from the first measurement made in August 2011 to August 2012 (year 1) and from August 2012 to August 2013 (year 2).

To estimate autotrophic and heterotrophic respiration for each site, the mean partitioning fractions determined from the experiment were multiplied by the total soil respiration (mean of two plots) obtained from the wider grid of soil respiration collars. Further details on these methods can be found in Chapter 5.

3.4.3. Relationships between total CO_2 efflux and soil environmental conditions

In order to determine the sensitivity of soil respiration to interannual changes in soil environmental conditions (considering the two recorded years), the ratio of soil respiration per collar in the same month of year 2 and year 1 was related to soil temperature and water content differences (within the same month and collar).

Soil water content (SWC) measurements were first calibrated considering the soil conditions in each site. The calibration was performed by determining the relationship between SWC estimated with the reflectometer and SWC estimated using a gravimetric method. Soil samples 0.0222-0.0244 m^3 were collected in each study site, brought to the laboratory and saturated with water. They were then left to dry in open air between 9 and 10 weeks, time during which their SWC and weight were measured every week using the hydrosense reflectometer and a hanging balance. The samples were finally oven dried and their weight recorded. The volumetric water content was determined using the equation (weight-dry weight)/volume of soil *100, which when divided by the density of water (1 g cm^{-3}) gave the SWC in %. The quadratic equation obtained regressing the (true) SWC determined through the gravimetric method against the SWC measured with the Hydrosense was used to calibrate the Hydrosense data collected in the field.

Further details on soil respiration and its relationship with soil environmental variables are supplied in Chapter 5.

3.4.4. Mass balance estimation of fine root productivity

Due to the importance of having a total NPP estimate in this carbon cycle study, a mass balance approach that uses heterotrophic respiration measurements and soil carbon inputs data was applied to estimate $NPP_{fine\ roots}$. Carbon (C) inputs to the soil (above-belowground mortality, fine root productivity and litterfall) are assumed to correspond with the belowground outputs (R_h, and dissolved organic C in water, DOC), including any alteration in soil C stocks (Malhi et al. 2009; Fenn et al. 2015). The period November 2011 to October 2012 was used, which also coincides with the NPP_{AG} estimates, allowing a total NPP assessment. We assumed quasi-equilibrium conditions on an annual timescale, i.e. no net accumulation or loss of belowground carbon. The use of only one year of data was mainly because we cannot assume multiyear steady state conditions in the coastal site, due to different amounts of litterfall in the two studied years (Chapter 4), and warmer and dryer conditions during the second summer. Further details on this methodology can be found in Chapter 5.

3.5. Relationships between stem radial change and environmental factors on an intra-annual basis using high precision dendrometer data

We installed automatic point dendrometers (Ecomatik, Germany) at DBH in five trees from each study area to monitor tree-radial changes and assess the relationship between environmental factors and tree radial contraction and increment (Figure 3.10). Trees were chosen from the dominant cohort, and they were healthy individuals located relatively close to each other (at the most 30 m apart).

We installed a soil temperature sensor close to the monitored trees and data from this sensor and the weather station in each site were used to correlate with stem radial change. All data were recorded every 30 minutes and hourly means were calculated.

Figure 3.10. Automatic point dendrometers installed in *Fitzroya* trees from the Coastal (left) and Andean sites (right, Photos: Aldo Farías and Yadvinder Malhi).

In order to analyse the dendrometer data as best as possible and with the most appropriate methodology according to the literature available, I did an internship at the Laboratoire d'écologie végétale et animale at the University of Quebec, Chicoutimi during July and August 2013. During the internship I was able to analyse my data using a specific routine created for this purpose (Deslauriers et al. 2011) and to discuss my data and results with the developers of this routine who have worked on this topic (dendrometers) for years. These researchers are the co-authors of my article.

More details on the general methodology for this objective are provided in Chapter 6.

3.6. Trends in tree-ring width and stable carbon isotope chronologies and their relationship with environmental factors

I used tree-ring width chronologies to assess any changes in tree growth and basal area increment (as a proxy of productivity) during past decades in each study site. Carbon isotope chronologies were used to evaluate any changes in discrimination and/or intrinsic water use efficiency that could reveal potential forest responses to environmental changes. I related both chronology types with climate variables and with changes in these variables during recent decades in each study area. Climate records used for this purpose were the longest, most complete and reliable ones close to each study area: Valdivia station (Isla Teja, 9 m a.s.l at 39° 48’ S, 73° 14’ W) close to Alerce Costero and Puerto Montt station (Tepual airport, 85 m a.s.l at 41° 25’ S, 73° 05’ W) close to Alerce Andino.

3.6.1. Tree-ring width chronologies

We collected two increment cores per tree from the largest and presumably oldest trees using a 5 mm diameter increment borer at around DBH in each study area (60 trees in each site). Collections were made in December 2011 and 2012. These cores were sanded and the tree rings measured to the 0.001 mm level. Cross dating of the samples was visual and the Schulman convention for the Southern Hemisphere (Schulman 1956), which assigns to each tree ring the date of the year when radial growth started, was adopted (Figure 3.11). Cross dating was later verified using the computer program COFECHA (Holmes 1983), which calculates correlations between tree-ring width series as a way of identifying absent or false rings and verifying cross dating errors.

Ring width measurements of each tree-ring sample were subsequently standardized using conventional methods in dendrochronology that fit a negative exponential curve or a linear regression to the series. The goal of this standardization was to remove variability that is not related to climate (e.g. due to tree age, Fritts 1976) and I carried it out using the ARSTAN44h2 software (Cook & Krusic 2013). In the standardization process the measured ring width in a particular year is divided by the value estimated by the adjusted curve, so the standardized series has a mean of one (1) and a relatively stable variance throughout its length (Fritts 1976). The quality of the generated mean chronology (mean of samples) was assessed using methods referred to in Chapter 7.

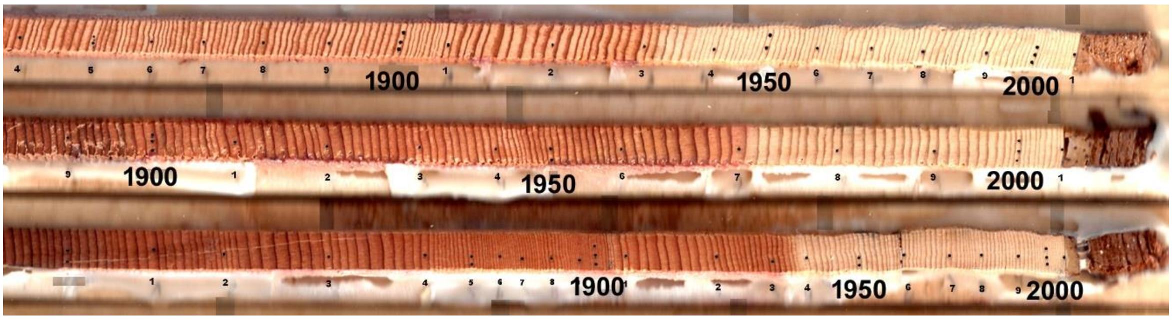

Figure 3.11. Tree-ring cores from *Fitzroya* from the Andean site showing visual cross dating mainly since the 1900s. For dating purposes, one dot indicates a decade, two dots 50 years and three dots 100 years. The outermost (most recent) ring is on the right.

I assessed changes in tree radial growth during the last century using the standard (detrended without autoregressive modelling) tree-ring width chronologies. In addition, basal area increments (BAI) were calculated for each tree and averaged to get a mean basal area chronology for each site. BAI is the yearly increase of a tree’s cross-sectional area, differing from radial growth increment in that it provides a two dimensional measure of the amount of wood added to the stem.

3.6.2. Carbon isotope chronologies

Five cores from different trees, dated using the tree-ring width chronologies from each site, were selected for isotopic sampling. Annual rings from these cores for the last 210 years (1800-2010) were sampled and pooled in order to determine trends in $\delta^{13}C$, discrimination (Δ), and intrinsic water use efficiency (iWUE).

Purified cellulose is a standard material for stable carbon isotope analyses (Taylor et al. 2008), mainly because different wood constituents have different isotopic ratios and variation in the content of these components across annual rings could potentially deteriorate the climate signal if whole wood is used for example (Rinne et al. 2005). However, it is very time consuming to extract cellulose and enough wood material is not always available for cellulose extraction. In order to test the relationship between carbon isotope values from whole wood, whole wood without extractives and alpha cellulose (the most stable cellulose) in *Fitzroya*, annual rings from ten tree-ring cores from the coastal site and for different periods were sampled and pooled. The periods analysed were 1851-1860, 1901-1910 and 1980-2010. These three periods were chosen to determine differences in the carbon isotopic signal in different decades along the studied 200 years. A longer period was used for the last three decades in order to perform and compare correlations with climate. Samples were ground to 40 mesh using a mill and resin extraction was carried out in a soxhlet extractor. Samples were run in a 2:1 toluene/ethanol mixture for 16 hrs, then they were dried for 15 hrs and run again in 100% ethanol for another 16 hrs. They were dried for another 15 hrs and finally run in boiling water for 8 hrs, according to the standards presented by Leavitt & Danzer (1993). The cellulose extraction was performed at the Tree-ring Laboratory at University of Arizona, since at the time of the analyses there were no facilities in Chile to perform this procedure on small samples. The isotopic analyses were carried out afterwards at the Laboratorio de

Biogeoquímica de Isótopos Estables at the Instituto Andaluz de Ciencias de la Tierra (CSIC-University of Granada).

Despite the great care placed on performing these tasks, there was an unintended and unfortunate contamination of the wood samples during grinding (which was not realized until later), and this prevents trusting these testing results. Therefore, they will not be presented in the thesis.

Due to costs, time and laboratory constraints, the isotopic analyses were performed using wood without extractives. Details on the processing of tree-ring width and isotope chronologies, as well as on the main analyses performed to determine trends and relate these chronologies with climate are presented in Chapter 7.

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Linking statement: Chapter 3 to 4

I start my empirical chapters by assessing the forest structure, aboveground biomass and primary productivity over one year in *Fitzroya* forests from my two study sites. This chapter addresses the first two specific objectives: **to assess the forest structure, species composition and characterise the environmental conditions associated with *Fitzroya* forests growing in the Coastal Range and the Andean Cordillera of southern Chile,** and **to assess biomass, aboveground net primary productivity (NPP_{AG}) and estimate carbon allocation and mean wood residence time in these two sites.** In addition, and together with information provided in Chapter 5, this chapter addresses the fourth specific objective: **to provide the first estimate of total NPP for *Fitzroya* forests in southern Chile using biometric estimates of aboveground components and indirect estimates of belowground productivity**. Thus, this and the next chapter provide a comprehensive assessment of the main components of the carbon cycle in these forests. I compare the carbon dynamics in these two sites placing the main findings within the context of other studies in temperate ecosystems.

I was the main author and wrote the paper, organised and conducted most of fieldwork with the help of research assistants and performed all the data analyses. Among the co-authors, Yadvinder Malhi provided the main supervision, advising on the study design and data analysis of the paper. Antonio Lara helped with the ecological interpretation of the data and contributed to the paper. The manuscript was submitted to *Plos One* in January 2015 and I was invited to submit a revised version, so I will evaluate the comments and prepare a response as required.

Chapter 4: The oldest, slowest rainforests in the world? Massive biomass and slow carbon dynamics of *Fitzroya cupressoides* temperate forests in southern Chile

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Abstract

The endangered *Fitzroya cupressoides* forests of southern South America include stands that are probably the oldest dense forest stands in the world in terms of mean tree age, with long-lived trees and high standing biomass. We assess and compare aboveground biomass, and provide the first estimates of net primary productivity (NPP), carbon allocation and mean wood residence time in medium-age stands in the Alerce Costero National Park (AC) in the Coastal Range and in old-growth forests in the Alerce Andino National Park (AA) in the Andean Cordillera. Aboveground live biomass was 113-114 Mg C ha^{-1} and 448-517 Mg C ha^{-1} in AC and AA, respectively. Aboveground productivity was 3.35-3.36 Mg C ha^{-1} year^{-1} in AC and 2.22-2.54 Mg C ha^{-1} year^{-1} in AA, values generally lower than others reported for temperate wet forests worldwide, mainly due to the low woody growth of *Fitzroya* in both sites. NPP was 4.21-4.24 and 3.78-4.10 Mg C ha^{-1} year^{-1} in AC and AA, respectively. Estimated mean wood residence time was a minimum of 1368-1393 years for *Fitzroya* in the Andes. Our biomass estimates for the Andean site place these ecosystems among the most massive forests in the world. Differences in carbon production between sites seem mostly apparent as differences in allocation rather than productivity. Residence time estimates for *Fitzroya* are the highest reported for any species and carbon dynamics in these forests are the slowest reported for wet forests worldwide. Although primary productivity is low in *Fitzroya* forests, they probably act as ongoing biomass carbon sinks on long-term timescales due to their low mortality rates and exceptionally long residence times that allow biomass to be accumulated for millennia.

4.1. Introduction

Forest biomass is driven by the long-term balance between growth rate and mortality [1]. The conservation of large stocks of biomass in undisturbed forests avoids significant carbon emissions to the atmosphere, so biomass quantification in primary forests is crucial under current climate change policy debates [2]. Net primary productivity (NPP) corresponds to the total amount of organic matter produced per unit time and is an important component of the global carbon cycle [3]. Changes in climate and atmospheric composition are likely to induce changes in NPP, therefore it is important to understand the magnitude, drivers and allocation of NPP in ecosystems [3].

A number of studies have assessed forest NPP, or more frequently aboveground NPP (NPP_{AG}), especially in temperate and boreal ecosystems from the Northern Hemisphere (e.g. [4-6]) and in tropical systems (see [7] for a compilation). However, very few studies have examined productivity in southern hemisphere temperate forests, such as those in southern South America [8-10].

One of the most compelling and least well-understood tree species of the southern hemisphere is *Fitzroya cupressoides* (Molina) I.M. Johnst. (Cupressacea) or alerce, an evergreen conifer from southern South America that may reach 5 m in diameter and 50 m in height, and can live more than 3600 years [11,12]. This is the second longest-lived tree recorded in the world [12] and the longest-lived tree that forms dense, tall stands. *Fitzroya* is endemic to the temperate rainforests of Chile and Argentina and has a disjunct distribution between 39° 50' and 43° S along three distinctive areas: the Coastal Range of Chile, the Andean Range of Chile and adjacent Argentina and locally in the Chilean Central Depression at ~41° S [13].

Research on the biomass and productivity of *Fitzroya* has previously been conducted only in the southern portion of the Coastal Range at Chiloé Island [9,10].

Reported values of aboveground NPP there varied between 4.6 and 6.9 Mg dry biomass ha^{-1} $year^{-1}$, with large differences in terms of wood productivity; between 2.7 and 5.1 Mg ha^{-1} $year^{-1}$ in the studies of [10] and [9], respectively. No studies have examined the carbon budget of the much older and higher biomass *Fitzroya* forests of the Andes, where year-long research is especially challenging because of difficult accessibility, adverse weather conditions and the high costs of sampling remote forests.

Because of the slow growth rates but long lifetime of *Fitzroya*, and its endemic and endangered status, it is interesting and important to assess the current ecological condition of these forests, focusing on their primary production. These forests are unique in terms of their longevity, so studies in these ancient temperate forests can potentially provide more general insights into the functioning and carbon cycle of old growth temperate ecosystems. Moreover, studies on these forests can contribute to the quantification of regional and more accurate global carbon budgets, improving the representation of southern hemisphere ecosystems and particularly of southern South America forests in global carbon studies [6].

The aim of this research is to primarily describe the biomass carbon dynamics of *Fitzroya* forests of medium and very old age growing in contrasting sites in the northern portion of the Coastal Range and the Andean Cordillera of southern Chile, respectively. Specifically, we examine and compare (i) aboveground biomass; (ii) NPP_{AG} and its allocation between wood and canopy tissue; (iii) total NPP and its above- and belowground allocation and iv) woody biomass residence time and its variation between species. In order to provide general information about the studied forests in each area, we also characterize the forest structure and species composition in each site.

Questions we specifically address include:

1. How do stand structure and aboveground biomass vary between the two sites?
2. How do patterns of NPP and its allocation vary between the two sites?
3. How do the productivity and carbon dynamics of these forests compare to other temperate ecosystems worldwide?

4.2. Methods

4.2.1. Study sites

We worked with an endangered tree species within National Parks, so the Chilean National Forest Service "Corporación Nacional Forestal, CONAF" granted the permission to work and develop the following reported activities in *Fitzroya cupressoides* forests within the two involved National Parks. Two 0.6 ha plots (AC1 and AC2) were installed in the Alerce Costero National Park (AC) at 40° 10’ S- 73° 26’ W in July-August 2011 (Fig. 1). This Park is located on the Coastal Range and the mean altitude of both plots is 850 m.a.s.l. Two similar 0.6 ha plots (AA1 and AA2) were installed in the Alerce Andino National Park (AA) at 41° 32’ S- 72° 35’ W (~ 760 m a.s.l) in August-October 2011 (Fig. 1). These two Ranges contain *Fitzroya’s* main populations and forests are highly contrasting in terms of disturbance regime, development stage and prevailing environmental conditions. Data analysed correspond to one year from November 2011 to October 2012.

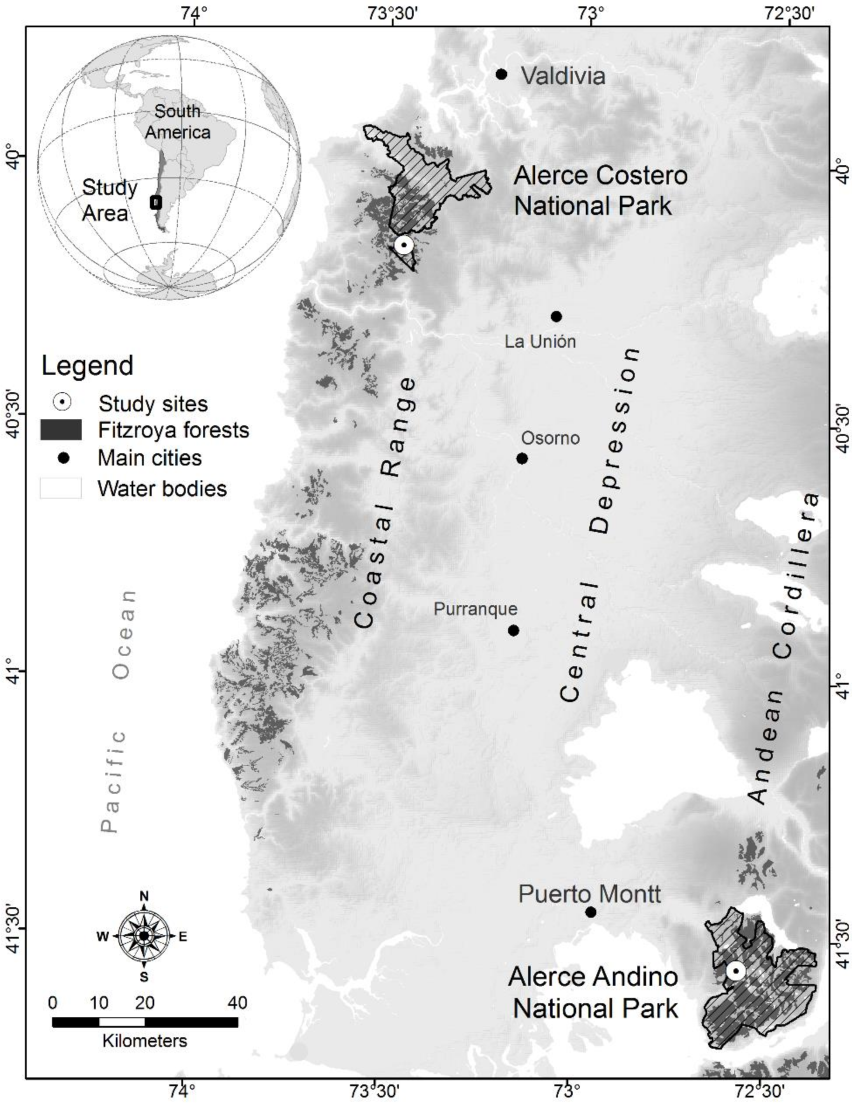

Fig. 1. Location map of the study sites. Location map of the study sites in southern Chile, indicating the distribution of *Fitzroya* forests north of 41° 45’ S. The map does not show stands located in the Central Depression near Puerto Montt due to their small area. Darker shaded areas correspond to higher altitudes in the Coastal Range and Andean Cordillera.

Fitzroya forests in the Coastal Range have developed following frequent low to mid-intensity fires and the landscape is commonly characterized by stands formed by living trees mixed with snags from older cohorts [14,15]. Most of fires in recent centuries have been caused by humans, although lightning may also be an influence [15]. Forests in the Coastal Range have also been affected by historical harvesting throughout the last few centuries, and by recent illegal cuttings since the end of the 1970s. In the Andes, *Fitzroya* forests are normally characterized by old growth even-aged stands with large trees, and have developed following large-scale disturbances such as volcanic ash deposition, lava flows and landslides [16,17].

The Coastal Range was not affected by Pleistocene glaciations and the soils originate from Pre-Cambrian to Paleozoic metamorphic rocks. Soils in this area are generally thin, poor in nutrients and severely podzolized [18]. Climate is characterized by high annual precipitation and a mild temperature range, and according to a non-automated rain gauge in the park, mean annual precipitation was 4180 mm during 1999-2010 [19]. The region has a Mediterranean climate influence, with approximately 47% of the annual precipitation occurring in winter (June to August) and ca. 9% during summer (December to February, [13, 18,19]). Fires have likely affected the study site during the last century (presence of fire scars in some trees and fire history reconstruction in the area, [15,20]).

The Andes at the study site latitude were extensively glaciated during the Pleistocene and most of the surfaces that were covered by ice at that time have been covered by recent andesitic volcanic deposits [11]. Soils where *Fitzroya* develops in the Andes are derived from volcanic parental materials, contain high organic matter, have high C/N ratios and low pH [21,22]. Climate conditions have not previously been described for the study area, but a nearby station at 240 m a.s.l (Lago Chapo ~18 km northeast of the study site) recorded a mean annual temperature of 10.3 °C and 4140 mm of annual precipitation [12].

4.2.2. Meteorological data

Automatic weather stations (Skye instruments, Powys, UK) were installed in clearings less than 1 km from the study plots in both areas. These stations recorded precipitation, relative humidity, temperature and total solar radiation every 30 minutes. Soil temperature was recorded with a Decagon sensor EC-T (Pullman, WA, USA) installed at a single point at 10 cm depth within the plots AC1 and AA1.

4.2.3. Soil characterization

After testing soil conditions using a steel soil sampler, two and three soil pits were excavated in AC1 and AC2 plots, respectively for physical and chemical characterization. In the Andes two soil pits were excavated in each plot. Chemical analyses were done separately for each surface horizon, where most of the roots develop. Analyses included the main macro- and micronutrients and were performed at the Laboratorio de Suelos Forestales at Universidad Austral de Chile.

4.2.4. Biomass and NPP_{AG} measurements

The main measurements in each 0.6 ha (60x100 m) plot were taken following and adapting the RAINFOR-GEM network protocol ([23], http://gem.tropicalforests.ox.ac.uk), where plots were subdivided in 15 subplots of 20x20 m.

Every tree ≥ 10 cm diameter at breast height (DBH, 1.3 m) was censused and tagged and its DBH measured. In order to have an estimate of *Fitzroya* woody biomass specifically for each study site, a volume equation was developed for each area. Volume calculated for each tree, multiplied by a biomass expansion factor (BEF) developed for the species, multiplied by the species wood basic density, provided the estimate of *Fitzroya* woody biomass including stem and branches. For the other tree species, biomass equations already available for Chile were used [24-26]. When a biomass equation did not exist for certain species, another one from a species of the same family or with similar structural characteristics was used.

In order to have an estimate of woody biomass for trees < 10 cm DBH, two 10x10 m plots were established within each plot and small trees (2-10 cm DBH) were censused using callipers. The same equations mentioned above were used to estimate biomass.

Aboveground coarse wood productivity (NPP_{ACW}) was assessed by determining the growth rate of existing surviving trees considering trees ≥ 10 cm DBH, as well as the smallest tree component (trees < 10 cm DBH) in the small plots scaled up to the hectare. A key difference from the normal protocol was that the slow growth rates and large tree diameters in *Fitzroya* made it difficult to measure annual growth by conventional forest inventories or even manual dendrometers. Instead we analysed tree rings to determine annual growth.

The estimate of the growth rate (mean growth for the last five years) was done by collecting two tree-ring cores from 15 *Fitzroya* trees per plot and from three trees per diameter class of the other species per plot. This sampling was restricted mainly because the plots are within National Parks and the National Forest Service (CONAF) established a maximum number of samples to extract in the associated permit. The mean growth rate per species was extrapolated to the trees that were not sampled. Tree-radial growth was added to diameter measurements and the new woody biomass was estimated with the allometric equations mentioned above. The difference in biomass corresponds to the annual woody productivity.

Branch turnover productivity (NPP$_{branch\ turnover}$) includes all woody material ≥ 2 cm diameter and was assessed by conducting censuses of fallen branches from live trees along two parallel 1x100 m transects in each plot, every three months.

Canopy productivity (NPP$_{litterfall}$) was estimated collecting litterfall at a monthly basis in fifteen 0.25 m^2 (50 cm x 50 cm) litterfall traps located approximately at the center of every 20x20 m subplot and placed at 1 m height above the ground. Litterfall from the understory was also collected in each site.

Further details on the overall methodology are provided in Methods in S1 File.

4.2.5. Total biomass and productivity

Total aboveground productivity was calculated for the period November 2011-October 2012, by summing the above-mentioned components:

$$NPP_{AG} = NPP_{ACW} + NPP_{branchturnover} + NPP_{litterfall} \qquad (1)$$

In a parallel paper, [27] estimated fine root productivity (NPP$_{fine\ root}$) from a mass conservation approach balancing soil heterotrophic respiration with organic matter inputs (carbon inputs to the soil were assumed to be equal to carbon outputs, including any change in carbon stocks, [28,29]). This was applied at whole site level rather than plot level using the following equation:

$$NPP_{fineroot} = R_h - Litterfall - (Mort_{AG} + NPP_{branchfall}) * F_{CWD} - Mort_{BG} + F_{DOC} + \Delta C \quad (2)$$

Where:

R_h: soil heterotrophic respiration for the period November 2011-October 2012.

Litterfall: mean annual amount of litterfall collected in both plots from each site.

$Mort_{AG}$: mean aboveground mortality.

$NPP_{branchfall}$: mean productivity associated to this component

F_{cwd}: fraction of coarse woody debris (CWD) entering the soil (not lost through in situ respiration).

$Mort_{BG}$: belowground mortality which totally enters the soil.

F_{DOC}: aqueous carbon leakage.

ΔC: net change in carbon stocks.

F_{cwd} has been rarely measured, [28] assumed a value of 0.25± 0.25 in a temperate broadleaved woodland in the UK, with a large (100%) uncertainty. Given the lack of information, the value adopted by [28] was assumed. $Mort_{BG}$ was determined as a percentage of aboveground mortality (percentage equivalent to the coarse root/aboveground biomass ratio found in *Fitzroya* forests from Chiloé Island, (6.9%, [30]). This is the only estimate of this ratio available for these forests and we applied a conservative uncertainty estimate of ±75% for this parameter. F_{DOC} and ΔC were assumed to be zero or insignificant terms on an annual basis. Values for the other parameters can be found in S1 Table.

In addition, we estimated coarse root NPP ($NPP_{coarse\ root}$) as a fixed fraction of NPP_{ACW}, using the coarse root/aboveground biomass ratio found in *Fitzroya* forests from Chiloé (6.9%, [30]). Total NPP was calculated as the sum of NPP_{AG} plus the belowground components.

All estimated productivity values are reported in Mg of carbon (C) ha^{-1} $year^{-1}$ (biomass values are reported in Mg C ha^{-1}). To convert dry biomass into carbon, the mean carbon content of aboveground biomass in Chilean temperate species (49.64%) was used [31]. All reported errors consider ±1 SE.

Error propagation was carried out using standard rules of quadrature, assuming that uncertainties are independent and normally distributed (e.g. [32]).

4.2.6. Mean wood residence time

This variable, an indicator of the mean time woody carbon remains in a system, was calculated using the most common approach as the ratio of mean standing woody biomass and mean woody productivity obtained through allometric equations [33]. In order to include only the main tree component, woody biomass and productivity included trees ≥ 10 cm DBH. This estimate must be considered an approximation, since this approach is valid just for systems where standing biomass stocks are near equilibrium. For aggrading systems residence time tends to be underestimated by this method; hence the calculated value is a minimum estimate for residence time.

4.3. Results

4.3.1. Climate and soil characterization

Precipitation was high year-round and moderately seasonal; total annual precipitation was 4450 mm in AC and ~6300 mm in AA (November 2011-October 2012). Mean annual air temperature was similar between sites, although lower in the Andes (7.5 ˚C and ~ 7.1 ˚C in AC and AA, respectively, S1 Fig.).

Soils are sandy loam and silty loam in AC and AA, respectively. Soils in AC are shallower and much poorer in nutrients (especially nitrogen and cations) than soils in AA. The C/N ratio is high in both sites, indicating a low decomposition rate leading to carbon accumulation. Finally, soils in both sites have a low pH and high exchangeable aluminium content (S2 Table). This feature indicates high toxicity for the roots, especially in AC, where the high aluminium content makes the limited nutrients even less available for the roots.

More details on climate and soil characteristics are presented in Results in S1 File.

Question 1. How do stand structure and aboveground biomass vary between the two sites?

4.3.2. Forest composition and structure

Tree density (trees ≥ 10 cm DBH) was 1415 and 1408 stems ha^{-1} and basal area was 89 and 87 m^2 ha^{-1} in AC1 and AC2, respectively. *Fitzroya* constituted around 85% of the tree stems in each plot and 93% and 94% of the total basal area in AC1 (83 m^2 ha^{-1}) and AC2 (82 m^2 ha^{-1}), respectively. The biggest trees were *Fitzroya,* which reached up to 86 cm DBH. The mean height of the canopy *Fitzroya* trees (≥30 cm diameter) was 14.4 m (±1.14) in AC1 and 14.1 m (±1.86) in AC2, with the maximum recorded height being 17.6 m in AC2. Stem volume for this species was 446 and 443 m^3 ha^{-1} in AC1 and AC2, respectively (see volume equations in S3 Table). The largest proportion of basal area in both plots was present in the 30-40 cm diameter class (S2 Fig.).

The second most abundant species in both AC plots was the evergreen *Drimys winteri* (Winteraceae). Two species from the *Nothofagus* genus (*Nothofagus nitida* and *Nothofagus betuloides*, Nothofagaceae) that frequently hybridize [34] were especially common in AC2. Other evergreen broadleaved tree species such as *Weinmannia trichosperma* (Cunoniaceae), *Embothrium coccineum* (Proteaceae) and *Tepualia stipularis* (Myrtaceae), accompanied *Fitzroya*. The understory was dominated by *Chusquea montana* (Poaceae), small trees of *Embothrium coccineum,* and by other evergreen species, such as *Gaultheria mucronata* (Ericaceae), *Gaultheria insana*, *Ugni candollei* (Myrtaceae), *Desfontainia fulgens* (Columelliaceae) and the fern, *Blechnum magellanicum*

(Blechnaceae). *Philesia magellanica* (Philesiaceae) was a rather widespread epiphyte commonly growing on *Fitzroya* trunks.

At the Andean site, stem density (trees ≥ 10 cm DBH) was lower at 782 and 720 trees ha^{-1} in plots AA1 and AA2, respectively and *Fitzroya* accounted for just 12% and 13% of this total number. However, *Fitzroya* accounted for 79% and 81% of total basal area, which reached 171 and 193 m^{2} ha^{-1} in AA1 and AA2, respectively. The largest tree was 235 cm DBH in AA2. The mean heights of *Fitzroya* trees were 31.80 m (±4.31) and 30.85 m (±4.82) in AA1 and AA2, respectively, with the maximum height recorded being 45.7 m in AA1. Stem volume for this species reached 1474 and 1777 m^{3} ha^{-1} in AA1 and AA2, respectively. More than half of the trees were distributed in the smallest diameter class (10-20 cm DBH) and the contribution of each size class to basal area was more or less stable up to 70 cm and increased significantly from 100 cm DBH due to the presence of *Fitzroya* (S3 Fig.). A notable feature was the almost complete absence of *Fitzroya* trees < 80 cm DBH, their domination of size categories > 100 cm, and the presence of trees with extremely large diameters. This condition, coupled with its slow growth rate, indicate that there has not been any significant recruitment of *Fitzroya* for several centuries.

Fitzroya was mostly accompanied by evergreen broadleaved species of which the most abundant were *Nothofagus nitida* (Nothofagaceae) and *Myrceugenia chrysocarpa* (Myrtaceae). Other species included *Laureliopsis philippiana* (Monimiaceae), *Amomyrtus luma* (Myrtaceae) and two evergreen conifers *Podocarpus nubigenus* (Podocarpaceae) and *Saxegothaea conspicua* (Podocarpaceae). *Saxegothaea* was especially abundant in both plots. The understory was dominated by *C. montana, D. fulgens, B. magellanicum* and *P. magellanica.*

The number of small trees (2-10 cm DBH) reached 1800 and 3050 trees ha^{-1} in AC1 and AC2, respectively, a high number already considering the density of trees ≥10 cm DBH. The main tree species represented in both plots was *Drimys winteri*.
The number of trees < 10 cm DBH was 950 and 1600 trees ha^{-1} in AA1 and AA2, respectively. The main tree species represented in both plots was *Saxegothaea conspicua*.

4.3.3. Aboveground biomass

The total aboveground woody biomass was 107.0 and 105.8 Mg C ha^{-1} in AC1 and AC2 respectively, with 3.77 % and 5.49 % of this in trees < 10 cm DBH. Total biomass was much greater in the Andes (423.1 and 488.5 Mg C ha^{-1} in AA1 and AA2 respectively), with trees < 10 cm DBH only accounting for 0.64 and 0.45 % (Table 1, Fig. 2). *Fitzroya* dominated biomass in both study sites with more than 70% of the total biomass. *D.winteri* and *Nothofagus spp.* were the second most important in biomass in AC1 and AC2, respectively. In the Andes, *N. nitida* was the second most important (Fig. 2).

Table 1. Aboveground biomass and productivity per plot. Total woody, canopy and understory biomass, yearly aboveground coarse wood productivity (NPP$_{ACW}$), canopy productivity (NPP$_{litterfall}$), branch turnover productivity (NPP$_{branch\ turnover}$) and total aboveground productivity (NPP$_{AG}$) for one year of data for Alerce Costero (AC1 and AC2) and Alerce Andino plots (AA1 and AA2). Estimates of fine root productivity (NPP$_{fine\ root}$) from [27], coarse root productivity (NPP$_{coarse\ root}$) and total NPP calculated in this study are also shown. SE is standard error of the mean

	Alerce Costero (AC)				**Alerce Andino (AA)**			
	AC1		**AC2**		**AA1**		**AA2**	
Component	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Woody biomass (≥ 10 cm) (Mg C ha^{-1})	103.0	10.3	100.0	10.0	420.4	42.0	486.3	48.6
Woody biomass (< 10 cm) (Mg C ha^{-1})	4.0	0.4	5.8	0.6	2.7	0.3	2.2	0.2
Canopy biomass (≥ 10 cm) (Mg C ha^{-1})	6.2	0.6	6.0	0.6	23.9	2.4	28.2	2.8
Canopy biomass (< 10 cm) (Mg C ha^{-1})	0.18	0.02	0.25	0.03	0.13	0.01	0.09	0.01
Understory biomass (Mg C ha^{-1})	0.70	0.15	0.70	0.15	0.35	0.13	0.35	0.13
Total biomass (Mg C ha^{-1})	**114.1**	10.3	**112.8**	10.0	**447.5**	42.1	**517.1**	48.7
NPP$_{ACW}$ (≥ 10 cm) (Mg C ha^{-1} year^{-1})	0.62	0.12	0.61	0.12	0.78	0.16	0.76	0.15
NPP$_{ACW}$ (< 10 cm) (Mg C ha^{-1} year^{-1})	0.19	0.04	0.39	0.08	0.12	0.02	0.14	0.03
Total NPP$_{ACW}$ (Mg C ha^{-1} year^{-1})	0.81	0.13	1.00	0.14	0.90	0.16	0.90	0.15
NPP$_{branch\ turnover}$ (Mg C ha^{-1} year^{-1})	0.039	0.01	0.038	0.01	0.15	0.05	0.40	0.22
NPP$_{litterfall}$ (Mg C ha^{-1} year^{-1})	2.27	0.33	2.16	0.31	1.09	0.06	1.16	0.09
NPP$_{litterfall\ understory}$ (Mg C ha^{-1} year^{-1})	0.23	0.07	0.16	0.02	0.08	0.02	0.08	0.02
Total NPP$_{AG}$ (Mg C ha^{-1} year^{-1})	**3.35**	0.36	**3.36**	0.34	**2.22**	0.18	**2.54**	0.28
Total NPP$_{fine\ root}$ (Mg C ha^{-1} year^{-1})	0.81	0.60	0.81	0.60	1.50	0.42	1.50	0.42
Total NPP$_{coarse\ root}$ (Mg C ha^{-1} year^{-1})	0.06	0.04	0.07	0.05	0.06	0.05	0.06	0.05
Total NPP (Mg C ha^{-1} year^{-1})	**4.21**	0.70	**4.24**	0.69	**3.78**	0.46	**4.10**	0.51

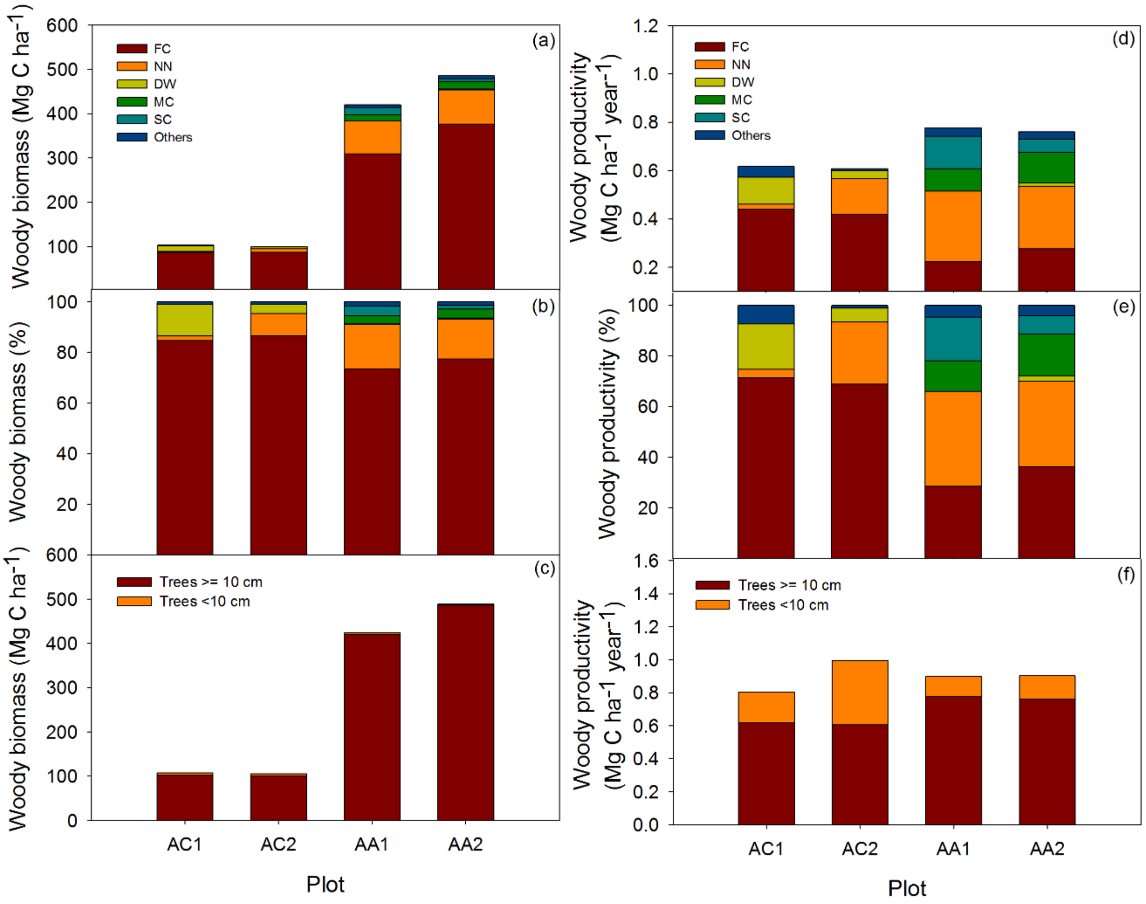

Fig. 2. Aboveground woody biomass and productivity per species and plot. Left panel: a) Aboveground woody biomass per species in all trees ≥10 cm diameter, b) proportion of the woody biomass presented in a) contributed by the different species in percentage (%) and c) woody biomass contributed by the large (trees≥10 cm DBH) and small (trees < 10 cm DBH) tree components within each plot. Right panel: d), e), f) the same as a), b) and c), respectively, but for aboveground woody productivity. FC: *Fitzroya cupressoides*, NN: *Nothofagus nitida*, DW: *Drimys winteri*, MC: *Myrceugenia chrysocarpa*, SC: *Saxegothaea conspicua*.

Most of the woody biomass in AC was mainly distributed between 30 and 50 cm DBH (S4 Fig.). *Fitzroya* was dominant throughout the diameter distribution in AC, except below 10 cm DBH, where *D.winteri* dominated biomass. In AA, *Fitzroya* entirely accounted for the very large diameter classes, while *N.nitida* mostly contributed to biomass between 30 and 90 cm, and *S.conspicua* and *M.chrysocarpa* at lower diameter classes (Fig. 3).

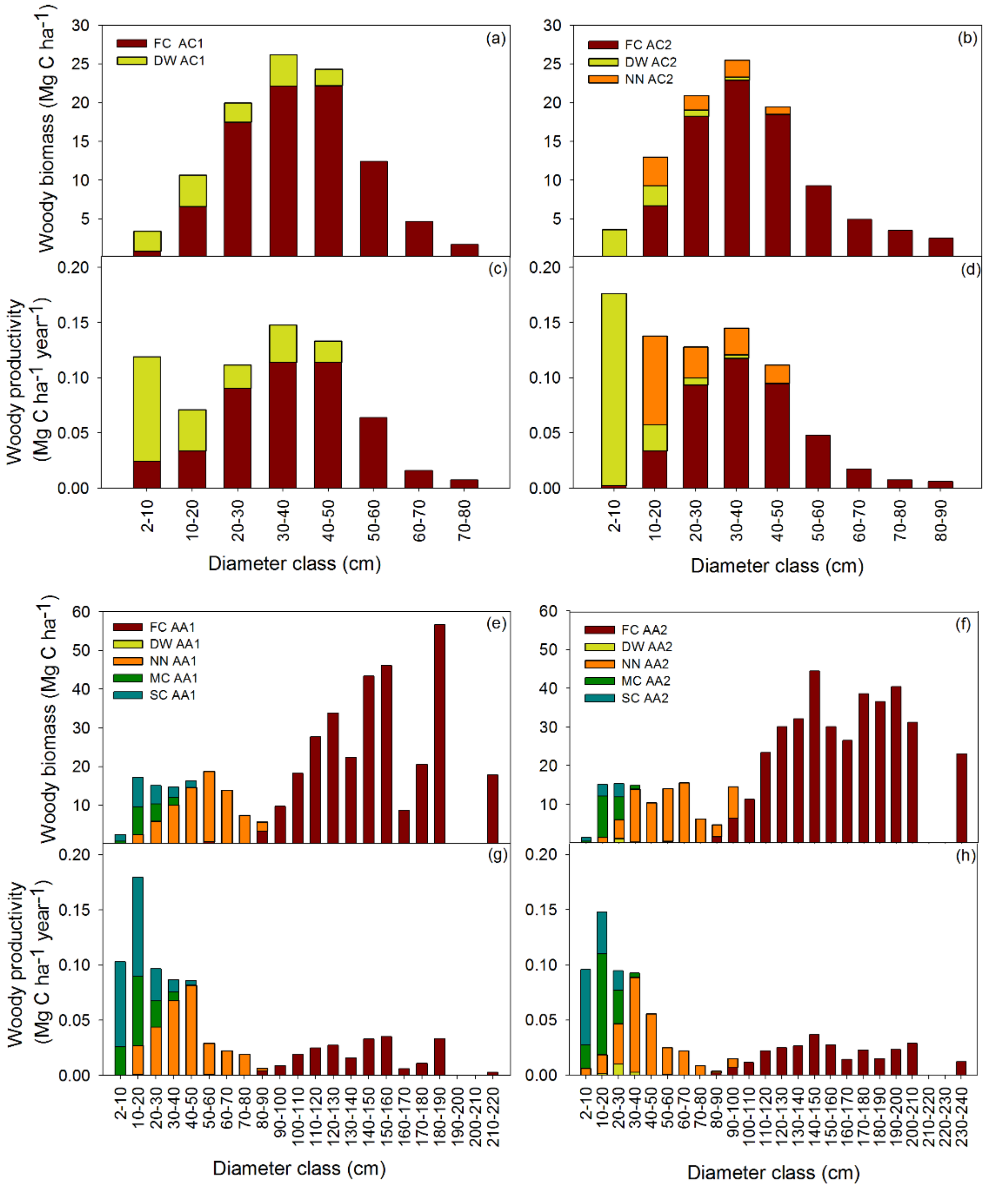

Fig. 3. Aboveground woody biomass and productivity per species and diameter classes. a), b), e), f) aboveground woody biomass for each of the most important species along different diameter classes in Alerce Costero and Alerce Andino plots. c), d), g), h) the same as in a), b), e), f) respectively, but for aboveground woody productivity. FC: *Fitzroya cupressoides*, NN: *Nothofagus nitida*, DW: *Drimys winteri*, MC: *Myrceugenia chrysocarpa*, SC: *Saxegothaea conspicua*.

Total canopy biomass reached 6.4 and 6.3 Mg C ha^{-1} in AC1 and AC2, respectively. 2.81 and 4.01% of this amount corresponded to trees < 10 cm (Table 1). Estimated canopy biomass reached 24.0 and 28.3 Mg C ha^{-1} in AA1 and AA2, respectively. 0.54 and 0.33% of this value corresponded to trees < 10 cm (Table 1).

Total aboveground biomass was 114.1 and 112.8 Mg C ha^{-1} in AC1 and AC2, respectively, while total biomass in AA reached 447.5 and 517.1 Mg C ha $^{-1}$ (Table 1). Total biomass from the understory was 0.61-0.62% and 0.07-0.08% of these values in AC and AA, respectively.

Question 2. How do patterns of NPP and its allocation vary between the two sites?

4.3.4. Aboveground productivity

Total NPP_{ACW} was 0.81 and 1.00 Mg C ha^{-1} $year^{-1}$ in AC1 and AC2, respectively, with 23 and 39 % accounted for by trees < 10 cm DBH. In the Andean site, total aboveground woody productivity was 0.90 Mg C ha^{-1} $year^{-1}$ in both plots, with 13 and 16% accounted for by small trees < 10 cm DBH (Table 1, Fig. 2). In AC there was no recorded mortality (trees ≥ 10 cm DBH) in the census interval and in AA1 and AA2 respectively, two and four non-*Fitzroya* trees ≥ 10 cm DBH died (0.20 %/0.09 % of woody biomass, S5 Fig.). *Fitzroya* was the species that, among trees ≥10 cm DBH, mostly contributed to productivity in AC and was the second most important after *N.nitida* in AA1 (Fig. 2).

An important proportion of total woody productivity appeared concentrated in the smallest tree component (< 10 cm DBH) especially in AC2 (Fig. 3 and S4 Fig.). In larger diameter classes, most of productivity concentrated in the 30-50 cm diameter class in AC and in the 10-20 cm diameter class in AA (Fig. 3 and S4 Fig.). In AC most of woody productivity in the smallest diameter classes (< 20 cm DBH) can be attributed to *D.winteri* and *N.nitida*. In AA most of productivity below 20 cm can be attributed to *S. conspicua* and *M.chrysocarpa* and to *N.nitida* between 30 and 90 cm. *Fitzroya* is the sole contributor above 100 cm DBH (Fig. 3).

Total annual branchfall ($NPP_{branch\ turnover}$) was almost the same in AC1 and AC2 and higher in AA, with more than double in AA2 than in AA1 (Table 1). Most of the branches, at least in the Andean site, fell during the winter period, probably because of windstorms or snow loads on branches (June-August; S6 Fig.).

Mean annual fine litterfall ($NPP_{litterfall}$ above 1 m) for the studied period was 2.27 ±0.33 and 2.16 ± 0.31 Mg C ha^{-1} year^{-1} in AC1 and AC2, respectively (Table 1). Litterfall reached 1.09 ± 0.06 and 1.16 ± 0.09 Mg C ha^{-1} year^{-1} in AA1 and AA2, respectively (Table 1). Litterfall from the understory (below 1.0 m) added an additional 10.1% and 7.4% to annual litterfall from vegetation above 1 m in AC1 and AC2, respectively. These values were 7.3% and 6.9% in AA1 and AA2, respectively (Table 1). For more details about litterfall seasonality and production see Results in S1 File and S7 and S8 Figs.

4.3.5. Total NPP

NPP_{AG} was 3.35 ± 0.36 and 3.36± 0.34 Mg C ha^{-1} year^{-1} in AC1 and AC2 and 2.22 ± 0.18 and 2.54 ± 0.28 in AA1 and AA2, respectively (Table 1). Total NPP was 4.21±0.70 and 4.24±0.69 Mg C ha^{-1} year^{-1} in AC1 and AC2 and 3.78±0.46 and 4.10±0.51 Mg C ha^{-1} year^{-1} in AA1 and AA2, respectively (Table 1).

In the AC sites, on average 24% of NPP was allocated to woody production (including coarse roots), 19% to fine roots, and 57% to canopy. In the AA sites, 31% of NPP was allocated to woody production (including coarse roots), 38% to fine roots, and 31% to canopy (Fig. 4). Hence, total NPP was somewhat similar in both sites, but in the younger site, more was allocated to canopy production and less to fine roots, with woody allocation being closer in both sites (Fig. 5).

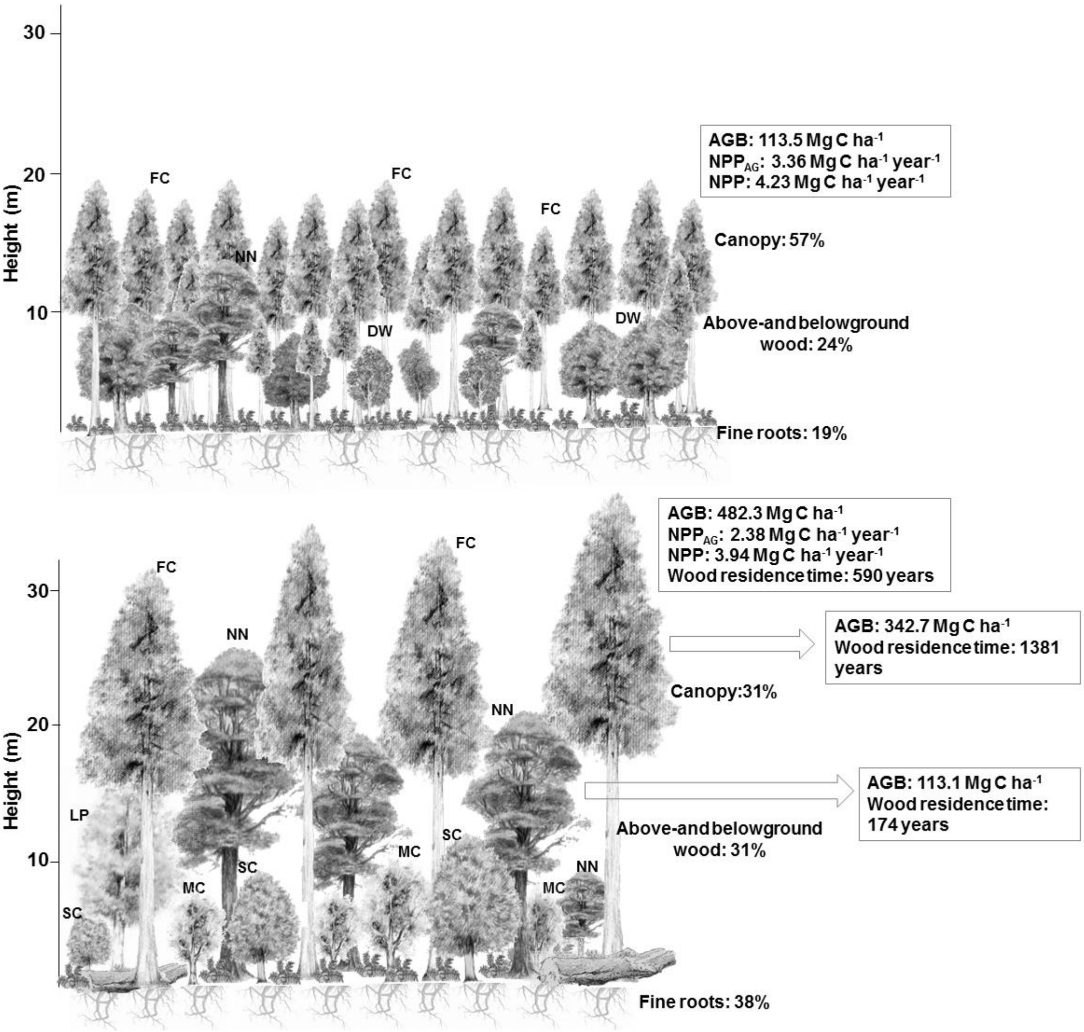

Fig. 4. Forest structure and carbon dynamics in Alerce Costero and Andino. Diagram exemplifying the structure of the forest in the coastal (upper panel) and the Andean site (lower panel). The main species in each forest are identified, the mean values for each carbon cycle component from both plots and the productivity allocated to canopy, wood and fine roots (in %) are shown. Arrows indicate separated values for the *Fitzroya* stand only and the *Nothofagus* dominated subcanopy forests in AA. FC: *Fitzroya cupressoides*, NN: *Nothofagus nitida*, DW: *Drimys winteri*, LP: *Laureliopsis philippiana*, MC: *Myrceugenia chrysocarpa*, SC: *Saxegothaea conspicua*. AGB: aboveground biomass, NPP$_{AG}$: aboveground productivity, NPP: total productivity.

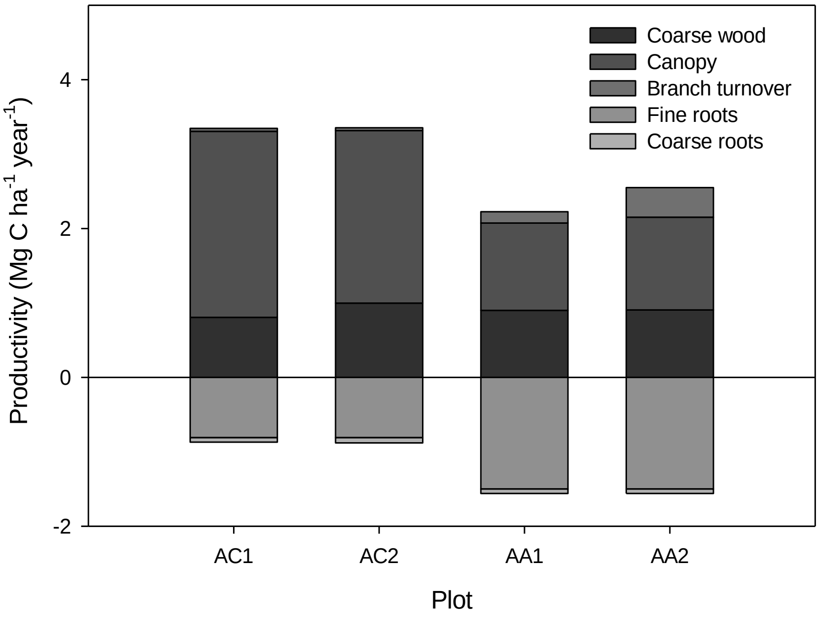

Fig. 5. Total productivity per plot. Total productivity in Mg C ha^{-1} year^{-1} for the one-year period November 2011-October 2012 in the four studied plots and its allocation to the different components. Belowground NPP is indicated as negative values.

Canopy productivity was higher than total aboveground wood productivity ($NPP_{ACW} + NPP_{branchturnover}$) in all plots, except AA2 (Table 1). However, branchfall is dominated by episodic events, and it seems likely that high branchfall in AA in one year is not representative of longer-term branch turnover in the old-growth forest.

4.3.6. Mean wood residence time

The mean wood residence time (for trees ≥ 10 cm DBH), calculated by dividing aboveground wood biomass by coarse wood productivity, was 166 ± 36 and 164 ± 36 years in AC1 and AC2, respectively. Estimates were 539 ± 123 and 640 ± 142 years in AA1 and AA2, respectively. When we considered the *Fitzroya* trees alone, they had mean residence time values of 198 ± 44 and 207 ± 46 years in AC1 and AC2 and of 1393 ± 311 and 1368 ± 306 years in AA1 and AA2, respectively. *N.nitida* stood out in the Andes as the species with the second longest residence time (average value of 277 ± 62 years, Fig. 6). The long residence times estimated for *Fitzroya* in AA are consistent with the minimum age of some trees in the stand (1200-1470 years old) derived from the tree ring data collected in the area.

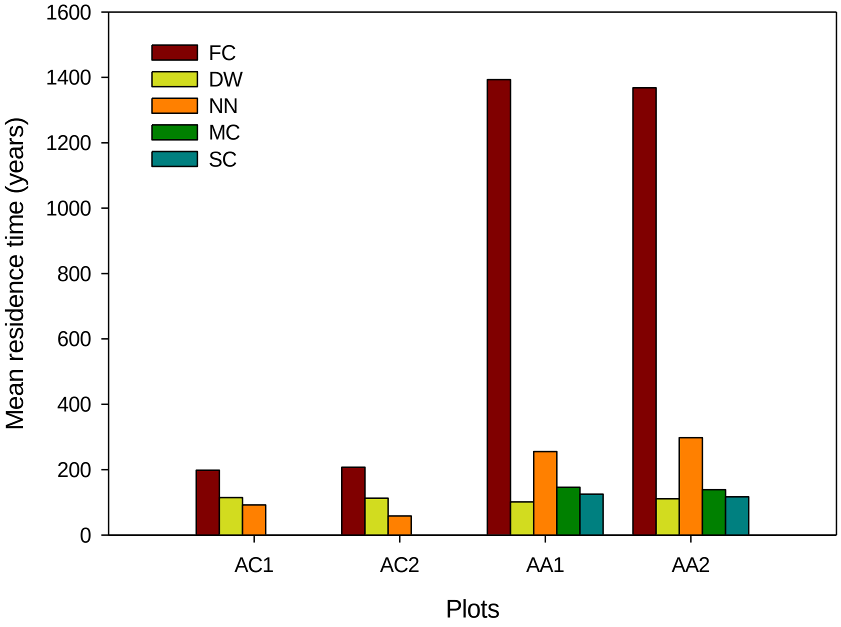

Fig. 6. Mean wood residence time per species and plot. Mean wood residence time for the main species in each plot. FC: *Fitzroya cupressoides*, NN: *Nothofagus nitida*, DW: *Drimys winteri*, MC: *Myrceugenia chrysocarpa*, SC: *Saxegothaea conspicua*.

Estimates of residence time are underestimates in the case of AC, as no mortality was recorded during the studied period, indicating that the forest stands have not reached quasi-equilibrium where mortality biomass loss equals woody biomass production. In AA there is not much difference between NPP_{ACW} and mortality, because of the large *Nothofagus* trees (20-64 cm DBH) that died during the interval (S5 Fig.). However, we recorded no mortality in the dominant *Fitzroya* in AA and hence this species, like the forest in AC, may still be experiencing long-term biomass accumulation. The Andean site appears characterised by a very old *Fitzroya* canopy that is still accumulating biomass, and a *Nothofagus*-dominated “sub-canopy” that is closer to dynamic equilibrium, with mortality balancing growth. In addition, trunks and snags of *Fitzroya* take many decades or even centuries to decompose. We did not observe any recently dead *Fitzroya* trunks at either AA plot, implying that there has been no mortality for probably several decades or centuries.

4.4. Discussion

Fitzroya forests in the Andes and in their northern distribution in the Coastal Range appear to be very different in terms of structure and species composition, but there has not been information until now on how different these forests can be in terms of biomass and productivity. This study provides for the first time combined estimates of aboveground biomass, NPP and carbon allocation in stands growing in these two areas.

4.4.1. Forest structure, biomass and productivity of *Fitzroya* forests in the study sites

Differences in forest structure between plots from the same site were slight, indicating the homogeneity of the studied forest sites. The difference in forest structure between AC and AA is mainly due to different disturbance regimes in both areas. Due to the approximate age of the oldest trees in AC (~ 300 years old) and the presence of some large diameter snags, it is plausible to assume that the studied forest was established after a fire occurred in 1681 [15,20]. *Fitzroya* regeneration in the Coastal Range has been reported to take place in open areas after stand devastating fires [15] and there are almost no particularly large trees and old-growth forests in the Coastal Range, due to fires and forest cuts since the 1500s [18]. In the Andes plots, *Fitzroya* is mostly present in large diameter classes and there is little regeneration as it is a relatively shade intolerant species [35]. In such a forest, the lack of small and medium *Fitzroya* trees indicates that most regeneration took place after a major disturbance (probably a landslide) over a thousand years ago. Similar distributions, characterized by an over-representation of large size-classes and restricted regeneration, have been reported for other shade-intolerant species mostly dependent on large-scale disturbance for recruitment [36]. Moreover, the high basal area of *Fitzroya*, its long-lived character and its pioneer and emergent status, suggests that these forests would be an example of the “additive basal area” phenomenon [36]. With their large diameters but relatively small crowns, they would exert only limited shade on the remainder of the forest below, acting essentially as giant slow-growing “poles” contributing large amounts of biomass, but having only moderate influence on light competition and ensuing effects on forest dynamics.

In AA, the *Nothofagus*-dominated forest below the *Fitzroya* canopy has a biomass of 112-114 Mg C ha^{-1}, woody productivity of 0.63-0.68 Mg C ha^{-1} year^{-1} and woody biomass residence times of 169 ± 38 - 178 ± 40 years. These values compare very well with biomass, productivity and residence times values found in evergreen *Nothofagus* forests in New Zealand (Table 2, 210 years of residence time, [37]) and with biomass values in montane mixed-broadleaf evergreen forests in Chiloé Island [10] and evergreen *Nothofagus* forests in Tierra del Fuego, Argentina ([38], Table 2). This would be consistent with the suggestion that the *Fitzroya* stand does not have a major influence in perturbing the productivity and dynamics of the *Nothofagus* forest that sits below. However, further studies on *Nothofagus* forests growing under similar conditions would be needed to draw firmer conclusions.

Table 2. Aboveground biomass and productivity in temperate forests worldwide. Estimates of aboveground biomass (AGB), aboveground coarse wood productivity (NPP$_{ACW}$), canopy productivity (NPP$_{litterfall}$) and total aboveground productivity (NPP$_{\mathrm{AG}}$) for different temperate forests worldwide. Values reported in Mg of dry biomass/productivity in the original studies were converted to Mg of C using the coefficient 0.5 in the case of studies in other countries and the same coefficient as in this study in the case of forests in Chile. NA indicates information not available

Forest[a]	**Site**	**AGB** (Mg C ha^{-1})	**NPP$_{ACW}$** (Mg C ha^{-1} year^{-1})	**NPP$_{litterfall}$** (Mg C ha^{-1} year^{-1})	**NPP$_{\mathrm{AG}}$** (Mg C ha^{-1} year^{-1})	**Reference**
Fc	Coastal Range, Chile (AC)	112.8-114.1	0.81-1	2.32-2.5	3.35-3.36	This study
Fc	Andean Cordillera, Chile (AA)	447.5-517.1	0.9	1.17-1.24	2.22-2.54	This study
Fc	Chiloé Island, Chile	285.9	2.53	0.89	3.42	[9,30]
Fc	Chiloé Island, Chile	268.4	1.34	0.94	2.28	[10]
Fc	Coastal Range, southern Chile	NA	NA	1.62	NA	[57]
Fc	Chiloé Island, Chile	NA	NA	1.01	NA	[63]
Mb	Chiloé Island, Chile	116.4	2.28-2.78	1.64-3.77	4.42-6.06	[9,10]
Ac	El Bolson, Argentina	78.4-99.9	1.05-1.4	1.7-2.3	2.95-3.6	[8]
Nb	Tierra del Fuego, Argentina	105-156	2.05-3.36	NA	NA	[38]
Aa	Trounson Forest Reserve, North Auckland	729	1.20	2.05	3.25	[54]
Ns	Craigieburn Range, New Zealand	122.6	0.59	2.03	2.62	[37]
Th	Western Coast Range, Oregon	746	2.0	4.0	6.5	[53]
Ps-Th	Western Coast Range, Oregon	355.4	4.15	1.1	5.25	[59]
Pm-Th	Oregon Cascades	781	NA	NA	NA	[52]
Ap-Pm	Oregon Cascades	440	4.9	1.6	6.5	[52]
Tm-Al-Pe	High Cascades summit, Oregon	NA	NA	NA	2.55	[60]
Pe-Al	Rocky Mountain National Park, Colorado	126.5	0.97	0.89	1.86	[61]
Ss	Humboldt Redwoods State Park	366-1650	NA	NA	2.65-9.40	[64]
Ss	Humboldt Redwoods State Park	1928-2320	2.5-3.5	0.5-2.5	3.5-5	[55]
EN	Pacific Northwest	228.4	NA	NA	7.35	[47]
MOT	Worldwide	5.45-1650	0.58-9.4	0.3-3.45	1.67-11.34	[56]
OT	US Pacific Northwest	464.7	NA	NA	NA	[51]
MOCTM	Worldwide	377	NA	NA	NA	[2]

[a]: *Fc: Fitzroya cupressoides, Mb: Mixed-broadleaf evergreen forests, Ac: Austrocedrus chilensis, Nb: Nothofagus betuloides, Aa: Agathis australis* (values reported are just for this species and not for the accompanying trees), *Ns: Nothofagus solandri, Th:Tsuga heterophylla, Ps-Th: Picea sitchensis-Tsuga heterophylla, Pm-Th: Pseudotsuga menziesii-Tsuga heterophylla, Ap-Pm: Abies procera-Pseudotsuga menziesii, Tm-Al-Pe: Tsuga mertensiana-Abies lasiocarpa-Picea engelmanii, Pe-Al: Picea engelmanii-Abies lasiocarpa, Ss: Sequoia sempervirens, EN: Evergreen needleleaf, MOT: Mature-old-growth temperate forests, OT: Old-growth temperate forests, MOCTM: Mature-old-growth cool temperate moist forests*

Our calculation of around four times more aboveground biomass in AA than AC constitutes a first estimate of the difference between forests growing in these two Ranges. This demonstrates the carbon storage capacity of *Fitzroya* forests under undisturbed conditions and in areas affected by recurrent fires. Biomass calculations may be underestimates, however, due to the low BEF estimated for *Fitzroya* from field measurements (1.066). This factor was low compared with mean values reported for other conifers (e.g. 1.18-1.21, [39]), but it was the only approximation available for the species. On the other hand, the underestimate might be somewhat counteracted by the fact we do not consider losses by heart rot due to lack of information.

The low woody productivity values reported in this study are indicated by the low radial growth rates found for all the species and especially *Fitzroya*. Mean annual rates for *Fitzroya* were 0.22 and 0.31 mm year^{-1} in AA and AC, respectively and for most of the other species, this rate never surpassed 1 mm year^{-1}. Reported mean growth rates for *Fitzroya* range from 0.28 to 2.99 mm year^{-1} [40], so values in this study are at the lower end of this range. Moreover, woody productivity was an important component in small diameter classes (DBH < 10 cm) especially in AC2, mainly due to the likely overestimation of woody biomass caused by using allometric equations just with DBH as the independent variable and the high density of these small trees.

When considering aboveground components, overall NPP_{AG}, and particularly canopy productivity, was lower in AA than AC, probably due to the different development stage of these forests. Older forests have been generally reported to be less productive than younger forests [41-43]. However, when belowground components were also included, we found little difference in NPP between the sites, suggesting that the observed differences in NPP_{AG} may be more driven by differences in allocation than in productivity. It is interesting to note that allocation to fine roots was not high in the Coastal site, where very poor soil conditions and high exchangeable aluminium contents are prevalent. This suggests root allocation is not in proportion to nutrient limitation. Fine root biomass in this site is around twice than in the Andes [27], implying that fine root residence time is longer in this area.

It has also been reported that foliage production decreases with age in conifer forests, which is consistent with our observations in the year reported here [44,45]. However, a subsequent year of litterfall measurements (data available online), provided almost the same amount in the Andes, but half the amount in the Coastal Range. Both sites experienced lower rainfall and warmer temperatures during the second summer (41% and 31% reduction in rainfall during January and February in AC and AA, respectively), but only in AC this did result in lower soil moisture status, suggesting that interannual variation in productivity is much higher in the younger site.

NPP_{AG} has been positively associated with growing season degree days and negatively associated with soil moisture in a climate gradient in Chiloé forests [10]. Thus, another possible reason for NPP_{AG} to be lower in the Andean site (particularly considering that branch turnover can be overestimated) is the rainier and cloudier climate in this site which may restrict productivity due to low radiation.

Question 3. How do the productivity and carbon dynamics of these forests compare to other temperate ecosystems worldwide?

4.4.2. Comparison with other forests in Chile and worldwide

4.4.2.1. Forest structure and biomass

The mean parameters found in AC (3215 and 4458 stems ha^{-1} and 92.5 and 91.4 m^2 ha^{-1} considering trees ≥ 2 cm DBH in AC1 and AC2, respectively) are different from the ones reported for *Fitzroya* forests in Chiloé. The density of the Chiloé forests was 9680 trees ha^{-1} (trees ≥ 2 cm) and the basal area was 138.2 m^{2} ha^{-1}, a very high value considering that *Fitzroya* trees are somewhat similar in size to the ones in AC [30].

Aboveground biomass values in AC (112.8-114.1 Mg C ha^{-1}) are smaller than the value reported for old-growth evergreen forests close to Valdivia (140 Mg C ha^{-1}, [46]) and lower than the ones reported for *Fitzroya* forests in Chiloé ([30], Table 2). In these Chiloé forests, more than the presence of large trees, tree density determines the high biomass reported.

Looking beyond Chile, the biomass values in AC are smaller than the mean reported for evergreen needleleaf forests from the US Pacific North West ([47], Table 2) and higher than the mean reported for secondary *Austrocedrus chilensis* forests in Argentine Patagonia (67.1 Mg C ha^{-1} and 156.8-199.8 Mg dry mass ha^{-1}, [8,48], Table 2). Biomass values in AC are also higher than the value reported by the IPCC for Temperate Oceanic forests of South America (180 Mg dry mass ha^{-1}, [49]).

On the other hand, the biomass values in AA (448-517 Mg C ha^{-1}) are higher than all the mentioned values above and higher than the one reported for an old-growth *Nothofagus* dominated forest in the Andes further north (435 Mg C ha^{-1}, [50]). Looking at other high biomass temperate forests, these biomass values are also higher than the mean compiled for mature and old growth cool temperate moist forests in a worldwide database ([2], Table 2) and the mean of both plots higher than the highest mean value reported for old-growth forests in the US Pacific northwest ([51], Table 2). Cool temperate moist forests as a biome; hold the highest aboveground biomass value per unit area worldwide ([2], locating *Fitzroya* forests from the Andes as one of the most massive forests in the world. The biomass values reported here for AA are amongst the highest reported for coniferous species. The other forests with high biomass are *Tsuga heterophylla,* *Pseudotsuga menziessi*, *Pseudotsuga-Tsuga* (all Pacific Northwest), *Sequoia sempervirens* (California) and *Agathis australis* (New Zealand), although this last estimate was based on limited sampling ([2,52-55], Table 2). The only forest stands with substantially higher biomass are *Sequoia* in California. However, *Fitzroya* is unique amongst the large conifers in combining such massive biomass with the lowest radial growth rate and productivity (likely due to poor soil nutrient conditions and high amounts of rainfall), implying that its exceptional longevity has a particularly important role in determining the high biomass of these stands.

4.4.2.2. Aboveground productivity per component

Woody productivity values in both sites are lower than the values reported for *Fitzroya* and mixed-broadleaf evergreen forests in Chiloé [9,10], and are also at the lower end of values reported for mature temperate forests in a global database ([56], Table 2).

Litterfall values in AC are a little higher than the mean reported for a *Fitzroya* site close to the study area [57] and much higher than the values reported for *Fitzroya* forests in Chiloé (Table 2). Values are however in the range reported for mixed-broadleaf evergreen forests in this island (Table 2).

Litterfall values in AC are within the range of values reported for warm temperate needleleaf evergreen forests (~3.3 to 6.9 Mg dry biomass ha^{-1} $year^{-1}$, [58]), but values in AA, fall below this range. Values in both sites are, on the other hand, within the range of measured values for mature temperate forests ([56], Table 2).

4.4.2.3. Total productivity, carbon allocation and mean wood residence time

Total NPP_{AG} values in AC (3.35-3.36 Mg C ha^{-1} $year^{-1}$) are in the range reported for *Fitzroya* forests in Chiloé by [9] and [10] (Table 2). The values in AA (2.22-2.54 Mg C ha^{-1} $year^{-1}$) approximate the one reported by [10], where lower litterfall production is compensated by higher woody productivity. However, studies in Chiloé do not include branchfall. Finally, values in both study sites are lower than total NPP_{AG} reported for mixed-broadleaf evergreen forests in Chiloé (Table 2).

Our NPP_{AG} estimates suggest that *Fitzroya* forests, especially in the Andes, have low productivity compared with other temperate wet forests. NPP_{AG} in the Andes for example is lower than the value reported for stands of another long-lived species, *Agathis australis* (Table 2). Total NPP_{AG} in both sites are within the range, but at the lower end of values reported for mature temperate forests worldwide [56] and they are lower than values reported for conifer forests in the US Pacific North West ([47,52, 53,59], Table 2). NPP_{AG} in both sites are on the range or higher though, than values reported for some high elevation forests in that country ([60,61], Table 2). Furthermore, the values in AA are lower than the lower bound NPP_{AG} estimate for old-growth *Sequoia* forests (Table 2).

Most of NPP_{AG} was allocated to the canopy, especially in AC. In contrast, most of other temperate forests appear to allocate more to stem rather than foliage productivity [56,62]. However, other mature southern hemisphere forests (*Nothofagus* and *Agathis* forests from New Zealand and *Austrocedrus* forests from Argentine Patagonia), also presented lower stem compared with foliage productivity ([8,37,54], Table 2). When allocation between aboveground components was separated by species (something not possible for below-ground allocation), most of carbon in *Fitzroya* was allocated to the canopy (81% in AC and 68% in AA) rather than wood (19% in AC and 32% in AA). In the case of other species, most of carbon was allocated to wood (69-72% in AC and 58-59% in AA) rather than the canopy (28-31% in AC and 41-42% in AA). Patterns are more extreme in the Coastal Range site and overall results demonstrate that the slow-growing *Fitzroya* has a low priority for allocation to wood. A recently reported positive relationship between woody productivity and allocation to stem growth, although for tropical forests, might help explain this lower woody allocation in *Fitzroya*. Increased allocation to stem growth, might be a way for forests that are more productive to obtain faster growth rates [33]. Trees in the sub-canopy, in particular those with broad crowns such as *Nothofagus,* would experience more intense competition for light, and hence may be expected to prioritise woody growth to overtop competitors, over leaf canopy growth.

Few temperate sites report total NPP. Our estimates of total NPP are lower than other reported for temperate forests in the Pacific Northwest: 6.8 Mg C ha^{-1} year^{-1} for an old-growth *Picea-Tsuga* forest, 7.7 Mg C ha^{-1} year^{-1} for a *Pseudotsuga* forest, 11.22 Mg C ha^{-1} year^{-1} for a *Tsuga-Pseudotsuga* forest [59] and 7.9 Mg C ha^{-1} year^{-1} for evergreen needleleaf forests [47]. Our estimates are also lower that the mean reported for evergreen temperate humid forests worldwide (7.8 Mg C ha^{-1} year^{-1}, [6]). The Coastal forest allocated a higher proportion of productivity aboveground (79%) compared with the Andean forest (59-62%). This finding is supported by the general notion that younger, aggrading forests allocate more carbon aboveground because of intense competition for available light, but does indicate that the poor soil conditions in AC do not increase allocation to roots (probably because of higher fine root biomass and residence times in this site).

Finally, total mean wood residence time in AA was considerably higher than the values estimated for other old-growth and mature temperate forests in the US Pacific Northwest and New Zealand (85 to 292 years, [37,41,53,59]). Among the reviewed studies, the only ecosystem that slightly surpassed the Andean stand was the forest investigated by [55], (ca. 717 years), which was dominated by *Sequoia* (> 80% of the number of trees). However, when we compare the *Fitzroya* trees separately, their residence times in AA greatly exceed those reported for other large forest stands.

Fig. 4 depicts an example of the forest structure in each site summarizing the main carbon cycling estimates for these forests.

4.5. Conclusions

We have described and contrasted the carbon stocks, productivity and residence times of two *Fitzroya* sites. The results show that these slow-growing forests show a non-equilibrium structure and dynamics hundreds of years after a disturbance, and *Fitzroya* may even continue to show a non-equilibrium structure and accumulate biomass more than a thousand years due to the lack of any observed mortality. The Andean *Fitzroya* forests are amongst the highest biomass forest sites and *Fitzroya* has perhaps the longest residence time ever observed in the world. Forests in the Coastal Range do not reach such high biomass values, mainly due to fires in the past, but probably have the potential to do so over centuries if appropriate conservation is given to the species in this area. Although *Fitzroya* forests only represent ca. 2% of the total native forest cover in Chile and a negligible portion of the temperate forests in the southern hemisphere, these ecosystems are unique in the combination of the amount of biomass carbon and the length of time it can be stored due to their longevity and protected status. This fact gives support for their consideration in national and international carbon mitigation initiatives, as well as for their adequate protection against fires and illegal cuttings. It is important to increase the enforcement of the law that declared *Fitzroya* a National Monument in 1976, as well as to set long term restoration programs to recover *Fitzroya* in the Coastal Range and Central Depression where extensive areas have been burned in the last centuries.

Fitzroya stands are likely to be ongoing biomass carbon sinks at centuries to millennia timescales, due to their low mortality rates and particularly long wood residence times. We encourage future research to further understand the ecosystem ecology and dynamics of these unique and ancient forest ecosystems.

4.6. Acknowledgements

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Chapter 4. Supplementary methods and results

Methods

Biomass stocks

According to the RAINFOR-GEM network protocol [1] a one-ha plot is commonly used to assess biomass and productivity. However, it was not possible to fit such a plot in the AC site, since there were no accessible forest patches of this size in the Park, and 0.6 ha plots were used instead. Similar 0.6 ha paired plots were used in AA, to facilitate comparison and consistency between the two sites.

Besides measuring diameter at breast height (DBH) of all trees ≥ 10 cm diameter, bark thickness was measured in three points around the trunk of every *Fitzroya* tree with a handmade tool especially designed for this purpose. This is mainly because *Fitzroya*, especially when it is older, develops a deep and spongy bark. Regarding heights, at least 10% of the number of individuals per diameter class (10 cm classes) per species were measured using a vertex IV (Haglof, Sweden). Heights for the rest of the trees were estimated when possible using a function that best fitted the data for each species and provided the expected normality and heterocedasticity in the residuals.

There is only one biomass allometric equation available for *Fitzroya*, developed using a non-destructive method in Chiloé Island, and that uses only DBH as the independent variable [2]. Therefore, in order to have specific biomass estimates for each study site, volume equations were developed for each area. Volume was calculated with the Smalian's formula for different trunk sections using diameter measured at different heights with a Spiegel Relaskop (Relaskop-Technik, Austria) and lengths of stems [3]. 35 and 40 trees were measured for this purpose in AC and AA, respectively. Different models relating volume with DBH and height were fitted [3] and the best equation was chosen according to goodness of fit and residual diagnosis. Two different volume equations, using diameter with and without bark were developed for each site and used for each tree.

An estimate of a biomass expansion factor (BEF) for *Fitzroya* was obtained from measurements of the diameter and length of all the branches up to 1 cm diameter in five trees fell in an illegal cutting. The affected stand was located in the Coastal Range further south from AC at 40° 38’ S, 73° 36’ W. Total volume was calculated using the Smalian’s formula for trunk and branch sections and summed for the different components, this total value was divided by stem volume in order to get the BEF. The estimated value for this factor was 1.066 (±0.01) and is the only existent approximation of this factor for the species. This value may not be that accurate for the Andean trees, since larger/older *Fitzroya* trees likely have larger and less branches, as well as a less symmetrical branch pattern than smaller trees [4]. Wood density was determined as the mean of three basic density measurements obtained through the water displacement method using three available cross sections from sites close to both study areas. This procedure was carried out at the Laboratorio de Maderas (Wood Laboratory) at Universidad Austral de Chile. Values for AC and AA were 0.411 (±0.008) and 0.444 (±0.015) g/cm^3, respectively.

Volume without bark, multiplied by the BEF and wood density provided an estimate of biomass without bark per each tree. The difference between volumes with and without bark obtained for each tree and an available estimate of bark density from the Andean site (0.203 ±0.019 g/cm^3), were used to calculate bark biomass. Total woody biomass for *Fitzroya* trees, was obtained through the sum of biomass without bark and the biomass of the bark.

For other tree species, when heights were effectively estimated through a robust height-DBH function (true in most cases), biomass equations that included height were used. In the few cases where the height-DBH relationship was not strong, biomass equations using only DBH were used (e.g. *Tepualia stipularis, Myrceugenia spp., Maitenus magellanica, desfontainia fulgens, Amomyrtus luma*). Since these biomass equations considered all the aboveground components, published values for the mean proportion of the tree occupied by bole and branches (all components except leaves) were used to the get woody biomass for each species [5]. Total woody biomass of the plot was calculated by summing the biomass per hectare of all trees 2-10 cm DBH and ≥ 10 cm DBH.

Canopy biomass in each plot was estimated using the mean proportion of biomass present in this component published for different species [5]. In the case of *Fitzroya*, the mean proportion found between foliage biomass and biomass in stem and branches using the equations developed by [2] was used in this study.

Finally, a limited assessment of biomass present in the understory was undertaken through sampling of the four corners of a random 20x20 m subplot from one plot in AC and one in AA. 1x1 m quadrats were established and all vegetation below one meter height was cut and brought to the laboratory to determine dry weight. Since this component was sampled in one plot per site, the same mean value was assumed for both plots.

Aboveground coarse wood productivity (NPP$_{ACW}$)

A small correction in productivity estimates was required for trees ≥ 10 cm that died within a year. It was assumed that trees that died did so on average half way through the census interval and then half of their growth was still considered as a contribution to productivity.

Branch turnover productivity ($NPP_{branch\ turnover}$)

$NPP_{branch\ turnover}$ represents a branch turnover term not usually captured in NPP measurements [6], though it may have some bias if old trees are senescing and shedding branches.

Each branch turnover productivity transect was subdivided in five 20 m sub transects, corresponding to the sides of each 20x20 m subplot. Particular attention was paid to account only for branches coming from live trees. When a new branch was found within a sub transect, it was lifted and cut when needed, to only include the portion within the transect. Branches collected in each sub transect were classified in decomposition categories and weighed in the field. A representative sample (e.g. at least half the number of branches or even all branches) per decomposition category and sub transect was weighed wet and brought immediately to the lab to determine its dry weight and the corresponding water content. This water content was later used to determine the dry weight of all the samples collected in the field. Branchfall from transects was extrapolated to one hectare.

Canopy productivity ($NPP_{litterfall}$)

Litterfall collection started in August and September 2011 in AC and in November 2011 in both Andean plots. Once collected, litterfall was immediately brought to the laboratory, separated into different components (leaves, needles, reproductive material, twigs and others), dried at 60 °C for 48 hours and weighed after removal from the oven. In order to assess litterfall from the understory vegetation, five litterfall traps per plot were installed just above the ground surface, in the four corner’s subplots and the center of each plot in AC. As the Andean site understory vegetation was less abundant, these traps were installed only in AA1. A second fine 1x1 m mesh was located at 1 m above the ground litter trap to exclude litterfall falling directly from above. These traps were installed in April 2012 and the first collection was made in May 2012. Therefore, monthly values from November 2012 to April 2013 had to be considered to obtain an annual estimate for this component. Since only one plot was sampled in AA, the mean value obtained for AA1 was extrapolated to AA2.

Monthly litterfall was obtained by calculating the mean of all litterfall traps per plot and extrapolating that value to one hectare. Annual productivity per plot was obtained averaging the annual sum of litterfall per trap. Total $NPP_{litterfall}$ was calculated adding litterfall collected from trees and from the understory.

Fine root productivity ($NPP_{fine\ root}$)

S1 Table shows the main parameters used to estimate fine root productivity according to the mass balance approach presented in the main manuscript.

Results

Climate characterization

Due to technical problems, meteorological data in the Andean site for May and most of June 2012 could not be recovered, so in order to have annual estimates gap filling was performed. Gaps were filled using linear regressions between monthly data at the study site and Puerto Montt (41° 25’ S, 73° 05’ W at 85 m a.s.l). This was done for precipitation, temperature and relative humidity using the period November 2011-October 2012.

There is a strong seasonality in solar radiation and temperature, with highest values observed during spring-summer (November to February) and the lowest values in fall-winter (May to August, S1 Figure). Solar radiation was significantly lower at the Andean site (on average 22% lower) and soil temperature showed similar variation as air temperature, being consistently lower in the Andes (S1 Figure). Highest precipitation values were recorded during fall-winter, but there was also a secondary peak in February 2012 (summer).

During winter months there was snow precipitation in both sites, particularly in the Andes where snow accumulates and lasts for many days. Relative humidity showed less marked seasonality in both sites with lower values in spring and summer.

Soils characteristics

In both sites soils are acidic, depths are ≤ 1 m and there is a dense fine root layer in the upper 30 cm. Soil characteristics are presented in S2 Table. Nutrient poor soils in the Coastal Range site are associated with the old parental material (metamorphic rocks), that has experienced a persistent process of nutrient lixiviation [7]. C/N ratios are especially high in the Andes, probably due to wetter conditions and lower soil temperatures in this site. Organic matter content is high in both areas, but especially high in AA.

$NPP_{litterfall}$

Total litterfall and needle production in AC and AA resembled each other in terms of seasonality (S7 Figure). For other litterfall components, seasonality was somewhat different in both sites due to a more diverse species composition in the Andes. In both sites, peaks in litterfall were in late summer (February) and late autumn (May, S7 Figure).

Litter fractions consisted of 90% and 83% *Fitzroya* needles, 6% and 12 % broadleaves, 1% reproductive material and 3% and 4% twigs in AC1 and AC2, respectively. Litter fractions for AA1 and AA2 corresponded to 43% and 50% *Fitzroya* needles, 44% and 38% broadleaves, 4 and 5% reproductive material and 9 and 7% twigs (S8 Figure).

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Chapter 4. Supplementary tables and figures

S1 Table. **Parameters to estimate fine root productivity.** Parameters used to estimate NPP$_{fineroot}$ through the mass balance approach presented in the manuscript. Mean values for both plots in each site plus/minus standard errors are presented. R$_h$ corresponds to soil heterotrophic respiration for the period November 2011-October 2012, Litterfall to the mean annual amount of litterfall collected in both plots from each site, Mort$_{AG}$ to the mean aboveground mortality, NPP$_{branchfall}$ to the mean productivity associated to this component, F$_{cwd}$ to the fraction of coarse woody debris (CWD) entering the soil and Mort$_{BG}$ to the belowground mortality which totally enters the soil (Alerce Costero, AC and Alerce Andino, AA).

Site	Rh (Mg C ha^{-1} year^{-1})	Litterfall (Mg C ha^{-1} year^{-1})	Mort$_{AG}$ (Mg C ha^{-1} year^{-1})	NPP$_{branchfall}$ (Mg C ha^{-1} year^{-1})	F$_{cwd}$	Mort$_{BG}$ (Mg C ha^{-1} year^{-1})
AC	3.23±0.56	2.41±0.220	0	0.038±0.008	0.25±0.25	0
AA	2.99±0.33	1.21±0.064	0.66±0.33	0.270±0.11	0.25±0.25	0.045±0.041

S2 Table. Soil characteristics in both study sites. Soil characteristics and nutrient composition in the upper horizons at plots AC1 and AC2 at the Alerce Costero site and plots AA1 and AA2 at the Alerce Andino site. Values shown are the averages of the soil profiles obtained in each plot

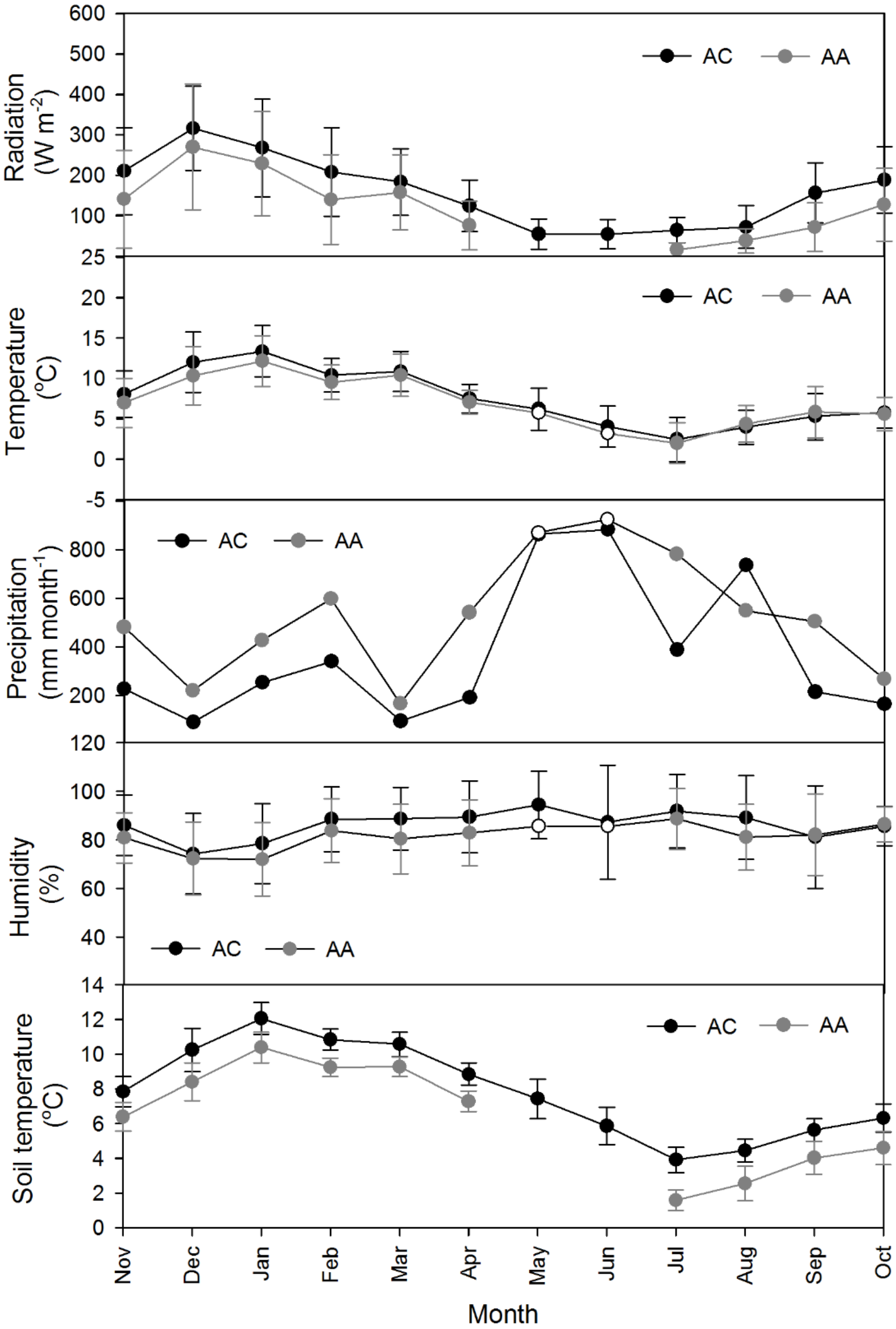
Plot	Effective Soil depth[a] (cm)	Surface horizon depth (cm)	Bulk density (g/cm^3)	pH	SOM[b] %	C/N	N (%)	P (Olsen) (ppm)	K (ppm)	Ca (ppm)	Mg (ppm)	Na (ppm)	Al[c] (ppm)
AC1	63	23	0.75	4.09	9.57	34.5	0.17	3.1	72	100	71	28	715
AC2	43	16	0.70	4.11	10.06	32.7	0.18	3.1	94.3	114.3	63.3	25.3	893
AA1	72	18	0.16	4.23	78.70	113.2	0.40	5.2	470	510	204	94	410
AA2	94	20	0.22	4.32	59.99	83	0.43	7.2	553	700	239	97	303

a: Soil depth where roots can potentially develop and extract water and nutrients without any apparent physical or chemical restriction.
b: Soil organic matter
c: Exchangeable Al

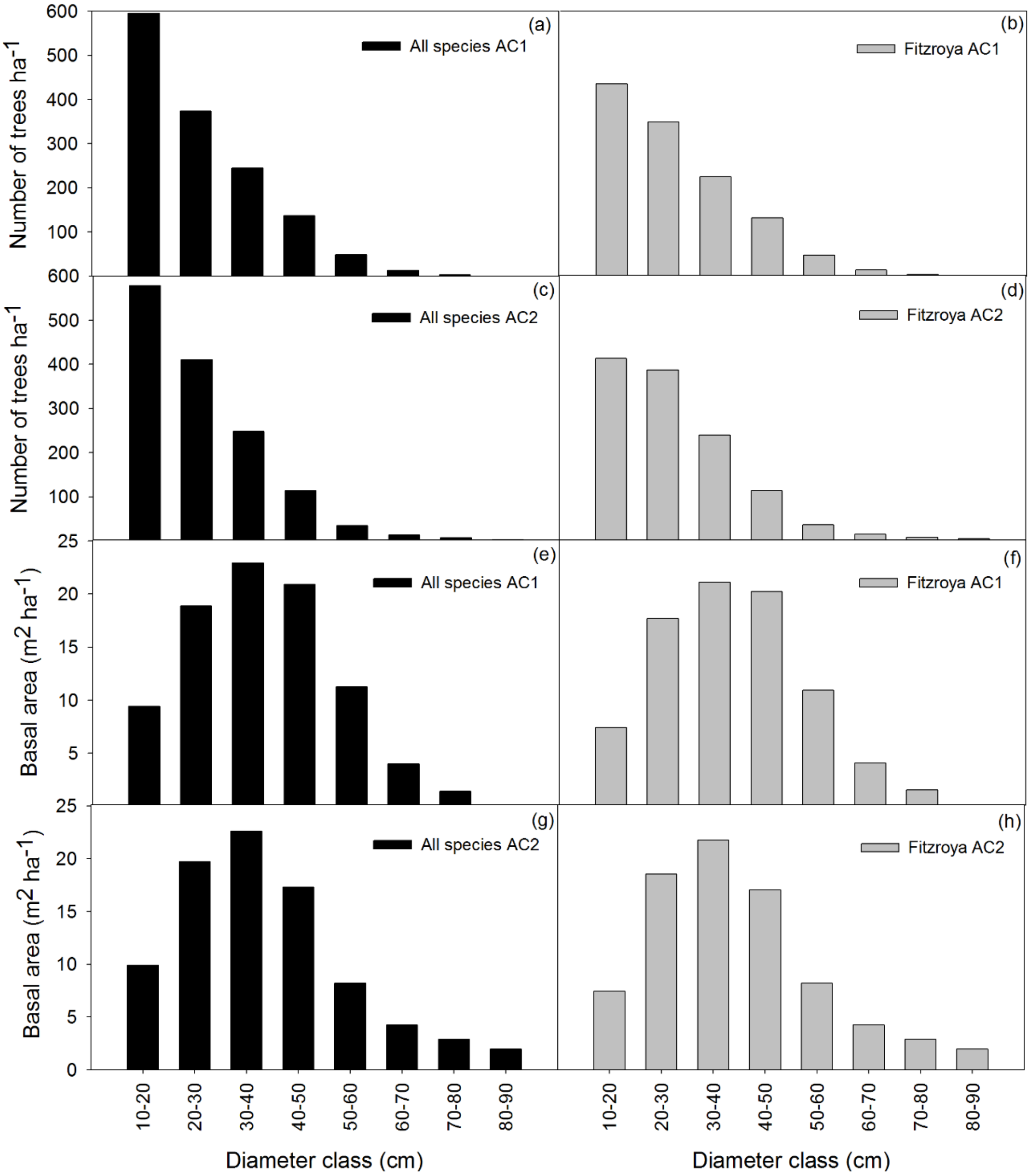
S3 Table. Volume equations for *Fitzroya cupressoides* in each site. Volume equations used for *Fitzroya* trees in Alerce Costero (AC) and Alerce Andino (AA). Statistics associated to the equations are also presented

Site	$V_{(withoutbark)}$[a]	R^2[b]	RSE[c]	DW[d]	$V_{(withbark)}$[a]	R^2[b]	RSE[c]	DW[d]
AC	$0.00023*DBH^{2.17614}$	0.96	0.1910	1.46	$0.00022*DBH^{2.18308}$	0.96	0.1936	1.41
AA	$0.00008001*DBH^{2.4823}$	0.99	0.2131	1.59	$0.00006877*DBH^{2.5071}$	0.99	0.2102	1.58

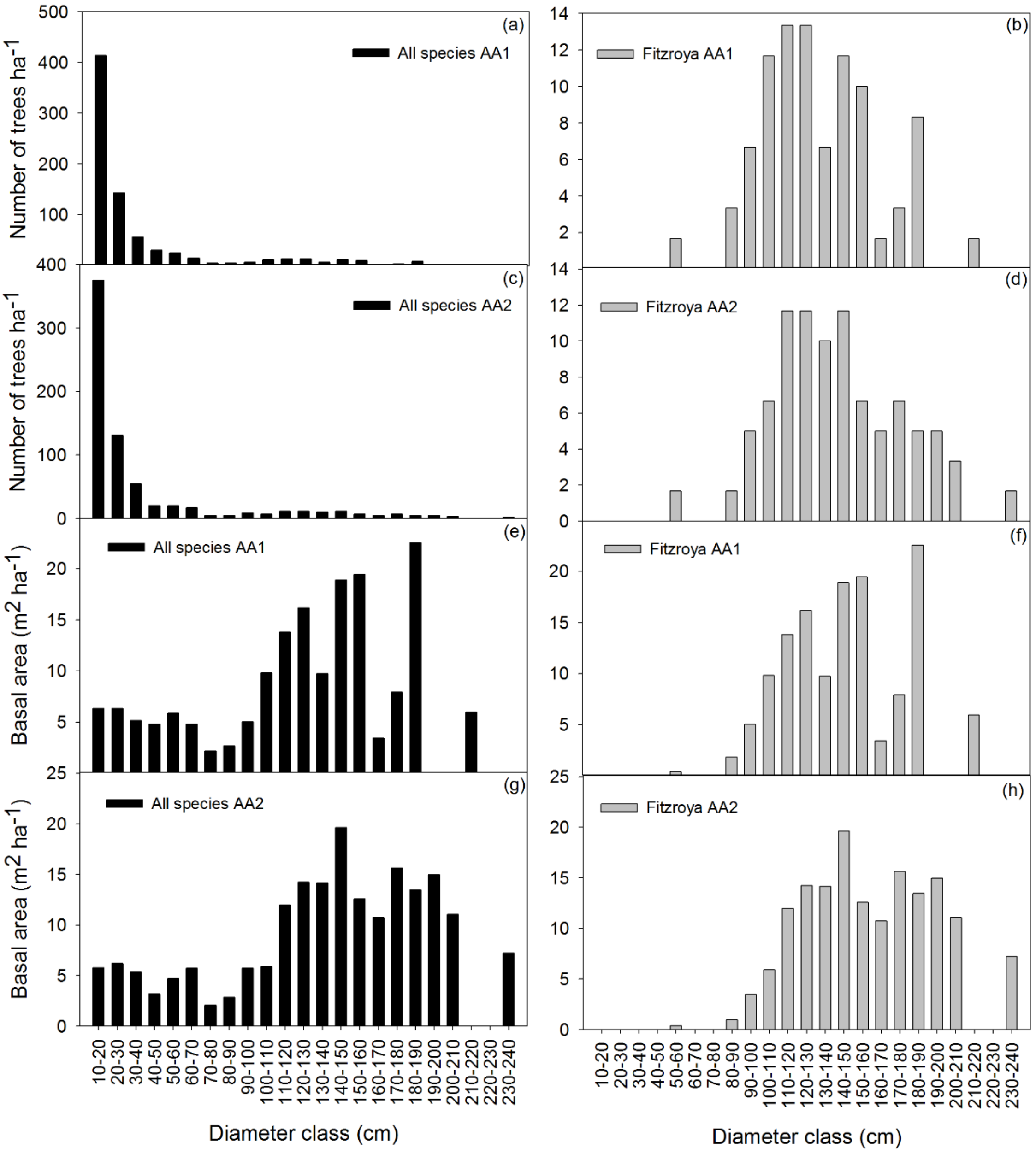
a: $V_{(withoutbark)}$: volume without bark in m^3, $V_{(withbark)}$: volume with bark in m^3. DBH is in cm.
b: Adjusted R^2
c: Residual standard error
d: Durbin Watson statistic. Residuals were not significantly autocorrelated according to this statistic.



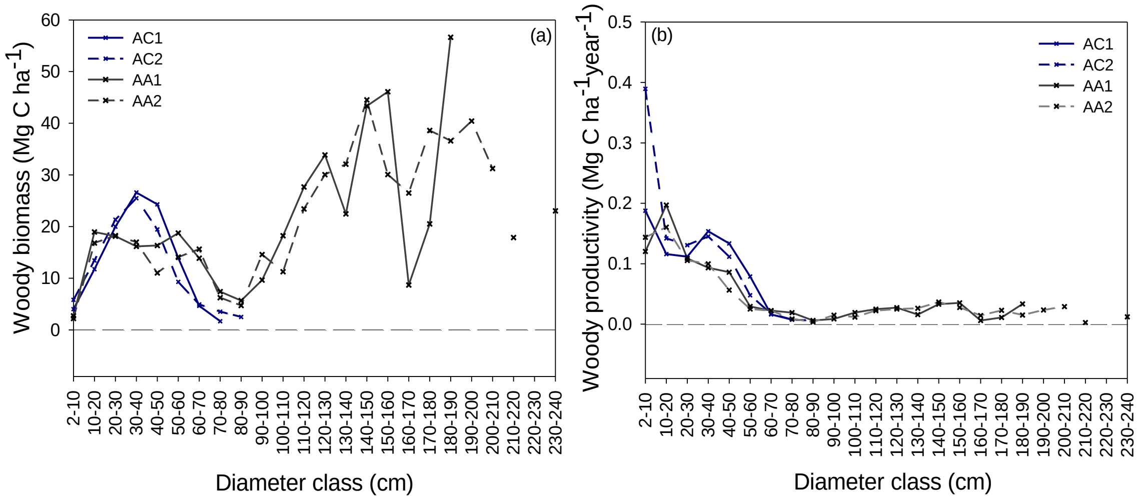
S1 Fig. Climate conditions in the study sites. Mean monthly radiation, temperature, relative humidity, soil temperature, and total monthly precipitation in Alerce Costero (AC) and Alerce Andino (AA) between November 2011 and October 2012. White dots in the figures indicate values estimated from climate data from the weather station at Puerto Montt. Error bars indicate ±1 SD.



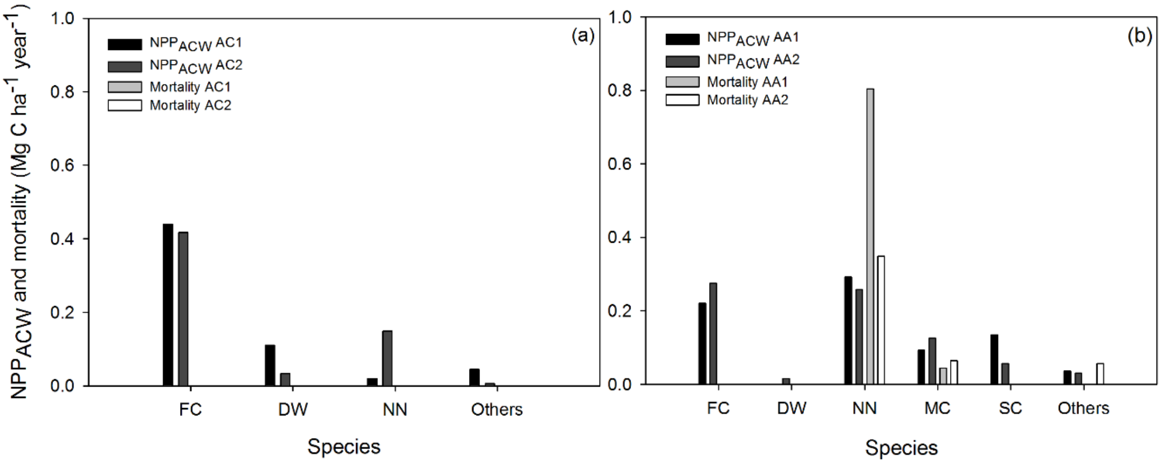
S2 Fig. Diameter distribution and basal area in Alerce Costero. a-d) Diameter distribution of all species and *Fitzroya* trees in Alerce Costero plots (AC1 and AC2, for trees ≥ 10 cm DBH). e-h) Basal area for the same components in both plots.



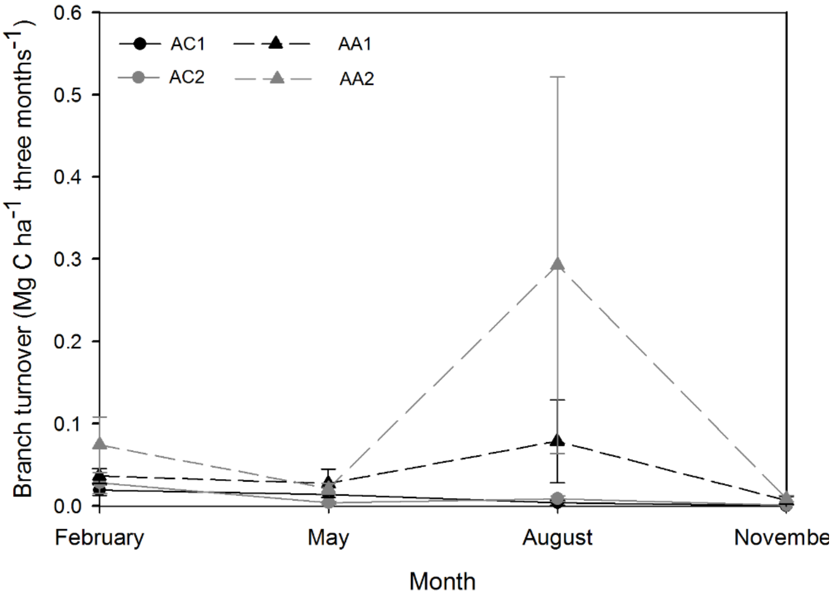
S3 Fig. Diameter distribution and basal area in Alerce Andino. a-d) Diameter distribution of all species and *Fitzroya* trees in Alerce Andino plots (AA1 and AA2, for trees ≥ 10 cm DBH). e-h) Basal area for the same components in both plots.



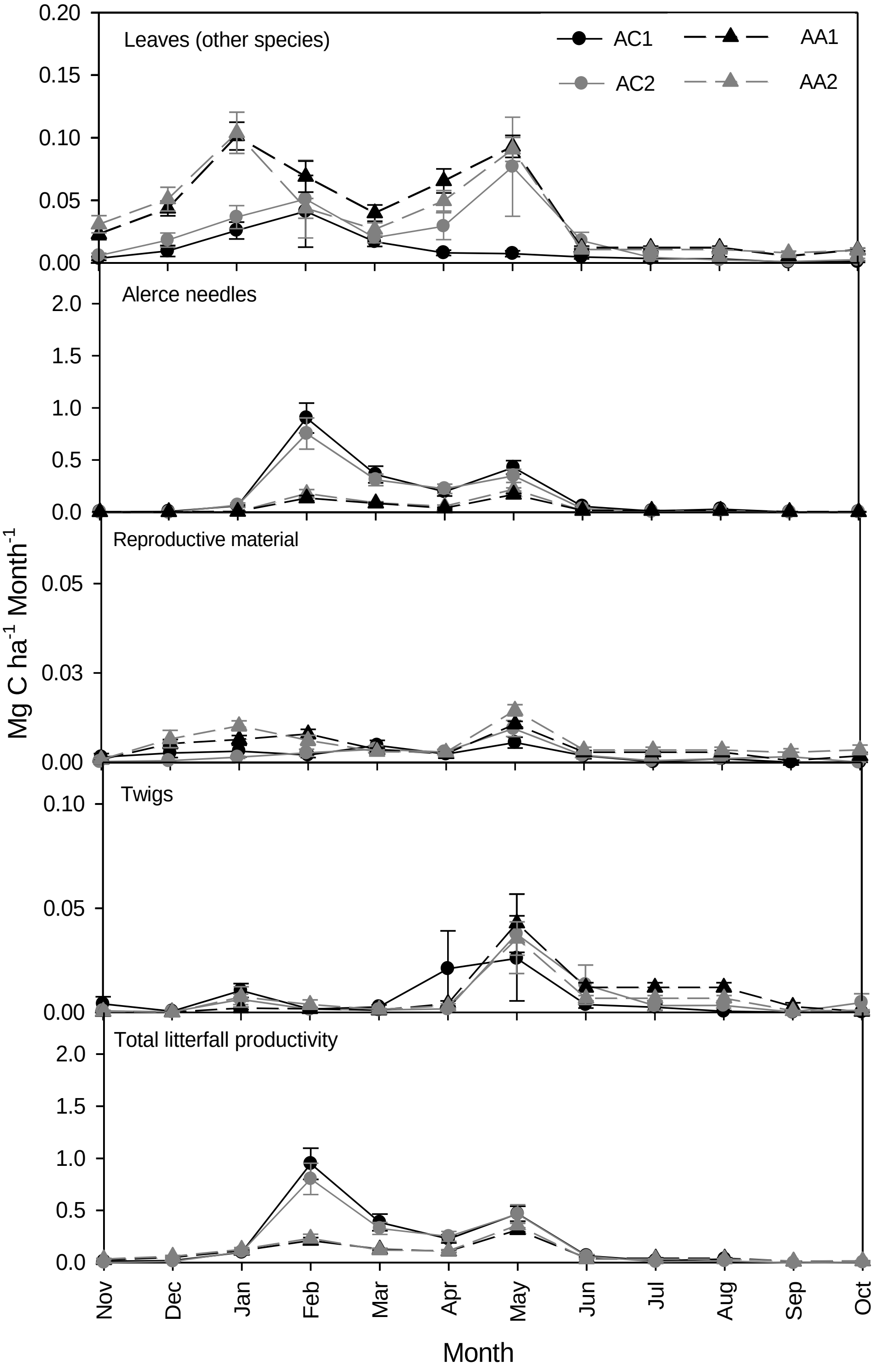
S4 Fig. Total woody biomass and productivity per diameter classes and plot. a) Total woody biomass for trees ≥2 cm DBH along different diameter classes in each plot, b) The same as a), but for total woody productivity.



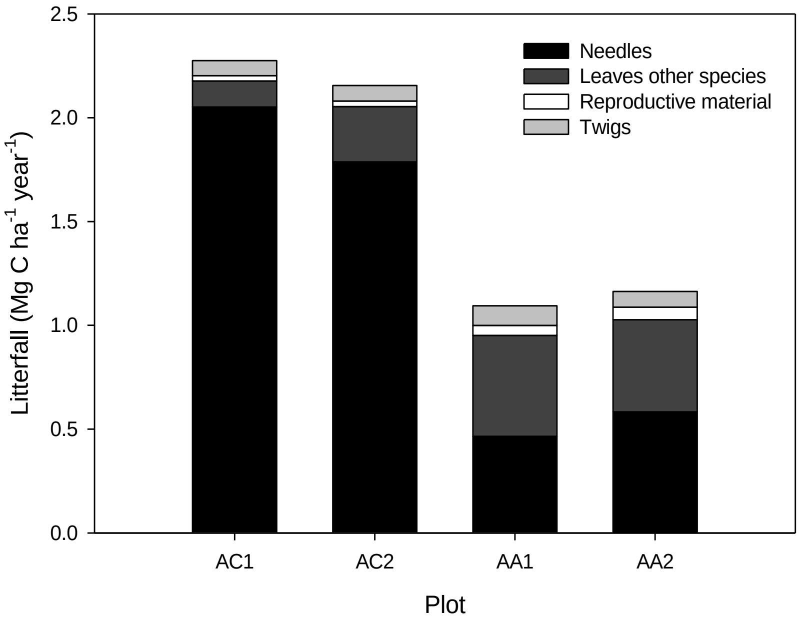
S5 Fig. Aboveground coarse woody productivity and mortality per species and plot. Aboveground coarse wood productivity (NPP$_{ACW}$) and mortality in trees ≥ 10 cm DBH in the Coastal (a) and Andean plots (b). FC: *Fitzroya cupressoides*, DW: *Drimys winteri*, NN: *Nothofagus nitida*, MC: *Myrceugenia chrysocarpa*, SC: *Saxegothaea conspicua*.



S6 Fig. Seasonal cycle of branchfall per plot. The seasonal cycle in branchfall (every three months) in each plot between February 2012 and November 2012. The month of February 2012 includes branches fallen in the period November 2011-February 2012. Error bars show ±1 SE.



S7 Fig. Seasonal cycle of litterfall and its components per plot. The seasonal cycle in canopy litterfall (bottom) and its components (non-*Fitzroya* leaves, needles, reproductive material and twigs) per plot between November 2011 and October 2012. Error bars show ±1 SE.



S8 Fig. Litterfall per component per plot. Litterfall per component (in Mg C ha^{-1} year^{-1}) per plot.

Linking statement: Chapter 4 to 5

The major aboveground components of the carbon cycle of *Fitzroya* forests were evaluated in the previous chapter, where I assessed the amount of biomass that *Fitzroya* forests can hold in the two sites and the amount of organic matter that these forests can produce during a year.

The logical next step is an assessment of the belowground components, mainly represented by soil respiration and fine root productivity. Thus, Chapter 5 addresses the third specific objective: **to assess soil respiration and its partitioning between autotrophic and heterotrophic components on a seasonal and annual basis in each of the study areas, and relate total respiration to soil environmental conditions.** This chapter represents the first attempt to understand the environmental drivers of soil CO_2 effluxes in these forests. It addresses this task by examining relationships between soil respiration and soil temperature and water content on an interannual basis. This chapter also deals with the understanding of belowground productivity and addresses the second part of the third objective: **to utilise a mass balance approach that uses heterotrophic respiration and belowground carbon inputs to estimate fine root productivity.** This fine root productivity is the one used in Chapter 4 to develop an estimate of total NPP.
I was the main author and wrote the paper, I also organised and conducted fieldwork during half of the studied period and research assistants continued data collection for the rest of the time under my close supervision and direction. I carried out all data analyses. Among the co-authors, Yadvinder Malhi was the main supervisor of this research, providing advice on the study design and data analyses. Antonio Lara contributed to the paper.

The manuscript was submitted to Biogeochemistry in September 2014, it was not accepted, but we were invited to resubmit the paper after corrections. I resubmitted the paper in January 2015.

Chapter 5: Soil respiration patterns and mass balance estimation of fine root production in *Fitzroya cupressoides* forests of southern Chile

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Abstract

The soil carbon dynamics of southern hemisphere temperate rainforests have rarely been studied. Here we report for the first time soil CO_2 effluxes and their partitioning for medium-age and old-growth *Fitzroya cupressoides* forests growing under contrasting environmental conditions in the Coastal Range and Andean Cordillera of southern Chile. *Fitzroya* is a high biomass and one of the longest lived species in the world. We characterized soil respiration patterns over almost two years. Annual soil respiration was a little higher in younger forests from the Coastal Range compared with Andean forests during the first studied year (6.37-6.66 vs 5.06-6.14 Mg C ha^{-1} yr^{-1}), and significantly higher during the second year mainly due to a warmer and drier summer (8.08-8.64 vs 4.98-5.35 Mg C ha^{-1} yr^{-1}). Therefore, warmer and drier conditions, likely to become more common in this region under future climate change, were associated with significantly higher respiration in the shallow soils of the coast, but not in the Andes. A higher proportion of autotrophic respiration was found in the coastal forest probably due to a much higher fine root biomass in this site. Fine root productivity an important contributor of belowground carbon fluxes in forests, was a little lower in the coastal site (0.81±0.60 yr^{-1} vs 1.50± 0.42 Mg C ha^{-1}), indicating a longer residence time of roots in forests from this area. Soil CO_2 effluxes from these ancient forests and their root productivity are at the lower end of values recorded for other mature and old-growth wet temperate forests worldwide. The longevity of these ecosystems as well as the particularly poor soils and rainy conditions where these forests grow may influence these facts. Interannual climate variability appears to be especially important for soil respiration in the Coastal Range site due to the more Mediterranean climate influence and restrictive soil conditions in this area.

5.1. Introduction

Soil respiration or carbon dioxide (CO_2) efflux from soils is the largest source of carbon from terrestrial ecosystems and plays a significant role in the global carbon cycle (Raich and Potter 1995, Wang et al. 2006). It frequently contributes 30 to 80% of total ecosystem respiration in forests (Davidson et al. 2006a). Soil CO_2 efflux can be a good indicator of overall ecosystem metabolism (Ryan and Law 2005), it is a crucial influence on net ecosystem C sequestration and atmospheric CO_2 concentrations (Metcalfe et al. 2011), and a better understanding of this efflux is key to improve the prediction of net ecosystem exchange responses to climate change (Lavigne et al. 2003).

Total soil CO_2 efflux is a combination of autotrophic (R_a) and heterotrophic (R_h) effluxes. The soil autotrophic component is contributed by living plant roots and their associated mycorrhizae and is often termed rhizosphere respiration, while the heterotrophic component mainly comes from microbes responsible for detritus and soil organic matter decomposition (Butler et al. 2012). Photosynthesis and plant carbon allocation mostly control substrate supply for autotrophic respiration. On the other hand, heterotrophic respiration is mostly controlled by the supply and quality of labile substrate in new detritus (Ryan and Law 2005). The proportion of soil respiration from autotrophic and heterotrophic components might vary largely with seasons and among ecosystems, with mean root respiration ratios (root respiration/total respiration) of ~ 43.7-48.6% across a range of studies (Hanson et al. 2000, Chen et al. 2011). Distinguishing between both soil respiration components allows a better interpretation and modelling of soil processes and provides a better understanding of the implications of environmental change on the climate feedback potential of soil respiration and on the carbon cycling and sequestration in forests ecosystems (Hanson et al. 2000, Subke et al. 2006, Trumbore 2006, Bond-Lamberty and Thomson 2010).

Soil respiration dynamics are primarily controlled by temperature, soil moisture and substrate properties, which influence the production and consumption of organic matter (Davidson et al. 1998, Raich and Tufekciogul 2000). The apparent annual temperature sensitivity of soil respiration is normally characterized by the Q_{10} function, but this can be affected by covariation of temperature with soil water content, root biomass and microbial biomass (Davidson et al. 1998). Given the complexity of factors that can affect soil respiration it is important to understand the environmental controls on soil effluxes at a site level, so potential responses of ecosystems to ongoing changes can be assessed (Fenn et al. 2010).

Most studies of soil respiration have focused on northern hemisphere or tropical ecosystems. There have been no detailed field studies of soil respiration in the temperate forests of southern South America, the largest area of temperate rainforests in the southern hemisphere (Armesto et al. 2009). More specifically, it is not clear how ancient and carbon massive ecosystems, such as *Fitzroya cupressoides* forests, possibly the oldest rainforests in the world in terms of mean stand age (Urrutia-Jalabert, 2015), are behaving in terms of soil emissions to the atmosphere. Similarly, there are no estimates of belowground productivity in these ecosystems, particularly of the productivity of fine roots ($NPP_{fineroot}$), which together with mycorrhizae, are important contributors to belowground fluxes in forests (Finér et al. 2011). Studying soil respiration in these ecosystems may provide more insights into the carbon cycle and general functioning of particularly old-growth rainforests.

We here investigate the soil CO_2 efflux emitted by two contrasting *Fitzroya* forests in southern Chile over almost two years. The study sites were selected from the two main mountain ranges where *Fitzroya* grows and stands were representative of the widespread condition of these forests in each area: a medium-age and an old-growth forest in the

Coastal Range and the Andes Cordillera, respectively. The difference in developmental stages is mainly because there are no old-growth forests in the Coastal Range due to fires and logging during past centuries. This will allow comparison of soil respiration in stands of different ages, but perhaps more interesting, in the same forest type growing under different environmental conditions. The climate in the Coastal Range has a more Mediterranean influence and soil conditions are more restrictive than in the Andes.

Since summer conditions were warmer and drier during the second studied year, we specifically evaluated the sensitivity of soil respiration to soil temperature and water content considering interannual variability in each study site. This assessment is especially relevant, because southern Chile is projected to experience significant climate warming (increase in summer temperature of up to 4° C) and drying (decrease in summer precipitation of up to 50%) by the end of this century (Fuenzalida et al. 2007).

Our specific objectives were to : a) examine seasonal patterns of total respiration (R_s), R_a and R_h in forests from both areas, b) quantify annual fluxes of R_s, R_a and R_h and the proportion of total respiration partitioned to these components in each site, c) assess the soil environmental factors that might influence total respiration changes between the two studied years in each site, d) use a mass balance approach to estimate $NPP_{fineroot}$ and compare belowground productivity in both areas.

5.2. Material and Methods

5.2.1. Study sites

Two 0.6 ha plots were installed in *Fitzroya* forests within the Alerce Costero National Park (AC) in the Coastal Range (40° 10’ S- 73° 26’ W) and within the Alerce Andino National Park (AA) in the Andes Cordillera (41° 32’ S- 72° 35’ W). These plots were established with the purpose of measuring primary productivity and soil respiration in

Fitzroya forests of different structure and origin, which were characteristic of these two Ranges. Plots AC1 and AC2 were installed at 850 m a.s. l, and AA1 and AA2 at 760 m a.s.l.

Climate in the coastal site is characterized by high annual precipitation (4,860 mm during 2012), a mean annual temperature of ~7.26°C (~3.5°C and 11.9°C in winter and summer 2012, respectively) and a Mediterranean influence where most of precipitation occurs in winter (~47% falling from June to August and ~9% from December to February, period 1999-2010, Veblen and Ashton 1982, Donoso et al. 2006, DGA 2011). Climate in the Andes is characterized by an even higher precipitation amount (~6,600 mm during 2012) and somewhat lower air annual temperatures than in AC (~6.89° C, and ~ 3.2°C and 10.7°C in winter and summer 2012, respectively). Summer precipitation (December-February) in AA was on average 1413 mm compared with 839 mm in AC (periods 2011-2012 and 2012-2013, Urrutia-Jalabert et al. 2015).

Soils in the coastal site have a sandy loam texture, are derived from Pre-Cambrian to Paleozoic metamorphic rocks and are generally thin, poor in nutrients and severely podzolized (Veblen and Ashton 1982, Urrutia-Jalabert 2015). The soils have poor drainage conditions, but a low water retention capacity, and can get particularly dry during rainless periods (Barichivich 2005, Gerding V, personal communication). Soils in the Andean site have a silty loam texture, are derived from volcanic parental material, have high C/N ratios and contain high organic matter (Peralta et al. 1982, CONAF 1985, Urrutia-Jalabert 2015, Table 1). Soils in this site remain wet all year round.

The forest stands in AC are dense and mostly dominated by *Fitzroya* in terms of number and basal area (Table 1). These stands have been affected by fires in the past (a stand forming fire in 1681 and fire release events probably in the 1800s and 1900s, Lara et al. 1999, Barichivich 2005, Urrutia-Jalabert 2015), so these slow-growing trees are not as large and old as expected for the species. The biggest trees are *Fitzroya* and reach up to ca. 86 cm in diameter. Other main species are *Drimys winteri* and two species from the *Nothofagus* genus (*N. nitida and N. betuloides*). The forest in AA is less dense and mostly dominated by *Fitzroya* in basal area (Table 1). The largest *Fitzroya* trees reach up to 235 cm in diameter and the species is mainly accompanied by evergreen species where the most abundant are *Nothofagus nitida, Myrceugenia chrysocarpa* and the conifer *Saxegothaea conspicua* (Urrutia-Jalabert 2015). Table 1 presents important features of the soils and forest structure in each plot.

Table 1. Soil features, nutrient contents and forest stand characteristics in each study plot (Alerce Costero (AC1 and AC2) and Alerce Andino (AA1 and AA2)). Soil characteristics correspond to the surface horizons (AC1:23 cm, AC2: 16 cm, AA1: 18 cm, AA2: 20 cm), where most of the roots develop.

Plot	Effective mean soil depth[a] (cm)	Bulk density (g/cm^3)	Soil organic matter (%)	C/N	N (%)	P (Olsen) (ppm)	K (ppm)	Density[b] (trees ha^{-1})	Basal area[b] (m^2 ha^{-1})	AGB[c] (Mg C ha^{-1})	Mean DBH[d] (cm)	Mean height[e] (m)
AC1	63	0.75	9.57	34.5	0.17	3.1	72	1415	89	114.1	26.8	14.4
AC2	43	0.70	10.06	32.7	0.18	3.1	94.3	1408	87	112.8	26.7	14.1
AA1	72	0.16	78.70	113.2	0.40	5.2	470	782	171	447.5	132	31.8
AA2	94	0.22	59.99	83	0.43	7.2	553	722	193	517.1	141.8	30.8

a: Soil depth where roots can potentially develop and extract water and nutrients without any apparent physical or chemical restriction.
b: These values include trees $\geq$ 10 cm diameter at breast height (DBH)
c: Aboveground biomass
d: Mean diameter at breast height (DBH) of *Fitzroya* trees
e: Mean height of *Fitzroya* trees $\geq$ 30 cm DBH.

5.2.2. Soil respiration measurements

Total soil CO_2 efflux was measured in permanent collars installed at the center of 15 20x20 m subplots within the plots in AC and AA. An infrared gas analyzer (IRGA) with a closed static chamber (EGM-4 IRGA and SRC-1 chamber, PP Systems, MA, USA) was used to measure total efflux for two minutes on every collar (11 cm diameter) inserted around 3-5 cm into the litter and/or organic layer. Due to the remoteness, difficult accessibility and mobility within the forests (especially in the Andean site) measurements were mainly taken between 9:00 and 17:00 hrs. The sampling frequency was once a month mostly from August 2011 to August 2013 in AC and from October 2011 to May 2013 in AA. Measurements in both sites were taken within a week of each other. There were gaps in the collected data especially during winter months in AA, where bad weather and dangerous road conditions prevented the access to the study site. In the coastal site, gaps in winter months were mostly due to instrument failure under cold conditions. Soil surface temperature (12 cm temperature probe, Electronic Temperature Instruments, West Sussex, UK) and volumetric water content (12 cm Hydrosense probe, Campbell Scientific Ltd., Loughborough, UK) were measured at the same time as respiration at a distance of less than 50 cm from each collar. The height of the collar was recorded after each measurement.

The partitioning of autotrophic and heterotrophic respiration was estimated using tubes installed at the four corners of each 0.6 ha plot. A long (40 cm) PVC collar was inserted at least 30 cm into the soil where roots were previously removed in order to measure heterotrophic respiration. Roots were manually removed and the root free soil was carefully reinstalled back within the collar without mixing the horizons nor compacting the soil. Soil respiration measurements started from one day (one plot in the Andes) to two weeks after installation. We did not observe high fluxes in the collars one day after installation in the Andean site, so we decided to keep these measurements. A short collar inserted 3-5 cm into the soil was installed next to each long collar to measure total respiration. The difference between the effluxes coming from these two collars was considered an estimate of autotrophic respiration. Soil temperature and water content were also monitored during each respiration measurement. These partitioning measurements were less continuous than those of total respiration, ranging from October 2011 to August 2013 in AC and from November 2011 to May 2013 in AA, but with a longer gap during winter 2012.

5.2.3. Annual soil respiration estimates

Soil respiration (µmol CO_2 m^{-2} s^{-1}) was calculated following the procedures presented in Marthews et al. (2012, http://gem.tropicalforests.ox.ac.uk/page/resources). A diurnal temperature correction was applied to convert spot measurements into estimates of daily mean respiration. Hourly soil respiration was calculated using hourly soil temperature measurements (from a temperature sensor in each site) and the Van't Hoff's formula described further below (Davidson et al. 2006b), assuming a temperature coefficient Q_{10} =2 for diurnal scale variation. This Q_{10} value was considered appropriate at a short-time scale in a cold climate ecosystem (Lavigne et al. 2003).

The diurnal correction was very small, due to the small diurnal amplitude of soil temperature variations, reaching at the most 1 °C during the warmest month in AA. Mean respiration rates per plot (µmol CO_2 m^{-2} s^{-1}) were obtained for the period November-May (Spring to Fall), when the four plots had almost complete measurements.

Annual carbon budgets (in Mg C ha^{-1} yr^{-1}) were calculated by linear interpolation of fluxes between measurement days. The annual periods considered were from the first measurement made in August 2011 to August 2012 (year 1) and from August 2012 to August 2013 (year 2). Gaps in winter months (when fluxes are low) were filled using the mean of fluxes in the months immediately before and after the gap. In AA2, there were more missing months during the winter-spring 2012 period, and estimated values from AA1 were used for this period. Furthermore, in order to have annual respiration values for the Andean site, and since fluxes in winter and beginning of spring were particularly low, values for August-September 2011 and June to August 2013 were assumed to be equal to October 2011 and May 2013, respectively. Annual soil respiration was calculated for each of the 15 collars, and sampling uncertainty was estimated as the standard error of the mean across these 15 collars.

Due to the low number of measurement points for respiration partitioning, both plots from the same site were analyzed together (a total of eight long and short collars per site). Some measurements from long collars had to be discarded due to waterlogging (two collars from AC that were too close to the basal schist and were observed to have almost permanent standing water were not considered for further analysis). In the other collars that had no visual standing water, we saw no evidence of suppressed fluxes at high soil moisture content to warrant their exclusion. To estimate autotrophic and heterotrophic respiration for each site, the partitioning fractions determined from the experiment were multiplied by the total soil respiration obtained from the wider grid of soil respiration collars, i.e. the partitioning data were only used to determine the proportion of total soil respiration assigned to R_h and R_a.

Error propagation was carried out for the partitioning components using standard rules of quadrature, assuming that uncertainties are independent and normally distributed (e.g. Malhi et al. 2014).

5.2.4. Relationship between CO_2 efflux and soil environmental conditions

Mid-summer (January and February) climate conditions in 2013 were particularly warmer and drier than conditions during the same period in 2012. Rainfall was 41% and 31% lower in AC and AA, respectively and mean temperature was 1.98 °C and 2.63 °C warmer in AC and AA, respectively. At a broader scale, maximum and minimum temperatures in southern Chile were above the climatological mean during January and February 2013; maximum temperatures during January in Valdivia (a lowland site close to AC) were up to 4.9° C warmer than the climatological mean (Quintana and Aceituno 2013). Due to these conditions, and since future climate is expected to be warmer and drier in the study areas (Fuenzalida et al. 2007), it was particularly interesting to assess any changes in soil respiration between these two years.

In analyzing total respiration responses to interannual changes in temperature and moisture in each site, we factored out the seasonal variation in climate, because seasonal patterns in phenology and microbial populations are likely to be uncorrelated to interannual and long-term relationships between environmental variables and respiration (Fenn et al. 2010). The difference in soil temperature and water content between the same collar and month in both years (year 2 – year 1) were used as independent variables in a model to explain changes in the ratio of monthly respiration between both years (resp. year 2/resp. year 1) for each site. We applied a linear mixed model considering differences in temperature (T_2-T_1), soil water content (H_2-H_1) and plot, as well as their interactions as fixed terms and "month" as a random effect. Logarithmic transformation was applied to soil respiration ratios to achieve linearity. We used the protocol for model selection proposed by Zuur et al. (2009), and the chosen model was evaluated for normality and homoscedasticity in the residuals. Goodness-of-fit of the models was assessed using the marginal and conditional R^2, specially developed for generalized linear mixed effects models by Nakagawa and Schielzeth (2013). Marginal corresponds to the variance explained by fixed factors and conditional to the variance explained by both random and fixed effects (Nakagawa and Schielzeth 2013). Relationships were calculated considering all the months as well as just the period November-May in each study site. To test the relationship between soil respiration and temperature and to compare with the wider literature, the Q_{10} coefficient was calculated for the studied period.

The exponential relationship between R_s and temperature derived from Van't Hoff (Davidson et al. 2006b) was used:

$R_2/R_1 = exp^{b(T_2-T_1)}$ corresponding to $\ln R_2/R_1 = b(T_2 - T_1)$

Where R_2 and R_1 correspond to soil respiration recorded in each collar during each month in year 2 and 1, respectively and the same applies to temperature (T_2 and T_1), and b is the regression coefficient for the difference in temperature that is used in the Q_{10} calculation. A linear mixed model with month as a random effect was also applied in this case. Q_{10} was calculated as:

$$Q_{10} = \ exp^{10b}$$

5.2.5. Mass balance estimation of root productivity

This study formed part of a broader research program that quantified the productivity and carbon cycle of *Fitzroya* forests in these two sites (Urrutia-Jalabert 2015). Given the availability of heterotrophic respiration measurements and carbon inputs to the soil, a mass balance approach could be applied to estimate fine root production. Carbon (C) inputs to the soil (root mortality and litterfall) are assumed to correspond with belowground outputs (R_h, and dissolved organic C in water, DOC), including any alteration in soil C stocks (Malhi et al. 2009, Fenn et al. 2015). In this analysis the period November 2011 to October 2012 was used, since R_h / R_a respiration measurements started this month in both sites and more reliable estimates can be obtained with less gaps in the annual data. We assumed quasi-equilibrium conditions on an annual timescale, i.e. no net accumulation or loss of belowground carbon. The use of only one year of data was mainly because we cannot assume multiyear steady state conditions in the coastal site, due to different amounts of litterfall in the two studied years (Urrutia-Jalabert 2015), and warmer and dryer conditions during the second summer. $NPP_{fineroot}$ (Mg C ha^{-1} yr^{-1}) in each study site was estimated as follows:

$$NPP_{fineroot} = R_h - Litterfall - (Mort_{AG} + NPP_{branchfall}) * F_{cwd} - Mort_{BG} + F_{DOC} + \Delta C$$

Where R_h is the soil heterotrophic respiration for the period November 2011-October 2012 in AC or AA. *Litterfall* corresponds to the mean annual amount of litterfall collected in both plots from each site. $Mort_{AG}$ corresponds to the mean aboveground mortality and $NPP_{branchfall}$ to the mean productivity associated to this component in both plots from each site. *Litterfall,* $Mort_{AG}$ and $NPP_{branchfall}$ were obtained from Urrutia-Jalabert (2015) and are listed in Table S1. F_{cwd} corresponds to the fraction of coarse woody debris (CWD) entering the soil (not lost through in situ respiration). $Mort_{BG}$ is the belowground mortality which enters the soil and was determined as 6.9% of aboveground mortality (equivalent to the coarse root/aboveground biomass ratio found in *Fitzroya* forests from Chiloé Island, Battles et al. 2002). We applied a conservative uncertainty estimate for this parameter (±75%), i.e. $Mort_{BG}$ 0.069± 0.052. Finally, we assumed that aqueous carbon leakage (F_{DOC}) and the net change in carbon stocks (ΔC) were zero or insignificant terms on an annual basis, given the overall carbon balance in each forest (Fenn et al. 2015).

F_{cwd} has been rarely measured. Malhi et al. (2009) estimated F_{cwd} to be 0.24± 0.15 in lowland Amazon rainforests and Fenn et al. (2015) assumed a value of 0.25± 0.25 in a temperate broadleaved woodland in the UK, with a large (100%) uncertainty. Given the lack of information, in our study we assumed the value adopted by Fenn et al. (2015), and since material derived from CWD is not a very large component in our plots, this assumption would not have important effects in the final estimations. We applied a rigorous uncertainty propagation procedure throughout using uncertainty propagation by quadrature, as in Malhi et al. (2009) and Malhi et al. (2014).

Additionally, mean fine root biomass (≤ 2 mm diameter) was estimated from five cores (11 x 30 cm) extracted from the four corners and center of each plot in August-November 2011. Live and dead roots were manually removed from the soil in six 10 minutes time steps, washed as soon as possible, oven dried for 48 hrs (at 60 °C) and weighed. Calculation of the total biomass was made following a method that fits a curve to the cumulative dry root mass in order to estimate the amount of unsampled roots (Metcalfe et al. 2007).

Fine root residence time was calculated dividing fine root biomass by productivity and fine root carbon use efficiency was calculated as the ratio between fine root productivity and the sum of this productivity plus belowground autotrophic respiration.

5.2.6. Statistical analyses

The significance of the difference in total soil respiration between sites was tested for each year considering measurements taken from November to May and a nested design, with plots nested within sites. A linear mixed model with sites as fixed and plots as random effects was used. This allows accounting for the soil respiration measurements within plots as possible pseudo-replicates. The same approach was employed to detect differences in R_s and soil temperature and water content between both summers (end of December to end of February, where the highest fluxes were recorded). A paired t-test was used to assess differences in soil heterotrophic and autotrophic respiration between both sites during each year. All statistical analyses were performed using the R statistical environment (R Development Core Team 2014) and the “lme” function from the “nlme” package was used to perform the linear mixed models (Pinheiro et al. 2014).

5.3. Results

5.3.1. Seasonal patterns in soil respiration

Seasonal variation in soil respiration showed the expected pattern of higher values during summer months in both study sites. There is not much information for winter months in the Andean site, but it is possible to observe that R_s values during these months were lower than during the rest of the year (Fig. 1). These patterns broadly correspond with soil temperature variations, with higher R_s values occurring when higher soil temperatures were recorded. Moreover, high R_s values in AC corresponded with periods of particularly lower soil water content, especially during January 2013, when the highest R_s value (~4.8 µmol CO_2 m^{-2} s^{-1}) coincided with the highest soil temperature (~14.7 °C) and the lowest soil water content recorded during the studied period (~24%, Fig. 1). In the Andean plots higher R_s during both summers was also associated with higher temperatures and lower soil water contents (Fig. 1). It is noticeable how soil conditions from the end of December 2012 to February 2013 were significantly different (warmer and drier) than the previous summer in the coastal site ($P<0.05$), leading to particularly high respiration values during this period. Soil respiration was significantly different between both summers in AC ($P<0.05$), a pattern that was not reproduced in the Andes ($P>0.05$).

Soil summer temperatures were higher in the coastal site (10.9°C -12.7°C during 2011-2012 and 2012-2013, respectively) compared with the Andes (10°C -9.8 °C during 2011-2012 and 2012-2013, respectively) and soil water contents were much lower in this former site along the studied period (monthly means of 24% to 43% in AC compared to 44% to 61% in AA, Fig. 1).

Autotrophic and heterotrophic respiration showed the same pattern as total respiration with higher values during summer in both sites, and especially during the second growing season in AC (2012-2013, Fig. 2). Monthly R_a was higher than R_h respiration for most of the studied period in AC, and this was especially the case for summer 2012-2013. The opposite was true in AA, where monthly heterotrophic respiration was higher than root respiration for most of the recorded period (Fig. 2).

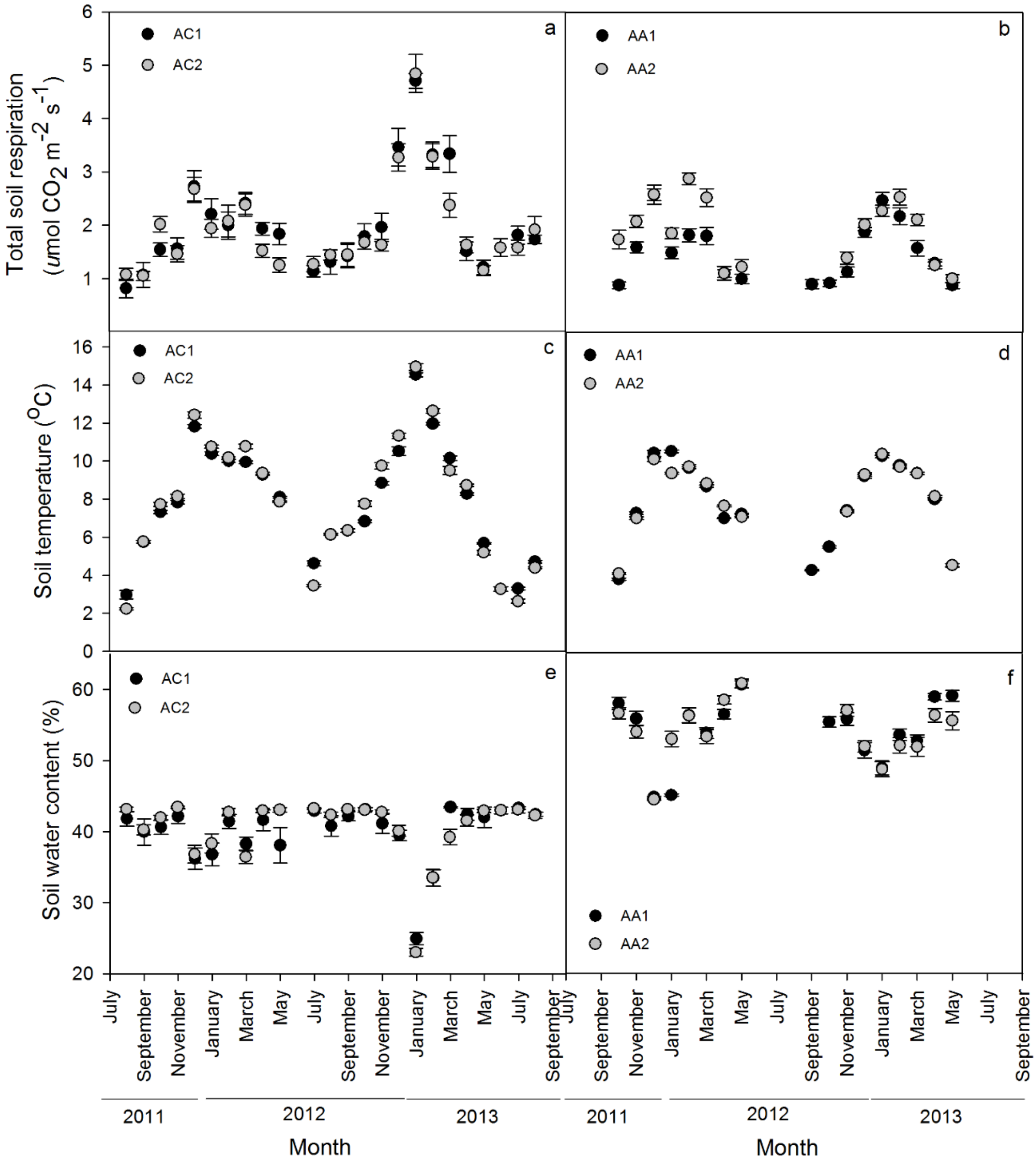

Fig. 1 a) Total soil respiration discrete measurements in Alerce Costero plots (AC1, AC2) and b) Alerce Andino plots (AA1, AA2) mainly from August 2011 to August 2013. c) and d) soil temperatures measured at the same time as soil respiration and e) and f) soil water content also monitored during each respiration measurement

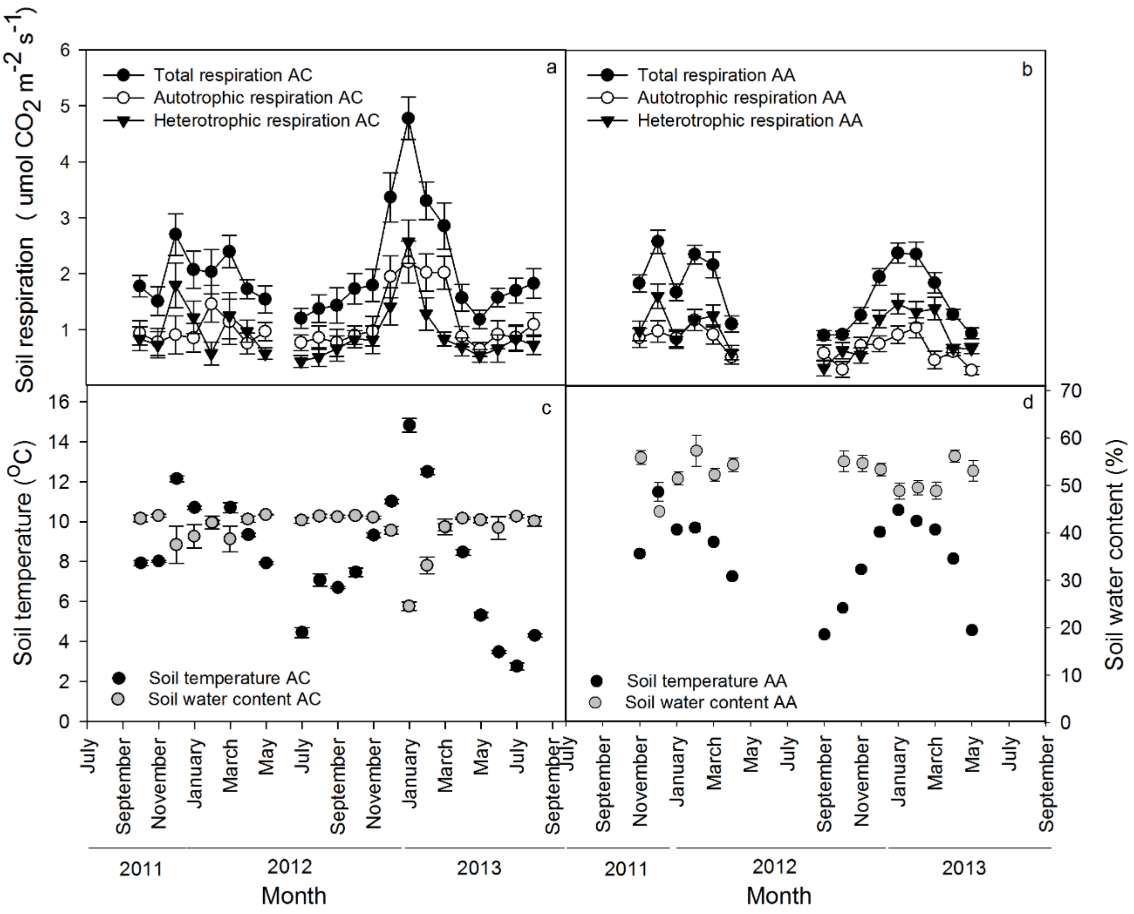

Fig. 2 a) Total, heterotrophic and autotrophic (total minus heterotrophic) respiration in Alerce Costero (AC) and b) Alerce Andino (AA) covering the period October/November 2011 to May/August 2013. c) Soil temperature and water content measured at the same points and time as soil respiration in AC; d) the same as c), but for AA

5.3.2. Total soil, autotrophic and heterotrophic respiration.

Annual R_S values were a little lower in AA (5.06-6.14 Mg C ha^{-1} yr^{-1}) than in AC plots (6.37-6.66 Mg C ha^{-1} yr^{-1}) during the 2011-2012 period and much lower (4.98-5.35 Mg C ha^{-1} yr^{-1} compared with 8.08-8.64 Mg C ha^{-1} yr^{-1}) during the 2012-2013 period (Table 2). Mean respiration values obtained for November-May were not significantly different between the two sites for the period 2011-2012, but they were significantly different during the following year, where mean values were much higher in the coastal site (2.59-

2.79 μmol CO_2 m^{-1} s^{-1}) than in the Andes (1.63-1.79 μmol CO_2 m^{-1} s^{-1}, $P<0.05$, Table 2). We estimated that respiration during November-May represented 68-70% and 71-74% of total annual soil respiration during 2011-2012 in AC and AA, respectively (Figure 3). For 2012-2013, these proportions were 71% and 71-73% in AC and AA, respectively (Figure 3). Annual R_a was a little lower in AA (2.68 ±0.36 Mg C ha^{-1}) than AC (3.38±0.64 Mg C ha^{-1}, difference not significant) during 2011-2012 and significantly lower during 2012-2013 (2.09±0.29 compared with 4.68±0.63 Mg C ha^{-1}, Table 2). Heterotrophic respiration was similar during the first year in both sites (~3.14 Mg C ha^{-1} yr^{-1}) and a little lower in AA (3.13±0.33 Mg C ha^{-1} yr^{-1}) than AC (3.65±0.54 Mg C ha^{-1} yr^{-1}) during the second period, although values were not significantly different. Finally, the proportion R_a/R_s was higher in AC (51.8-56.2%) than AA (46.1-39.9%, Table 2) for the two studied years.

Table 2. Total annual and mean November to May soil respiration (R_s) in each plot for the two studied periods. Annual autotrophic and heterotrophic respiration are also presented for each site (Alerce Costero (AC) and Alerce Andino (AA)) and the proportion of autotrophic to total soil respiration.

Plot	Annual R_s (Mg C ha^{-1} yr^{-1})		Mean November-May R_s (μmol CO_2 m^{-2} s^{-1})		Annual autotrophic respiration (Mg C ha^{-1} yr^{-1})		Annual heterotrophic respiration (Mg C ha^{-1} yr^{-1})		Proportion autotrophic /total respiration (%)	
Period[a]	1	2	1	2	1	2	1	2	1	2
AC1	6.66±0.58	8.64±0.73	2.09±0.23	2.79±0.25	3.38±0.64	4.68±0.63	3.14±0.54	3.65±0.54	51.8±10.7	56.2±8.70
AC2	6.37±0.47	8.08±0.54	1.89±0.20	2.59±0.22						
AA1	5.06±0.33	4.98±0.29	1.62±0.13	1.63±0.11	2.68±0.36	2.09±0.29	3.14±0.37	3.13±0.33	46.1±6.73	39.9±5.92
AA2	6.14±0.31	5.35±0.25	2.03±0.13	1.79±0.11						

a: Period 1 corresponds to 2011-2012 and period 2 to 2012-2013.

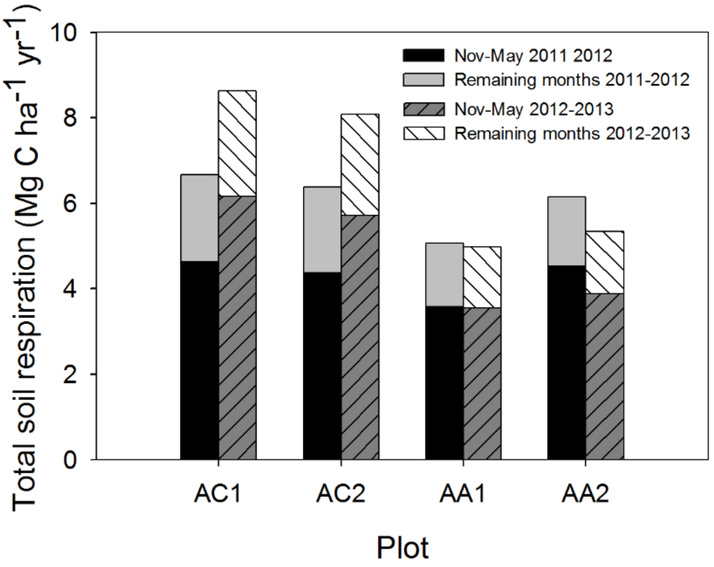

Fig. 3 Estimated total soil respiration during the period November-May and total annual soil respiration for the four plots (Alerce Costero (AC1 and AC2) and Alerce Andino (AA1 and AA2)) during the two studied years

5.3.3. Sensitivity of soil respiration to soil temperature and water content

The outputs from the linear mixed model in AC included both the difference in soil temperature (T_2-T_1) and water content (H_2-H_1), as well as the interaction between both terms as variables significantly explaining the ratio of monthly soil respiration between both years (Table 3). The goodness of fit of this model was $R^2_{marginal} = 0.28$ and $R^2_{conditional} = 0.36$, and was much higher when just considering the period November-May which mainly excludes winter in the analysis ($R^2_{marginal} = 0.42$ and $R^2_{conditional} = 0.47$, Table 3). The interaction term mainly indicates that low soil humidity conditions during year 2 intensified the effect of warmer temperatures on higher respiration. If the ratio of respiration between years were ascribed to temperature alone, $R^2_{marginal}$ values were much lower than when also considering soil water content effects (0.20 and 0.32 for the whole year and the period November-May, respectively, Table 3). In addition, the Q_{10} values were 3.49±1.01 and 4.39±1.40 considering either all months or the period November-May only, respectively (Table 3). In AA none of the environmental terms significantly explained soil respiration, so results are not presented. Warmer soil temperatures and lower soil water contents during the second year produced significantly higher respiration in the coastal site, but not in the Andes where soil conditions were not particularly different between both summers and pronounced changes in respiration were not observed (Fig. 4).

Table 3. Models of total soil respiration (natural logarithm of monthly ratio between year 2 and year 1, R_2/R_1) in relation to the difference in soil temperature and water content between both years for Alerce Costero (AC). Models only including the difference in temperature as an independent variable, in order to calculate a Q_{10} value, are also presented. Models including all data available from August 2011 to August 2013 and just including the period November-May are shown. N (number of data points), the residual variance, random intercept variance, as well as Q_{10} and $R^2_{marginal}$ and $R^2_{conditional}$ are included. a, b, c and d correspond to the intercept, and coefficients for the difference in temperature (T_2-T_1), soil water content (H_2-H_1) and the interaction between them (T_2-T_1)(H_2-H_1), respectively.

Site	N	a	b	c	d	Residual variance	Random intercept variance	Q_{10}	Marginal/conditional R^2
AC	287	0.130	0.116	0.019	-0.009	0.194	0.024	-	0.28/0.36
AC	290	-	0.125	-	-	0.203	0.057	3.49	0.20/0.38
$AC_{Nov-May}$	186	0.101	0.132	0.015	-0.008	0.171	0.018	-	0.42/0.47
$AC_{Nov-May}$	189	-	0.148	-	-	0.178	0.049	4.39	0.32/0.47

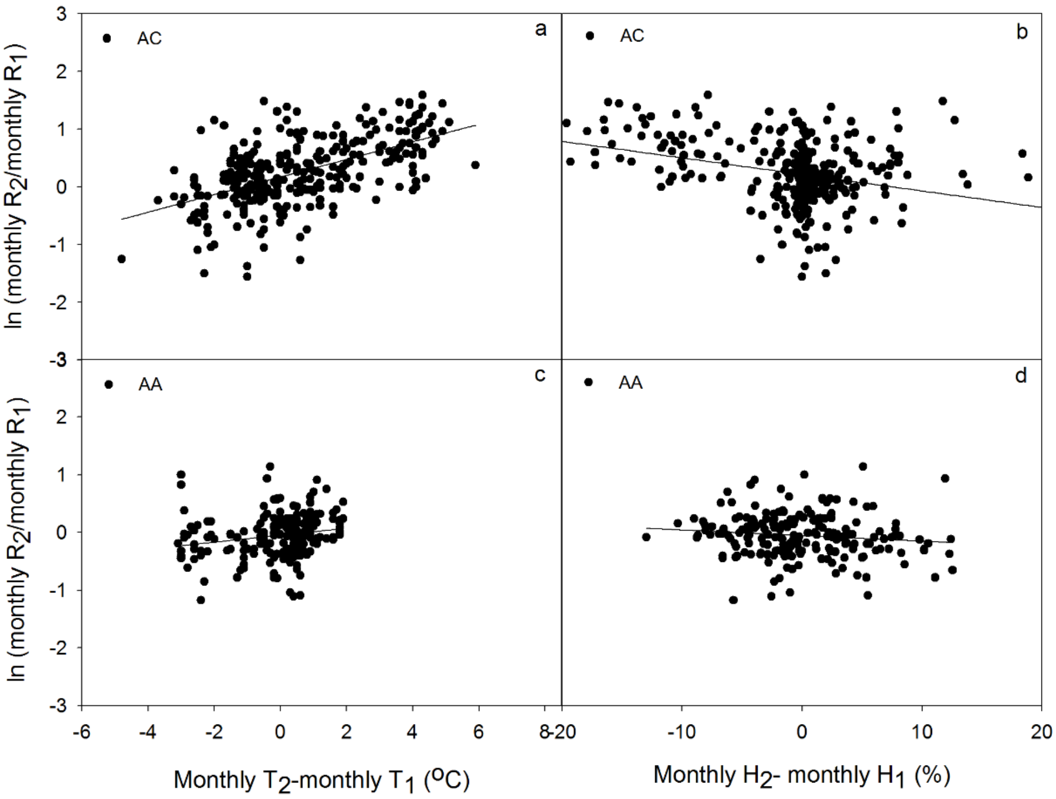

Fig. 4 a) and c) relationship between soil temperature (T_2-T_1) and soil respiration (ln (R_2/R_1)) in Alerce Costero (AC) and Alerce Andino (AA), respectively. b) and d) relationship between soil water content (H_2-H_1) and soil respiration (ln (R_2/R_1)) in AC and AA, respectively. R_2 and R_1 correspond to soil respiration recorded in each collar during each month in year 2 and 1, respectively and the same applies to temperature (T_2 and T_1) and soil water content (H_2 and H_1). The straight lines reflect the direction of the relationship between the environmental variables and the natural logarithm (ln) of (R_2/R_1). The statistics of the combined effect of (T_2-T_1) and (H_2-H_1) on ln (R_2/R_1) in AC are presented in Table 3

5.3.4. Fine root production estimation

Our mass balance-based estimates of $NPP_{fineroot}$ in AC and AA for the period November 2011-October 2012 were 0.81±0.60 and 1.50± 0.42 Mg C ha^{-1} yr^{-1} in AC and AA, respectively (Table 4). Due to the relatively large error term, calculated differences between sites are not significant.

The estimates of fine root biomass were almost the double in the Coastal Range site than in the Andes (~21-22 vs 10-12 Mg C ha^{-1}, respectively, Table 4). Therefore, mean estimates of fine root residence times are much larger in AC than AA (Table 4). The low productivity but high fine root biomass suggests very long fine root lifetimes in these forests, especially in the Coastal Range site.

Table 4. Fine root biomass, productivity and fine root mean residence time in each of the study sites (Alerce Costero, AC and Alerce Andino, AA). The two values shown for biomass and residence times correspond to the two plots per site.

Site	Fine root biomass (Mg C ha^{-1})	Fine root productivity (Mg C ha^{-1} yr^{-1})	Fine root residence time (years)
AC	21.3 ±1.1-22.2±1.6	0.81±0.60	26.4± 19.6-27.4± 20.4
AA	10.1±1.4- 12.1±2.2	1.5± 0.42	6.7±2.1- 8.04± 2.7

5.4. Discussion

In this paper we have assessed seasonal patterns of total soil respiration and its autotrophic and heterotrophic components in *Fitzroya* stands from the Coastal Range and the Andean Cordilleras of southern Chile. We have estimated annual fluxes for these components and assessed the soil environmental variables that were mostly related with total respiration. Finally, we have employed a mass balance approach that uses heterotrophic respiration and soil carbon inputs, to estimate fine root productivity in each study site.

5.4.1. Seasonal and annual CO_2 effluxes in total respiration and its partitioning

Seasonal variability in soil respiration in both sites was characteristic of patterns observed in other temperate systems, where higher soil respiration correspond with warmer summer conditions (Curiel Yuste et al. 2004, Sulzman et al. 2005, Fenn et al. 2010, Ngao et al. 2012). Soil water content patterns in our study sites were characteristic of temperate sites with some Mediterranean influence (especially in the coastal site) where summers are drier than the rest of the year. Soil water content was higher in AA, compared to AC all year long. This would be expected given the larger amounts of annual and summer precipitation, the reduced transpiration under frequent cloudy conditions, as well as the greater retention properties of the soils at this site.

General weather conditions were warmer and drier during summer 2012-2013 than in the previous year, but this translated into significantly warmer soil temperatures and lower soil water contents only in AC. There was a notable increase in soil respiration in AC over the warm and dry summer, probably due to warmer soils and dry conditions that increased the diffusion and contact surface for oxygen in pore spaces in these usually wet soils, increasing the rates of aerobic respiration (Davidson et al. 1998). Sites that are normally wet but occasionally dry considerably during summer can experience a large release of CO_2 with an increase in microbial and autotrophic respiration (Davidson et al. 1998). High soil water contents reduce the emission of CO_2, not only because of low production rates, but also because of lower diffusion, hence the increase in CO_2 effluxes under drier conditions could also be reflecting a higher diffusion rate (Schwendenmann et al. 2003, Wood et al. 2013).

However, large reductions in water content can also reduce CO_2 effluxes (Singh and Gupta 1977). Thus, most studies in temperate forests report lower respiration rates when drier conditions occur, due to slower nutrient transport and reduced and less active microbial populations (Davidson et al. 1998, Savage and Davidson 2001, Irvine and Law 2002, Lavigne et al. 2004, Fekete et al. 2014). Low soil moisture has been shown to limit soil respiration and masks the dependence of soil respiration on soil temperature in warm temperate forests and Mediterranean ecosystems (Reichstein et al. 2003, Tedeschi et al. 2006).

Soil CO_2 effluxes generally show a curvilinear response to soil moisture, with low respiration rates at low and high water contents and varying thresholds at which the relationship respiration- soil moisture varies from positive to negative (Singh and Gupta 1977). Soil water contents along the year surpassed 24% and 44% in the Coastal and Andean sites, respectively. These values are well above the 12%-15% threshold below which a positive response of soil respiration to water content has been reported for temperate forests in the US and Europe (Davidson et al. 1998, Ruehr et al. 2010). This would indicate that in these normally wet *Fitzroya* sites water content probably rarely falls below a threshold that would negatively influence soil respiration even during summers. Given that significant differences in soil respiration between stands of different ages are only present during the second year, differences between sites are likely influenced by the more Mediterranean climate influence and poorer soil conditions in the stand from the Coastal Range. Shallower soils, lower water retention capacity and less humid conditions during summer would influence the sensitivity of soils in AC.

Heterotrophic respiration also increased and peaked at the time when warmer and drier conditions were observed in AC. However, R_h was a smaller fraction of total R_s compared with the previous year, a finding consistent with other studies which report a decline in heterotrophic respiration during particular dry summer conditions, mainly because of the decrease in decomposition in dry litter layers (Scott-Denton et al. 2006, Trumbore 2006).

R_a on the other hand, has been reported to be less sensitive to dry conditions, since the metabolism of roots seems less affected by these conditions than the metabolism of microorganisms (Epron et al. 2001, Scott-Denton et al. 2006).

Annual total soil respiration estimates during the first year in both sites (5.06-6.66 Mg C ha^{-1} yr^{-1}) were lower than values reported for an old-growth coniferous forest in Oregon (7.27- 8.41 Mg C ha^{-1} yr^{-1}, Sulzman et al. 2005) , lower than the mean value recorded for evergreen (mostly coniferous) forests along an altitudinal gradient in Europe (7.72 Mg C ha^{-1} yr^{-1}, Rodeghiero and Cescatti 2006) and similar to fluxes modelled for an old growth Ponderosa Pine forest in the drier eastern Cascades, Oregon (4.83-5.97 Mg C ha^{-1} yr^{-1}, Irvine and Law 2002). Values during both years were within the broad range of values reported for temperate coniferous forests from the northern hemisphere (4.27-18.05 Mg C ha^{-1} yr^{-1} , Hibbard et al. 2005), and much lower than values reported for mature and old-growth stands in rainy sites of western Oregon (10.8-20.7 Mg C ha^{-1} y^{-1}, Campbell and Law 2005).

Higher annual respiration amounts in AC than AA were primarily due to higher R_a in this coastal site, which was significantly higher during the second year. Higher autotrophic respiration in AC than AA are consistent with differences in root biomass between sites. Positive correlations have been reported between R_a and root biomass across different temperate forests in China for example (Wang and Yang 2007).

R_h was generally higher than R_a in the Andes and there were no significant differences with the coastal site in the two studied years. We expected lower R_h in AA than in AC, mainly because litterfall was lower (1.21±0.064 vs 2.41±0.220 Mg C ha^{-1} yr^{-1}, Table S1). However, the accumulation of organic matter inputs throughout time due to larger and older trees and higher rates of mortality in these forests could result in relatively higher heterotrophic respiration (Saiz et al. 2006, Urrutia-Jalabert 2015). Additionally, R_h rates per unit of soil carbon are lower in the Andean site (0.025 year^{-1}) than in the Coastal Range (0.048 year^{-1}) considering data for the first studied year; giving a mean soil carbon residence time of 21 and 40 years in AC and AA, respectively. This is due to the larger soil carbon stocks in the upper horizon in the Andes (124.7 Mg ha^{-1}) compared with the coastal site (64.8 Mg ha^{-1}), reflecting the slower dynamics of the old-growth stand (i.e. soil carbon in AA cycles at half the speed of AC).

5.4.2. Limitations in total respiration estimates and its partitioning

One possible bias to consider is that total soil respiration in these sites may be underestimated because of collar insertion cutting roots and reducing root biomass and autotrophic respiration (Heinemeyer et al. 2011). However, roots were not cut with a knife in our setup, and are likely to just have been displaced downwards rather than cut with collar insertion. Most soil respiration studies with which we compare our results apply a similar methodology and do not correct for this under-estimate, so relative comparisons are still valid.

Another bias to consider is potential waterlogging in the partitioning experiment, caused by the deliberate removal of roots, which may suppress heterotrophic respiration. This was a concern especially in Alerce Costero due to the shallow soils on top of schist. We tried to minimize the effect of waterlogged soils on R_h estimates not considering the partitioning units that had water build-up within the collars. It is still possible that other collars were waterlogged below the soil surface, however we did not monitor soil moisture within individual collars. While recognizing this as a potential bias, we note that our R_a / R_h ratio estimate in both sites of around 1:1 is typical of the range found in other temperate forests (Epron et al. 2001, Irvine and Law 2002, Lavigne et al. 2003, Ruehr and Buchmann 2010).

Finally, it is possible that soil disturbance caused by root removal at the set-up of the partitioning experiment would cause increased fluxes immediately after installation, followed by a gradual decline (Hanson et al. 2000). Two days have been reported to be enough for CO_2 rates to stabilize after root removal in larger soil volumes than the ones used in our experiment (Edwards 1991). However, we did not observe high fluxes one day after collar installation and once analyzing trajectories in R_h over the two years, we found no evidence of such a pattern in the flux data (Figure S1).

5.4.3. Sensitivity of soil respiration to soil temperature and water content

Our study allowed the assessment of differences between sites in their soil respiration response to drier and warmer weather conditions, which are expected to increase in frequency under climate change. The soil respiration ratio between both years (R_2/R_1) was sensitive to soil temperature differences, as well as to soil water content differences and the interaction between them in AC. A strong influence of differences in temperature was expected, because most biological processes correlate with soil temperature in moist temperate ecosystems (Wang and Yang 2007). Soil moisture on the other hand, appears to be the main driver of soil respiration in arid systems like the one in northern Chile (Perez-Quezada et al. 2012). In sites with adequate soil water content (such as AA), the soil moisture sensitivity is low. However, in AC in the summer, the strong decline in soil water content (covarying with a sharp increase in temperature) leads to a strong increase in respiration and a high apparent Q_{10}. The apparent Q_{10} values found in AC (3.49-4.39) were higher than values reported for other coniferous forests growing under milder conditions (1.49-3.0, Campbell and Law 2005, Sulzman et al. 2005), but they were within the range of values reported for temperate forests in a global database (2.1-6.3, Bahn et al. 2010), and close to the values reported for a mixed hardwood forest in northeastern US (3.4-5.6, Davidson et al. 1998). Occasional drier conditions in usually wet sites can lead to higher Q_{10} values (Davidson et al. 1998). This is because in wet ecosystems substrate availability can increase at warmer temperatures when drier soils have a larger effect on redox conditions rather than on the diffusion of substrates (Davidson et al. 2006b). It is important to note that Q_{10} values in this study are approximations, since they were calculated with interannual temperature variations much lower than 10 °C and relationships can change when considering larger ranges.

In contrast with the Coastal Range site, at the Andean site interannual changes in soil temperature and water content were not large enough to cause a significant response in soil respiration. This means that this site seems more resilient to climate warming and drying due to overall wetter summers and better soil conditions.

5.4.4. Soil respiration and productivity

Our mean fine root biomass estimates (22.2±1.6 and 21.3±1.1 Mg C ha^{-1} in AC1 and AC2 and 10.1±1.4 and 12.1±2.2 Mg C ha^{-1} in AA1 and AA2) are higher than the mean recorded for temperate coniferous forests in a global study (~4.1 Mg C ha^{-1}, Jackson et al. 1997). These values are also higher, or in the case of the Andean site, in the range of indirect biomass estimates for old-growth wet forests from the Pacific Northwest (5.2- 12.6 Mg C ha^{-1}, Smithwick et al. 2002). High fine root biomass values in AC are probably associated with the predominantly low nutrient conditions and poor water retention properties of the soils (Table 1). This is consistent with the widely reported negative correlation between root biomass and soil fertility conditions (Yuan and Chen 2010). Given the higher fine root biomass and the lower (or somewhat similar) root productivity in the coastal site compared with the Andes, the fine root residence time appears much longer in the younger forests from the Coastal Range.

Mass conservation requires that on medium-long timescales (annual to multi-year scales) soil heterotrophic respiration is closely related to productivity. If soil carbon stocks are not showing significant increase or decline or substantial lateral export, the soil (and litter) heterotrophic respiration is equal to inputs of organic material through leaf and root litter, plus breakdown of woody necromass, which are in turn and in total closely related to long-term NPP. Higher rates of respiration have been reported in more productive sites (Lavigne et al. 2003, Curiel Yuste et al. 2004), with primary productivity an important driver of soil respiration at short (hours, daily) and annual time scales (Kuzyakov and Gavrichkova 2010). In this study, the slightly higher soil respiration in AC is probably linked to the higher litterfall and overall productivity reported for this site (Urrutia-Jalabert 2015); however, we observed high interannual variability in AC, so a longer time series would be required to better match soil respiration to productivity in this area.

Fine root production estimates in both sites are below the mean reported for temperate forests in a global study (~4.28 Mg dry mass ha^{-1} yr^{-1}, Finér et al. 2011), and these low values are consistent with the overall low estimates of aboveground productivity reported for these forests (3.35-3.36 and 2.22-2.54 Mg C ha^{-1} yr^{-1} in AC and AA, respectively, Urrutia-Jalabert 2015). Fine root production may be low due to the high exchangeable aluminium content in both sites that can prevent cell division and root elongation (Lambers et al. 2008, Urrutia-Jalabert 2015).

Fine root production was also well below total belowground carbon allocation (TBCA) as defined by Raich and Nadelhoffer (1989), suggesting that fine root production was considerably lower than root respiration. Fine root carbon use efficiencies reached 0.19 and 0.36 in AC and AA, respectively. The Andean value is close to the fine root production/TBCA ratio obtained for some deciduous and coniferous forests in the US (0.33, Nadelhoffer and Raich 1992), and both estimates are much lower than the mean belowground carbon use efficiency (including fine and coarse roots) found for mature forests (0.47, Chen et al. 2011) and for forests in a temperature gradient worldwide (0.5, Litton and Giardina 2008). One possible explanation for the low fine root carbon use efficiency particularly in the coastal site, could be the high fine root biomass found in these forests, which could cause higher rates of maintenance respiration per unit ground area (McDowell et al. 2001, Chen et al. 2011). The apparent extraordinary longevity of the fine roots, particularly in the AC site may also require greater investment in root maintenance and defence against herbivores and pathogens, rather than investment in growth. It is also possible that if there was any underestimate of R_h (caused by non-apparent waterlogging, for example), this would lead to an underestimate of fine root productivity and thereby of fine root carbon use efficiency.

5.4.5. Final remarks

To our knowledge there have been no other medium-term (more than a year) field studies on the soil carbon fluxes in temperate ecosystems of southern South America. Therefore, this study contributes to understanding how these humid ecosystems may in principle respond to interannual variability. Our study points out the potential for strong interannual variability in the CO_2 effluxes emitted especially by *Fitzroya* forests growing in the Coastal Range of southern Chile, but less variability in the more humid soils of the Andean Range. Warmer and drier weather conditions, which could be more common in the future (Fuenzalida et al. 2007), significantly influence the soil environment at the Coastal Range and lead to higher soil respiration. Thus, moderate drying leads to a large soil CO_2 efflux in this particularly wet site, although much stronger drying under the projected climate change scenario could cause the opposite effect (Davidson et al. 1998). On the other hand, soil respiration seems more stable and resilient to these interannual changes in old-growth forests from the Andes, where soils do not get particularly affected by more extreme weather conditions. More years of research and monitoring are needed for a better long-term understanding of these responses.

Our measured CO_2 effluxes in *Fitzroya* forests are at the lower end of values reported for other mature and old-growth wet temperate forests in the world. *Fitzroya* forests also show slower fine root dynamics. These features could probably be due to the longevity as well as the very rainy conditions and poor soil conditions (resulting in low productivity) where this species grows. There seems not to be a significant difference in soil respiration between forests of different ages growing under different environmental conditions, but differences appear when climate changes and the site with more restrictive conditions increases CO_2 effluxes. Therefore, the underlying environment is a key factor to consider when evaluating forests responses to a changing climate.

Our study makes a novel contribution in the monitoring and understanding of carbon fluxes in temperate rainforests affected by summer droughts and growing under restrictive soils. It would be important to determine if soil respiration increases in AC are accompanied by higher fine root production or if they just would imply that these forests behave as net carbon sources under warm and dry conditions. Further studies should focus on performing long-term measurements in both sites to have a more accurate estimation of the effects of changes in climate on soil autotrophic and heterotrophic effluxes, and to examine the effect of soil respiration variability on interannual variations in net ecosystem exchange.

5.5. Acknowledgements

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Chapter 5: Supplementary information

Tables and Figures

Table S1. Parameters used to estimate fine root productivity (NPP$_{\textit{fine root}}$) in Alerce Costero (AC) and Alerce Andino (AA). Litterfall corresponds to the mean annual amount of litterfall collected in both plots from each site (period November 2011-October 2012), Mort$_{AG}$ to the mean aboveground mortality, NPP$_{branchfall}$ to the mean productivity associated to this component, F$_{cwd}$ to the fraction of coarse woody debris (CWD) entering the soil and Mort$_{BG}$ to the belowground mortality which totally enters the soil

Site	Litterfall (Mg C ha^{-1} yr^{-1})	Mort$_{AG}$ (Mg C ha^{-1} yr^{-1})	NPP$_{branchfall}$ (Mg C ha^{-1} yr^{-1})	F$_{cwd}$	Mort$_{BG}$ (Mg C ha^{-1} yr^{-1})
AC	2.41±0.220	0	0.038±0.008	0.25±0.25	0
AA	1.21±0.064	0.66±0.33	0.270±0.110	0.25±0.25	0.045±0.041

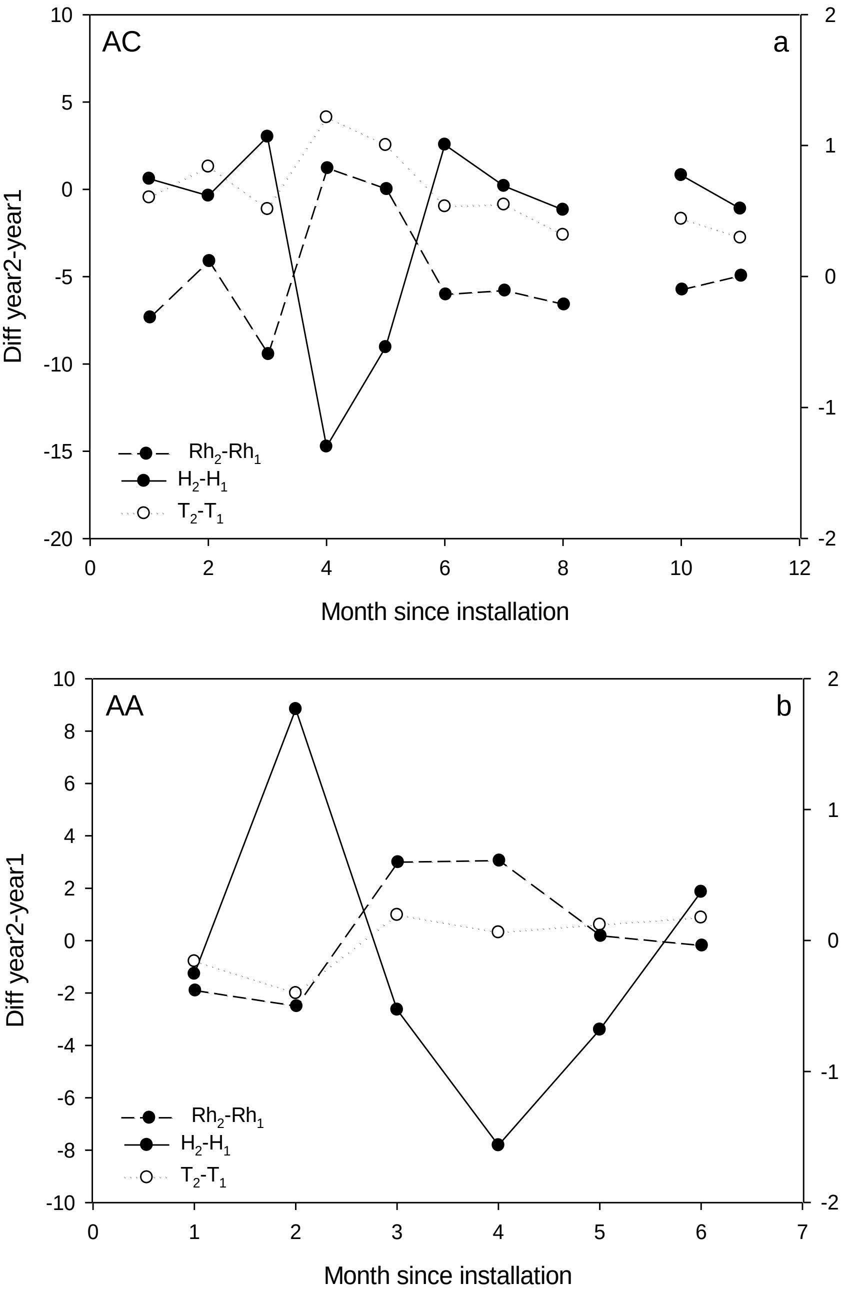

Figure S1. a) Differences in heterotrophic respiration (Rh_2-Rh_1) between the same month in year 2 and year 1 (right axis) and differences in soil temperature (T_2-T_1) and soil water content (H_2-H_1, left axis) in Alerce Costero (AC). The first month corresponds to October (2012-2011). b) The same as a) but for Alerce Andino (AA) and the first month corresponds to November (2012-2011). Variations in heterotrophic respiration differences since month of installation do not follow a response after disturbance pattern and mostly follow variations in temperature and water content differences.

Linking statement: Chapter 5 to 6

The previous two chapters examined the above (Chapter 4) and belowground (Chapter 5) carbon dynamics in *Fitzroya* forests. In addition, Chapter 5 assessed the main soil environmental variables that influenced belowground carbon fluxes in these forests. I next wanted to understand the environmental factors related to variation in aboveground carbon fluxes, and most specifically in woody productivity. Therefore, the last two chapters explore these relationships using tree-ring/radial growth and climate data at a high (intra-annual) and medium/low (annual/decadal) temporal resolution.

Chapter 6 deals with the understanding of tree radial growth-climate relationships at a high temporal resolution. This is required, because the variables that usually appear to drive interannual growth changes in dendrochronological studies are not necessarily the ones that drive cellular growth. This chapter addresses the fifth specific objective: **to evaluate which climatic factors are mainly related to *Fitzroya* stem radial change on an intra-annual basis using high precision point dendrometers in trees from the two study areas.** This paper investigates the environmental variables that are mostly related with stem radial contraction and increment in this species using radial change and environmental data collected over almost two years.

I was the main author and wrote the paper, I collected data in the field with the help of research assistants. Dr. Sergio Rossi and Prof. Annie Deslauriers gave guidance and instruction on data analysis and Sergio Rossi provided the scripts in SAS to analyse the dendrometer data. Yadvinder Malhi also provided guidance on data analysis. I wrote the manuscript and conducted the analyses, with inputs and comments from all co-authors including Antonio Lara.

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Chapter 6: Environmental correlates of stem radius change in the endangered *Fitzroya cupressoides* forests of southern Chile

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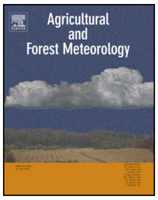

Environmental correlates of stem radius change in the endangered *Fitzroya cupressoides* forests of southern Chile

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ABSTRACT

Relationships between environmental factors and stem radius variation at short temporal scales can provide useful information regarding the sensitivity of tree species' productivity to climate change. This study used automatic point dendrometers to assess the relationship between environmental variables and stem radius contraction and increment in ten *Fitzroya cupressoides* trees growing in two sites, the Coastal Range (Alerce Costero National Park) and the Andean Cordillera (Alerce Andino National Park) of southern Chile. The growing season in each site, determined using stem daily cycle patterns for each month, was longer in the Coastal Range site than in the Andes. Warmer and sunnier conditions were positively related with daytime tree radius contraction in both areas, and relationships were stronger in the Coastal Range site where more pronounced shrinking events were associated with prolonged warm and dry conditions compared to the Andes. Stem increment was positively related with precipitation and humidity in both sites, reflecting the positive effect of water on cell turgidity and consequent enlargement. Relationships between stem radius change and environmental variables considering longer temporal scales (7 to 31 days), confirmed a stronger association with humidity/vapor pressure deficit and precipitation, rather than with temperature. Although *Fitzroya* grows in particularly wet and cool areas, current and projected drier and warmer summer conditions in southern Chile may have a negative effect on *Fitzroya* stem increment and carbon accumulation in both sites. This effect would be more critical in the Coastal Range compared with the Andes though, due in part to more limiting soil conditions and less summer precipitation in this area. Long-term research is needed to monitor different aspects of the response of these endangered ecosystems to this additional threat imposed by climate change.

6.1. Introduction

Climate change is likely to have considerable effects on tree growth and forest productivity (Boisvenue and Running, 2006); however, the directionality of these changes remains unclear. Positive effects on growth may occur due to CO_2 fertilization of photosynthesis (although there is an ongoing debate about the extent of this effect on forests), as well as because of an increase in growing season length due to higher temperatures (Allen et al., 2010). Increases in productivity might be observed in cold climates due to warming, where water is sufficient to compensate for greater vapor pressure deficits, and also in water-limited systems due to precipitation increases. Negative effects on growth may occur due to increased evaporative demand due to warmer temperatures and deficits in precipitation (Fischlin et al., 2007). The specific response of forests is likely to vary from site to site, so the mechanistic assessment of current tree growth-climate relationships can inform our understanding of species' sensitivities to climate change.

In southern Chile, summer temperatures are projected to increase up to 4 °C and precipitation is projected to decrease up to 50% by 2100 in a medium-high greenhouse gas emission scenarios (Fuenzalida et al., 2007). In fact, a pronounced decrease in annual precipitation has been observed in the region during the last

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century in combination with an increase in the frequency of droughts, especially during the last 50 years (Trenberth et al., 2007; Christie et al., 2011; González-Reyes and Muñoz, 2013). These changes are likely to have a particular impact on the growth of endemic tree species, commonly adapted to high precipitation and cool climate conditions.

Among the most compelling and least well-understood ecosystems in southern South America are *Fitzroya cupressoides* forests. *Fitzroya*, or alerce, is the second longest-lived tree in the world, with a maximum life span of >3600 years (Lara and Villalba, 1993). *Fitzroya* is endemic to the temperate rainforests of southern South America and mainly grows in the Andes of Chile and adjacent Argentina and in the Coastal Range of Chile between 39°50′ and 43°S (Veblen and Schlegel, 1982; Lara et al., 2002). It is a giant conifer that can reach heights of >50 m and diameters >5 m (Donoso et al., 2006), thus representing a huge potential for long-term carbon sequestration and storage under undisturbed conditions. It is currently listed as endangered in the IUCN Red List of Threatened Species (IUCN, 2013).

Despite the importance of *Fitzroya* given its long lifespan, slow growth, and conservation status, it has been poorly studied in terms of its physiology and growth responses to environmental conditions and climate change. Dendroclimatological studies have found that *Fitzroya* tree-ring growth is positively related with summer precipitation, and mainly negatively related with summer temperature, especially from the previous growing season (Villalba, 1990; Villalba et al., 1990; Lara and Villalba, 1993; Neira and Lara, 2000; Barichivich, 2005). It is likely however, that these are not the direct drivers of stem productivity, since this type of study focuses on growth processes at long time spans, leaving a gap in the understanding of the causal chain between cellular and radial growth (Köcher et al., 2012). Since inter-annual radial growth variability is the result of a combination of average climate conditions, as well as specific events, it is clear that the effect of short duration climatic events on radial growth in this species cannot be detected using a dendrochronological approach (Duchesne and Houle, 2011).

Cell division and enlargement, which are the processes that generate growth, are considerably more sensitive to changes in water content than photosynthesis (Muller et al., 2011). Irreversible growth occurs in a cell when a certain pressure threshold in the tissue is exceeded, so when there is water deficit in the tree, this inhibits cell division and particularly cell expansion (Hsiao and Acevedo, 1974; Lambers et al., 2008). Besides the positive effects of water, it has also been reported that temperature would be important in determining the growth rate of metabolic processes in the cambium, as temperature is minimum at night, when hydraulic conditions are more suitable for growth (Drew et al., 2008). Probably the only straightforward way to monitor growth at a short time scale, and therefore assess the direct environmental correlates of radial increment in particular species, is through the use of high precision dendrometers. This monitoring can provide valuable information regarding subtle differences in climate sensitivity among species or populations, and potential long-term limitations to forest productivity caused by climate change (Pérez et al., 2009).

High precision automatic dendrometers can provide information on variation in water storage throughout the year, as well as seasonal growth (Deslauriers et al., 2007a), and they have been widely used to describe stem growth phenology and to evaluate growth-climate relationships in various ecosystems (e.g. Downes et al., 1999; Deslauriers et al., 2003, 2007b; Mäkinen et al., 2003; Bouriaud et al., 2005; Biondi and Hartsough, 2010; Köcher et al., 2012).

To date, the only study that has assessed *Fitzroya* stem increment-climate relationships at a daily time scale was carried out using band dendrometers in Chiloé Island (at the southern distribution of *Fitzroya* in the Coastal Range, Pérez et al., 2009). The authors reported that daily stem growth was positively related to precipitation and negatively related to radiation. Nevertheless, it is not clear if these relationships hold for populations located toward the north in the Coastal Range, as well as in the Andes, where forests are much older and environmental conditions are different.

We investigated environmental correlates of stem radial contraction and increment of *Fitzroya* trees growing in two distinct environments in southern Chile (the Coastal Range and the Andean Cordillera). These sites were chosen because they contain the main populations of this species and the forests greatly differ in their structure, disturbance regime and environmental conditions.

The studied stands are representative of the widespread condition of forests in each range, with old and large trees in the less disturbed Andean area and younger and smaller trees in the Coastal Range, where there has been a multi-century influence of fires. We sought to resolve the following questions: (1) How do the stem radial change patterns compare between trees growing in these two areas?, (2) What environmental variables are related to daily stem radial contraction and increment in both sites?, (3) How can we better interpret the coarse-scale dendroclimatological relationships previously reported for this species?, and (4) Considering the findings from the previous objectives, what are the implications of climate change for *Fitzroya* stem growth and carbon sequestration in these two areas?

6.2. Methods

2.1. Study sites and tree selection

The study was conducted in the Alerce Costero National Park, close to the northern distribution of *Fitzroya* in the Coastal Range at 850 m.a.s.l (40°10′S, 73°26′W) and in the Alerce Andino National Park in the Andean Cordillera at 760 m a.s.l (41°32′S, 72°35′W, Fig. 1). Mean annual precipitation and temperature in 2012 were 4860 mm and 7.26 °C in the Coastal Range site and ca. 6600 mm and ca. 6.89 °C in the Andes (Urrutia-Jalabert, 2014).

The effective soil depth in Alerce Costero is generally thin (29 to 67 cm), and soils are brown-earths and severely podzolized (Veblen and Ashton, 1982; Urrutia-Jalabert, 2014). Soil texture in the upper horizon is mostly sandy-loam and organic matter content is ca. 10%. The studied forest is medium-age, dense (1415 trees ha^{-1}, considering trees ≥10 cm diameter at breast height (DBH)) and predominantly dominated by *Fitzroya*. Sampled trees were dominant and ranged between 35.5 and 47.9 cm DBH and 14.4 and 15.8 m height. In Alerce Andino, the effective soil depth is larger than in the Coast (56 to 100 cm), soils are derived from volcanic material (silty-loam texture) and contain a high amount of organic matter in the upper horizon (ca. 80%, Urrutia-Jalabert, 2014). The studied forest is old-growth, less dense than in the coast (782 trees ha^{-1}) and *Fitzroya* is the most important species in terms of basal area. Sampled trees were dominant and ranged between 82.5 and 161.5 cm DBH and 33.2 and 35.6 m height.

2.2. Dendrometer data collection

From Spring 2011 (October–November) to Fall 2013 (May 2013), stem size variation was recorded in five dominant trees per site every 30 min and averaged over each hour using automatic point dendrometers (DR model, Ecomatik, Munich, Germany) installed at breast height. The instrument consists of a displacement transducer that is anchored to the tree using two screws. The instrument resolution is 2.6 μm and thermal expansion is <0.1 μm K^{-1}. The temperature variation does not affect the sensor measurements, and due to construction the thermal expansion of the framework is negligible. To reduce the influence of bark expansion and

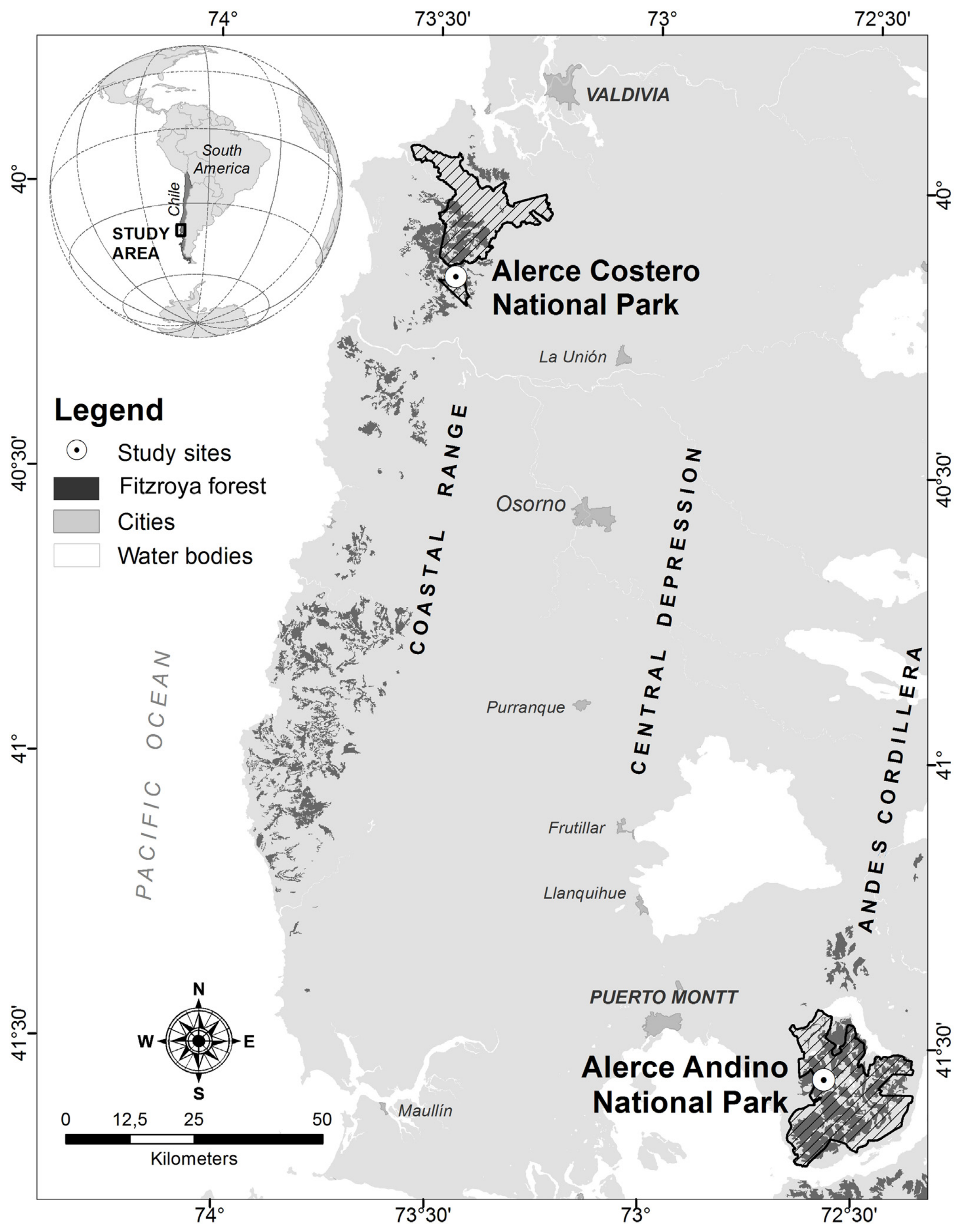

Fig. 1. Study sites in the Alerce Costero and Alerce Andino National Parks in southern Chile. The approximate location of the three major physiographic units in the area is shown (Coastal Range, Central Depression and Andean Cordillera). The distribution of *Fitzroya* forests north of 41°45′S is also displayed.

contraction, the outermost part of the bark was removed taking care to not damage the cambium. Raw measurements of every dendrometer were carefully checked and noisy or unexplained data, such as periods with constant or sudden extreme values, were removed for further analyses.

2.3. *Environmental data*

A weather station (Skye Instruments, Powys, UK) recording precipitation, temperature, relative humidity and total radiation was installed <1 km from the monitored trees at each site. In addition, one soil temperature sensor (Decagon EC-T, Pullman, USA) was installed close to the monitored trees in each site at 10 cm below the surface. Data were recorded every 30 min and hourly means were calculated. Vapor pressure deficit (VPD, hPa) was calculated from the hourly means of temperature and relative humidity (Jones, 1992).

2.4. *Growing season estimation*

Since only the growing period is recommended to be used to examine correlations with environmental parameters (Deslauriers et al., 2007a), some studies have assessed this period using microcoring techniques and subsequent cell analyses (Deslauriers et al., 2003; Rossi et al., 2006). Alternatively, growing season estimates have commonly relied on meteorological parameters (e.g. the period between the last spring and the first fall frost), phenological observations and satellite data, among others (Zhou et al., 2001;

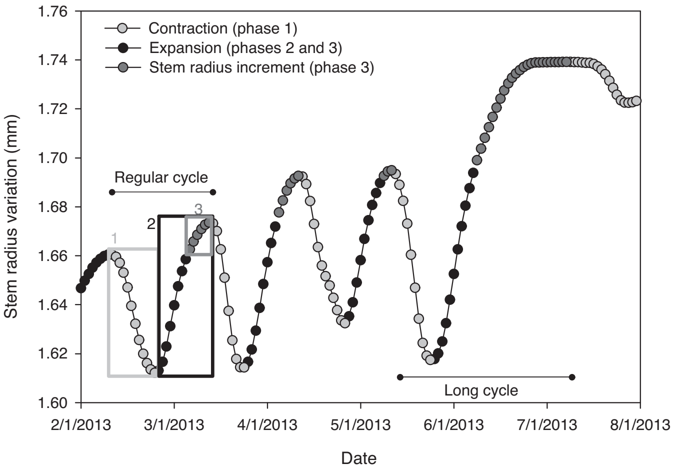

Fig. 2. Cycles in stem variation divided in three distinct phases: contraction (phase 1), expansion (phases 2 and 3) and radius increment (phase 3). Each dot represents an hourly measurement and the cycles are an example of data recorded during the first week of January 2013 (2nd to 8th of January) in one tree from the Coastal Range site.

Menzel et al., 2003). Here, in order to assess site-specific periods according to patterns of stem variation recorded by trees, an analysis of the daily cycle was performed. During the growing season, a clear pattern of daytime contraction and nighttime expansion (with high amplitudes) should be observed; while during the dormant season, amplitudes should be much lower in temperate climates or cycles can even be inverted in cold climates (Turcotte et al., 2009; Devine and Harrington, 2011; King et al., 2013). Therefore in each site, the amplitude of the daily cycle (from 0 to 23 h) was calculated for each month including the mean of all dendrometers for the whole sampling period.

The daily cycles of each month were scaled to start in zero and a K-means cluster analysis with $k = 2$ was used to divide the year in two periods: non-active and growing season. This clustering procedure is a partitioning method that finds a single partition for a group of objects; where objects within each cluster are more alike to one another than to objects assigned to other clusters (Legendre and Legendre, 2012). To confirm the definition of clusters, a hierarchical cluster analysis using the R package “pvclust” was performed (Suzuki and Shimodaira, 2006). Data recorded during the months defined as the growing period were used for subsequent analyses focused on the relationship between stem radius change and climate.

2.5. *Extraction of stem radius variation*

In order to extract the stem radius variation during the growing period, the stem cycle approach was used in this study (Downes et al., 1999 modified by Deslauriers et al., 2003). This approach uses stem shrinking and swelling to divide the stem cycle into three different phases: contraction, expansion and stem radius increment (Downes et al., 1999; Deslauriers et al., 2003; Fig. 2). Contraction (phase 1), includes the period between the morning radius maximum and the afternoon minimum and expansion (phase 2) includes the total period between the radius minimum to the next morning maximum. Stem radius increment (ΔR or phase 3) corresponds to the portion of the expansion phase from the time the stem radius surpasses the morning maximum until the following maximum, and has been considered as an estimate of growth (Deslauriers et al., 2003; Deslauriers et al., 2007a). When the previous cycle maximum was reached a positive stem radius change ($\Delta R+$) was calculated. When this maximum was not reached, a negative stem radius change ($\Delta R-$) was defined; however, only positive values were used for further analyses. The duration of each phase (h, hours) was also estimated. Environmental variables were also processed according to each phase division in order to match them with stem data. Analyses were carried out using a routine specially developed for this purpose by Deslauriers et al. (2011) using the SAS software (SAS Institute, Cary, NC).

The stem circadian cycle commonly lasts around 24 h, but rain events can result in longer or shorter cycles (Deslauriers et al., 2003, 2007a,b, Fig. 2). To perform additional analyses, we defined regular (24 h ± 3 h), short (<21 h) and long (>27 h) cycles (Deslauriers et al., 2007b; Turcotte et al., 2009).

2.6. *Relationship between stem radius change and climate variables*

In order to find the environmental correlates of stem radius change, bootstrapped correlations were calculated between stem contraction (magnitude of phase 1) and stem radius increment (magnitude of phase 3) and the environmental variables occurring during each phase (average or sum (precipitation) of values for the respective phase). The Kendall tau-b correlation coefficient (T) was used since these relationships did not comply with all assumptions for a parametric test and the data contained tied observations (tied ranks). To make variables independent from each other and avoid using non-stationary data in the correlation analyses, the first difference was used for contraction and for all the climate variables, except precipitation. Mean correlations were significant if after 1000 bootstrapped iterations their absolute values were at least two times their standard deviations (SD) (Deslauriers et al., 2003). Data for the two estimated growing seasons (2011–2012, 2012–2013) were used.

Correlations were performed considering all cycles, as well as regular cycles alone, allowing to primarily assess the effect of long cycles on the relationship between climate and stem radius change. In addition, phase duration could be highly dependent on environmental factors and the effect of these factors on stem increment could be indirect through phase duration (Deslauriers et al., 2007b). As such, partial correlations were performed for all cycles’ data using duration as a partial correlate.

Finally, to examine the relationships between stem radius change and environmental variables at a longer time-scale, and thereby establish a better link with dendrochronological findings, correlations were also performed using time windows of 7, 21 and 31 days. For this purpose, the daily maximum radius was obtained and the first difference (difference between the maximum stem radii of two subsequent days) was used as a proxy of daily stem radius change (all data, including positive and negative values were used). A moving average for 7, 21 and 31 days was calculated for the mid-point of each window position and the deviations of each daily value from the mean average were calculated for the dendrometer and environmental data to perform correlations using these anomalies.

In order to better understand covariance and redundancy of the environmental variables mostly related with stem radial change, a principal components analyses (PCA) was performed using all the variables during each time period. Variables were logarithmically transformed as necessary to comply with linear relationships for the PCA.

6.3. Results and discussion

3.1. *Patterns of stem radius change*

Radius variation in all trees showed characteristic seasonal patterns in both sites (Fig. 3). Most of variation and stem increment

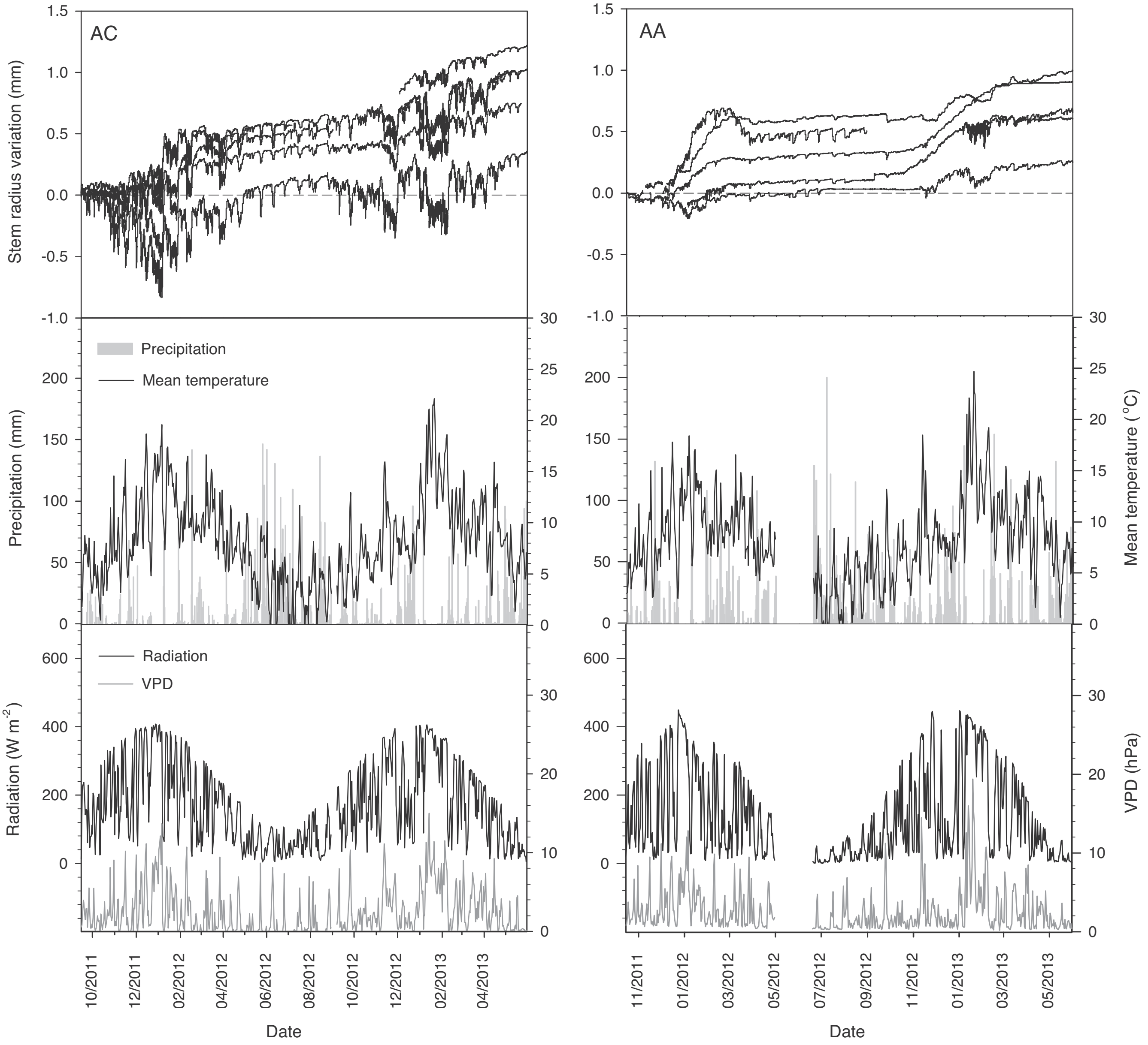

Fig. 3. Stem radius variation of the five trees monitored in each site and climate data from Alerce Costero (AC, left) and Alerce Andino (AA, right) for the whole studied period. Higher variability in stem radius is displayed during austral spring-summer months, when mean temperature, radiation and vapor pressure deficits (VPD) are higher and precipitation is slightly lower.

were observed during the period of higher temperature, radiation and VPD in spring and summer (~November–December through March). Precipitation was abundant all year long, with lower values recorded during summer (average of 839 and 1413 mm during December–February in Alerce Costero and Alerce Andino, respectively, Fig. 3). The amplitude of stem variations was lower during winter months in both sites and generally higher in the Coastal Range than in the Andes throughout the year. Trees responded synchronously in both study sites.

Stem radius especially in two trees from the Coastal Range site and in one tree from the Andes decreased during the summer of the first year, reaching the lowest values at the end of December 2011 and beginning of January 2012. This decrease corresponded with a rainless period of 15 days, accompanied with high values of radiation and temperature. The magnitude of this shrinking event was higher in the Coastal Range than in the Andes. An important shrinking was also observed in most trees of both sites during the second half of January 2013. This corresponded to a period of very little precipitation during 17 days, with the warmest temperatures registered during the whole studied period (mean values of 16.9° and 16.1 °C in Alerce Costero and Alerce Andino, respectively). January was a particularly dry and hot month, where minimum and maximum temperatures in Valdivia (at a low altitude close to Alerce Costero) were up to 2 °C and 4.9 °C warmer than the climatological mean (1961–1990), respectively (Quintana and Aceituno, 2013). The amplitude of this decrease was also larger in the Coastal Range site than in the Andes, and the two periods with strong shrinking patterns were the longest ones with almost no precipitation and warm temperatures in both areas. Strong stem shrinking in the middle of the summer was also reported for *Fitzroya*, but not for other evergreen broadleaf species in Chiloé, when a strong El Niño event (1998) affected the region and a long rainless and warm period (26 days) hit Southern Chile (Pérez et al., 2009). According to these authors, radial growth of *Fitzroya* is negatively affected by increased evaporative demand during rainless and sunny periods.

3.2. Growing season estimation, cumulative radial increment and cycle characterization

The evident higher stem activity during spring-summer compared to fall-winter resulted in a clear pattern of physiological activities suitable for wood formation and helped to estimate a potential period of growth in both study sites. According to the K-means cluster analyses, the growing season which was characterized by higher stem daily amplitudes, was estimated to occur from November to March in the Coastal Range and from December to February in the Andes (Fig. A1, Appendix). The remaining months in both sites were assigned to the second cluster and could be considered as part of a dormancy or “less active” period (Fig. A1). These results were confirmed by the hierarchical cluster analysis (results not shown). In spring and summer, the increases in temperature and radiation drive greater evaporative demand during the day that contribute to depletion of stem water reserves and, combined with the refilling at night, increase the amplitude of diurnal cycles (King et al., 2013). The lower daily amplitude observed during fall and winter seems characteristic of mild or maritime temperate regions, where there are no pronounced freeze-thaw events that could affect stem variation (Turcotte et al., 2009; Devine and Harrington, 2011; King et al., 2013).

The shorter growing season in the Andes agrees with the difference in climate between both sites, with air temperature, and particularly soil temperature and radiation being most of the time lower in the Andes than in the Coastal Range (Fig. A2, Appendix). Mean summer air temperature (December–February, 2012 and 2013) was 11.9° and 11.1 °C in Alerce Costero and Alerce Andino, respectively. Mean radiation was 259 and 218 W m^{-2} and mean soil temperature was 11.1° and 9.4 °C in the Coastal Range site and the Andes, respectively. Latitude and a more Mediterranean climate influence in the Coastal Range site would explain these differences.

The shorter growing season in Alerce Andino is also in agreement with Donoso et al. (1990) who stated that the growing season should be significantly shorter in the Andes than in the Coastal Range due to more intense snow precipitation during winter and long-lasting snow cover in spring.

The mean cumulative radial increment in trees from Alerce Costero was 0.41 (±0.21) and 0.25 mm (±0.02) for the growing seasons 2011–2012 and 2012–2013, respectively. In Alerce Andino, the mean cumulative radial increment was 0.31 mm (±0.23) and 0.25 mm (±0.13) for 2011–2012 and 2012–2013, respectively. These results indicate that tree growth in both sites was lower during the second growing period (2012–2013), likely due to the strong decreases in stem radius recorded in most trees during January 2013 in both areas.

In terms of cycle characterization, in the Coastal Range site 75% of the cycles were classified as regular cycles, 17% as long cycles and 8% as short cycles. The longest cycle in this site lasted 128 h (Fig. A3, Appendix). In the Andes, 70% of the cycles were classified as regular cycles, 20% as long cycles and 10% as short cycles. The longest cycle event lasted 126 h (Fig. A3).

On average, the contraction phase was longer in Alerce Costero (9.3 h ± 2.5) than in Alerce Andino (8.3 h ± 2.8, $p < 0.05$). The mean time when this cycle started in the morning was 9:48 h (±2:04) in the Coastal Range site, and 8:48 h (±2:06) in the Andes; it was later in Alerce Costero mainly due to lower air temperatures and higher relative humidity conditions in the early morning in this site, because of the greater oceanic influence on this area.

The duration of the expansion phase varied considerably due to rainfall events, ranging from 1 to 118–119 h in the Coastal Range site and the Andes, respectively. The mean start time of this event was 19:24 (±1:48) in the coast and 17:12 h (±2:22) in the Andes. The longest increment phases were observed to last 108 and 115 h in Alerce Costero and Andino, respectively.

The longer contraction phase in Alerce Costero than Alerce Andino was mainly due to the higher radiation and air temperature experienced by trees during the day (from 09:00 to 19:00 h) in the former site (540 W m^{-2} and 14.3 °C) compared with the latter (465 W m^{-2} and 13.1 °C) during summer (December–February).

Additionally, a higher amplitude of contraction was found in the Coastal Range (0.06 mm) compared to the Andes (0.01 mm, $p < 0.05$), indicating that trees in the coast would utilize their internal stem water reserves faster than in the Andes (King et al., 2013). One possible explanation for this would be the difference in soil conditions between both sites: the Alerce Costero site is characterized by shallower soils with lower water retention capacity, which is accentuated by the sandy texture and less organic material in this area compared to Alerce Andino (Barichivich, 2005; Gerding, personal communication). These characteristics would result in a greater resistance to water flow from the soil, causing higher amplitudes of stem variation and a higher use of the stem water pool during the day (Sevanto et al., 2005). Soil volumetric water content measurements in each site during summer (at a monthly basis at 12 cm depth) partly reflect the differences that exist between areas: mean values reached 39% and 50% in Alerce Costero and Andino during 2011–2012 and 32% and 51% during the drier summer (2012–2013). A particularly strong drop in water content was observed during January and February 2013 in the Coastal Range site (Urrutia-Jalabert, 2014). An additional explanation for the difference between sites, would be that conditions are commonly cloudier and rainier (~40% more precipitation during December–February) in Alerce Andino than in Alerce Costero, and daily contraction amplitudes have been reported to be lower on overcast or rainy days (Devine and Harrington, 2011; King et al., 2013). The higher amplitude of contraction in the Coastal Range site could not be explained by a higher amount of bark in trees from this site, since trees have much thinner bark in the coast than in the Andes and in any case most bark was removed at the dendrometer contact point. Finally, an alternative explanation might be that larger and taller *Fitzroya* trees in the Andes have a greater sapwood capacitance compared to the trees from the Coastal Range which would depend more on soil water availability (Scholz et al., 2011).

3.3. Relationships between environmental factors and stem radius change

Considering all and just regular cycles, stem contraction during the day in both sites was positively related with mean and maximum temperatures, VPD and radiation (Fig. 4). Humidity on the other hand, was negatively related with contraction ($T = -0.58$) and precipitation also was negatively related with this variable, but just in Alerce Costero when considering all cycles (Fig. 4). This is in agreement with what was found by Devine and Harrington (2011) for young Douglas fir (*Pseudotsuga menziesii*) and supports the above reported statement that warmer and drier conditions are usually associated to strong stem shrinking patterns. Relationships were stronger in the Coastal Range site than in the Andes, meaning that trees in the former site would be more sensitive to environmental conditions that make the stem contract.

Stem radius increment mainly occurs at night or early morning, as was corroborated in this study. It has been reported that

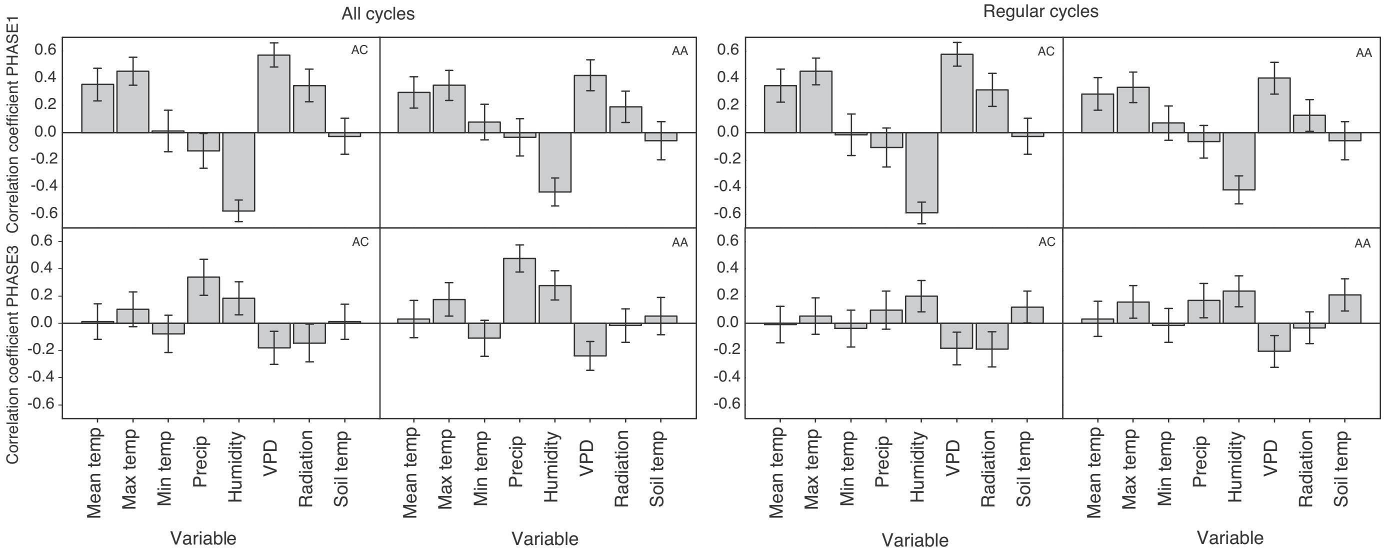

Fig. 4. Left panel: Kendall tau-b correlations between environmental variables in Alerce Costero (AC) and Alerce Andino (AA) and stem contraction (top panel) and increment (bottom panel) considering all cycles. Right panel: Kendall tau-b correlations between environmental variables in Alerce Costero and Alerce Andino and stem contraction (top panel) and increment (bottom panel) considering just regular cycles. Significant correlations are present when the error bar does not cross zero.

cell enlargement takes place mostly at night or on rainy days, when turgor is high and cambium is supplied with optimal water availability (Dünisch and Bauch, 1994; Downes et al., 1999; Deslauriers et al., 2003; Steppe et al., 2006; Gruber et al., 2009). During the day, more water is lost through transpiration than absorbed through the roots, so an internal water deficit affects trees and transpiration would negatively affect radial expansion (Tardif et al., 2001).

Stem increment had a significantly positive correlation with precipitation and humidity considering all cycles in the Coastal Range. VPD and radiation on the other hand, had a negative correlation with this factor. In the Andes, the pattern was more or less the same, but maximum temperature was also significantly and positively correlated with stem increment and radiation was not correlated with this factor (Fig. 4). Radiation was not important, probably because it is lower in this site. When performing correlations just considering regular cycles in the Coastal Range site, correlation with precipitation was not significant implying that the relationship between precipitation and stem increment is just seen when long precipitation events occur in this site. Additionally, a higher negative correlation with radiation was observed and a slightly significant positive relationship was obtained with soil temperature. In the Andes, relationships remained significant for the same variables, although correlation with precipitation decreased considerably. In addition, a positive correlation with soil temperature also appeared in this site ($T = 0.21$, Fig. 4). Since VPD is derived from and strongly related to air humidity, relationships between these variables and stem radius change were usually similar in magnitude, but in opposite directions.

The positive relationship between maximum night temperature, soil temperature and stem increment particularly in the Andes, can be explained by generally colder conditions in this site compared to the Coastal Range. A number of studies have reported a positive relationship between night temperatures and stem increment (Deslauriers et al., 2003; Xiong et al., 2007; Drew et al., 2008). Night-time temperatures have been found to have a greater effect on tracheid expansion than daytime temperatures (Richardson and Dinwoodie, 1960; Richardson, 1964; Dünisch, 2010). Thus, low night temperatures were reported to negatively affect the expansion of differentiating tracheids in *Podocarpus latifolius* (Dünisch, 2010). The positive relationship between soil temperature and stem increment on the other hand, could be associated to a positive effect of warmer soil temperatures on root water uptake and stem rehydration and the consequent beneficial effect on internal water balance (Tardif et al., 2001; Pérez et al., 2009).

Precipitation and humidity have been usually reported to positively affect stem increment in different conifer and broadleaved species (Deslauriers et al., 2003, 2007b; Duchesne and Houle, 2011; Krepkowski et al., 2011; Köcher et al., 2012). The direct effect of precipitation on radial growth is to increase the water status in the stem, inducing high water potentials that favor cell enlargement (Steppe et al., 2006). Humidity on the other hand, also contributes reducing the negative pressure in the conducting system, helping to increase turgor (Köcher et al., 2012). In the same sense, high VPD acts to inhibit cell enlargement and growth, due to its indirect effect on cell turgidity (Pantin et al., 2012).

It is relevant to highlight the importance of precipitation and humidity conditions for maintaining the water status in the stem in order to induce cell enlargement and radial growth in *Fitzroya*. This occurs even in our very rainy sites which receive more than 800 mm of precipitation during summer.

Fig. 5 shows correlations between environmental factors and duration of the increment phase (phase 3), as well as partial correlations between environmental factors and stem radius increment using duration as the partial correlate (considering all cycles). Precipitation showed the highest correlation with duration in both study sites, so when rainfall was higher duration of the increment phase was longer. Humidity also had a significant positive correlation with duration of the increment phase in Alerce Andino, but not in Alerce Costero. VPD and minimum temperature had a negative correlation with duration of cycles in both areas. In the Coastal Range site, radiation also had a negative correlation with the duration of the increment phase (Fig. 5).

Partial correlations were performed given the positive and significant relationship between duration and stem radius increment ($T = 0.52$ in both sites). Only humidity and VPD remained as variables with significant correlation with stem radius increment in the Coastal Range site.

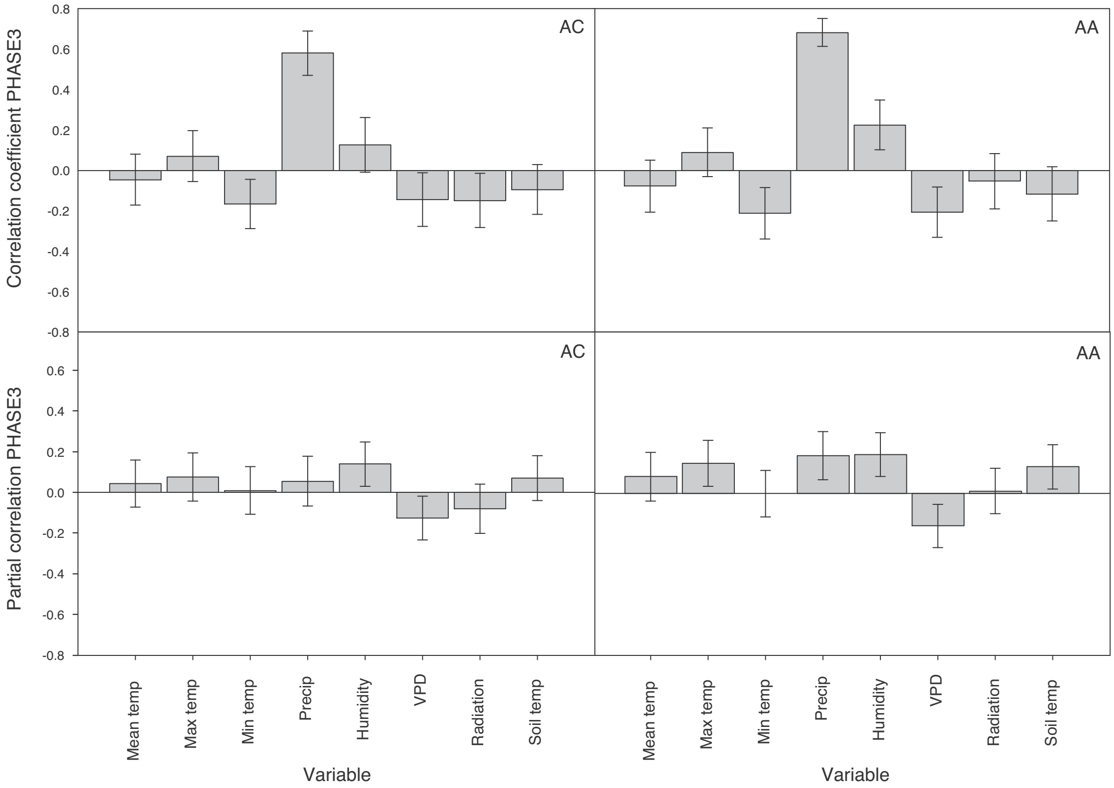

Fig. 5. Top panel: Kendall tau-b correlation coefficients between environmental factors and duration of the increment phase in Alerce Costero (AC) and Alerce Andino (AA). Bottom panel: Partial correlations between environmental factors and stem increment considering duration as the partial correlate. Significant correlations are present when the error bar does not cross zero.

The other variables that were reported as significant in Fig. 4 (precipitation and radiation) would have an indirect relationship with stem increment through phase duration. In the Andes, correlations remained significant for all the reported variables in Fig. 4 (Fig. 5). The fact that the positive effect of precipitation is mediated through duration in Alerce Costero, can be explained because soils do not have good water retention capacity, so more rainy days are needed in order to induce growth. On the other hand, the negative relationship between stem increment and radiation was mainly mediated through duration in this site. More radiation shortens the expansion/increment phases, reducing the favorable period for growth. Radiation increases transpiration and water loss from the tree, causing less cell turgidity and consequently less cell enlargement (Fritts, 1958).

Finally, since prevailing weather conditions mostly during the expansion phase have been shown to affect stem radial increment (Deslauriers et al., 2003), correlations were performed between environmental conditions during this phase and stem increment. This was done just using regular cycles to minimize the effect of large differences in duration between phases. The only variables that were related with increment were humidity ($T = 0.24$) and VPD ($T = -0.19$) just in Alerce Andino, but these correlations were lower than when using environmental variables from the increment phase.

General findings in this study are in agreement with Pérez et al. (2009), who reported a positive effect of precipitation, soil hydration and temperature and a negative effect of photosynthetic active radiation on the radial increment of *Fitzroya* from Chiloé. Strong shrinkage events were equally experienced by trees from Alerce Costero and Chiloé, located in both extremes of the Coastal Range.

3.4. *Insights and interpretation of findings in dendrochronological studies*

Relationships between stem radius change and environmental variables considering longer time scales revealed that correlations with precipitation, humidity and VPD remained significant and even increased in the case of humidity and VPD, compared with correlations using the daily increment (considering all cycles) in both sites. Moreover, negative correlations with mean and maximum temperature were also significant, as well as with radiation in both areas (Table A1, Appendix).

The PCA plots (component 1 vs. component 2) for the different time scales highlight the close positive association between stem radius change and precipitation and humidity (Fig. 6). A negative association was particularly strong with radiation at longer time scales in both sites. For conciseness, and since patterns for 21 and 31 days were the same, Fig. 6 shows only the “daily”, 7 and 31 days results. “Daily” here and in the rest of the text refers to the time scale given by the stem cycle. Principal components 1 and 2, explained 58.5, 73.6 and 78.8% at a “daily”, 7 and 31 days scale in the Coastal Range site. In the Andes these values were 60.6, 76.3 and 79.5%, respectively.

Climate-tree growth relationships established in dendrochronological studies have a limited explanatory power in terms of an implicit growth mechanism (Zweifel et al., 2006), so a better understanding of the processes behind these relationships in *Fitzroya* can be obtained using this high-resolution study. The reported negative relationships of *Fitzroya* tree-ring width with summer temperature (Villalba, 1990; Villalba et al., 1990; Lara and Villalba, 1993; Neira and Lara, 2000; Barichivich, 2005) mainly appeared when longer time scales were considered in this

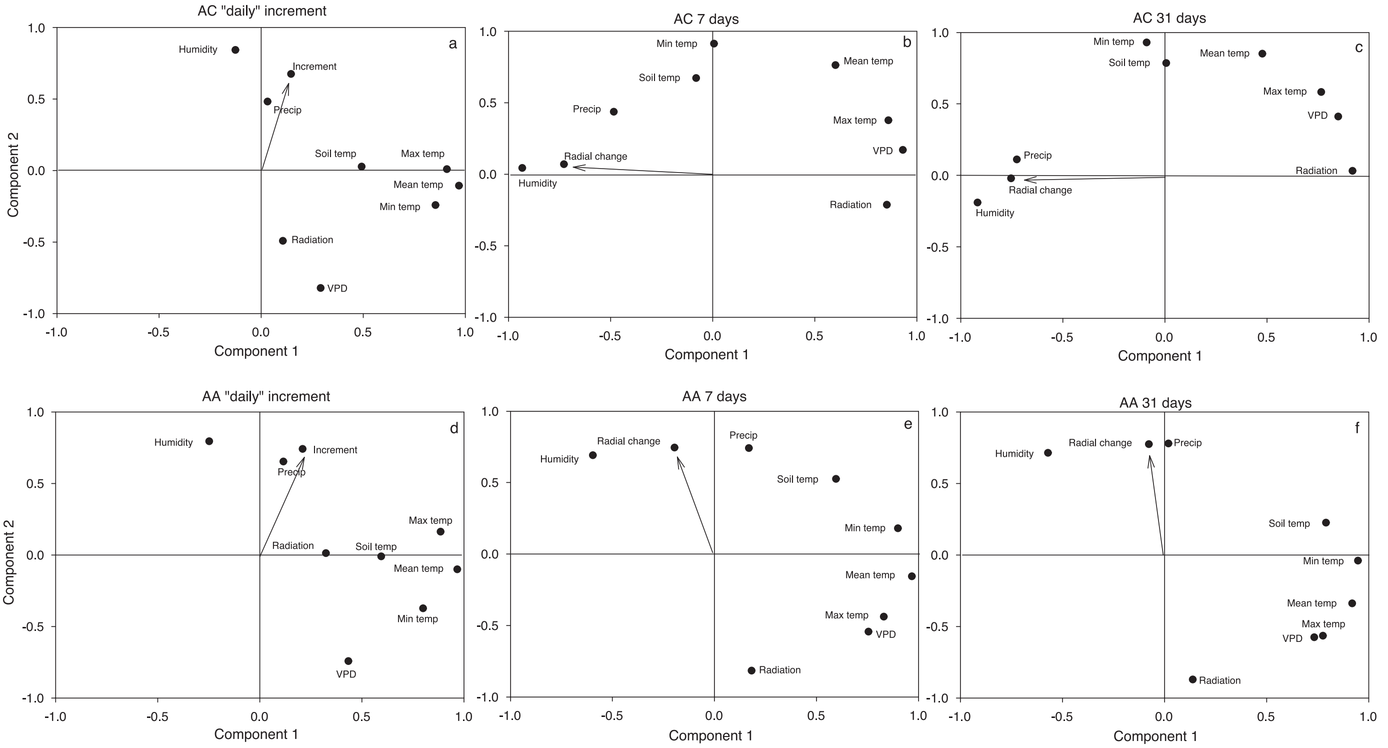

Fig. 6. Principal component analysis between (a) stem radius increment and environmental variables at a "daily" basis in Alerce Costero (AC), (b) stem radius change and environmental variables at a time scale of 7 days in the same site and (c) stem radius change and environmental variables at a time scale of 31 days also in Alerce Costero. (d)–(f) the same as (a)–(c), but for Alerce Andino (AA). Arrows in each plot point toward the stem increment/stem radius change variable. Environmental variables closely located to the stem radius variables or directly opposite covary more strongly with the response variables (closer variables are positively related and opposite variables are negatively related).

study. This relationship can be understood through the strong shrinking events recorded in *Fitzroya* when long warm, sunny and particularly dry periods occur. This would reduce the number of days with appropriate climate conditions for growth to take place, producing a smaller tree ring.

The correlation and the PCA examined together indicate that variables directly influencing the stem water status, namely humidity, precipitation and VPD, had a stronger relationship with stem radius change. In the second place, radiation and, to a lesser extent, maximum temperature were negatively related with this variable. It is likely that these negative correlations are a by-product of the strong correlation between humidity and radiation, and between VPD and maximum temperature, rather than a direct causal relationship among radiation/temperature and growth rate. It is noteworthy that mean temperature had a weaker relationship with stem radius change and minimum temperature did not have any significant relationship at all. This suggests that maximum temperature would matter, because of its links with VPD, rather than through direct effects on plant metabolism. Hence the results support the primary influence of humidity conditions on cell growth rates on the studied time scales. The negative association between tree-ring width chronologies and summer temperature appears to be mediated through the effect of temperature on VPD.

The reported negative effect of previous summer temperature and precipitation on the other hand, can be because warm temperatures are usually associated with dry conditions in the study area, and hence carbon assimilation can be reduced if stomata close (McDowell et al., 2008). Less carbon assimilation would result in less carbon reserves and a smaller tree-ring during the next season. Moreover, high temperatures during the previous growing season combined with higher respiration rates can reduce the starch reserves that can be used for the following growth period (Deslauriers et al., 2014).

The use of multiple environmental variables in this study, although correlated among themselves, provided important insights that would not be possible to obtain with a priori restriction to a few variables.

3.5. Stem growth sensitivity to climate change

Findings of this study allow some tentative inferences to be made regarding the vulnerability of *Fitzroya*'s growth rates to climate variations. Current and projected climate change, characterized by decreased precipitation and warmer temperatures in southern Chile (González-Reyes and Muñoz, 2013; Fuenzalida et al., 2007), may have a negative effect on the carbon sequestration capacity and long-term storage of *Fitzroya* populations from both study sites. However, *Fitzroya*'s radius variation currently appears to be especially sensitive to dry and warm conditions in Alerce Costero, meaning that forests growing under similar restrictive site conditions in the Coastal Range are more vulnerable to experience stem shrinking and lower growth compared with trees from the Andes. Strong stem shrinking is experienced by *Fitzroya* trees in the Coastal Range site even during years that are not as extreme as El Niño years, which indicates that restrictive soil conditions and a more Mediterranean climate influence can make *Fitzroya* tree growth more vulnerable to future climate change. In addition, precipitation seems to be related with stem increment on a daily basis in the Coastal Range site only when long rainfall events take place, so less precipitation in the future, may negatively affect this variable. A significant negative trend in the tree-ring width (using detrended series to discount the effect of growth changes due to tree aging, Fritts, 1976), as well as in the basal area increment chronology has been observed in this site especially in the last 40 years (Urrutia-Jalabert, 2014). This likely reflects the effect of decreased precipitation and increasing maximum temperatures on cell enlargement and consequent stem radial growth (significant trend in summer maximum temperature in Valdivia for the period 1960–2009, Urrutia-Jalabert, 2014). This negative trend in growth has not been seen so far in the older Andean forest, reinforcing the higher sensitivity of trees from the Coastal Range to current changes in climate (Urrutia-Jalabert, 2014).

It is important to emphasize, however, that further studies should address measurements of leaf water potential, sapwood capacitance and non-structural carbohydrates in *Fitzroya* trees, especially during dry periods, to assess to what extent they are affected by these conditions. In the case of trees from the Andes, and since it has been reported that absolute daily reliance on stored water across different species is higher in larger trees; stored water might help avoiding embolism in a future drier climate (Scholz et al., 2011).

6.4. Conclusions and implications

This study is the first to assess, at a high resolution level, the relationship between stem radius contraction and increment and environmental conditions in *Fitzroya* trees growing in the Andes and Coastal Range of southern Chile. The high resolution approach that we used was unique in allowing us to track the seasonal course of stem radius variation throughout the studied period and estimate a growing season for each area based on the definition of stem daily cycles. Moreover, we could explore the stem daily cycle in detail, understand the differences between sites and define the contraction and increment phases for subsequent correlation analyses. We found that stem radius contraction was positively related with radiation, temperature and VPD in both sites, so sunnier, warmer and less humid conditions conducive to higher transpiration rates, were associated to stronger stem contraction and shrinking events. The amplitude of these events was more pronounced in Alerce Costero than Alerce Andino, reflecting a higher sensitivity of this site to these growth-adverse conditions. Stem increment on the other hand, was primarily related with precipitation and humidity in both sites, reflecting the positive effect of water on stem water potential and especially cell enlargement. Relationships with humidity/VPD were stronger when considering longer time scales (7 to 31 days), and VPD appears to be the driver of the previously reported negative correlations between tree-ring width chronologies and temperature. Projected climate change in southern Chile is likely to impose restrictions to *Fitzroya*'s stem radius increment and carbon uptake, especially in the Coastal Range. This is somewhat surprising given the high amounts of annual precipitation that fall in *Fitzroya* sites. Long-term monitoring is needed in order to assess the responses of these forests total productivity to climate variations. Future research on *Fitzroya* forests should concentrate on multi-scale assessments ranging from cellular-scale analyses to determine the environmental variables that mostly influence xylogenesis, to ecosystem-scale studies to assess the actual condition of these forests and their interaction with climate (e.g flux towers). This knowledge is fundamental to better understand the vulnerability of these unique ecosystems and their carbon sequestration capacity to climate change.

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Appendix A. Appendix

A.1. Results

Figs. A1–A3 and Table A1

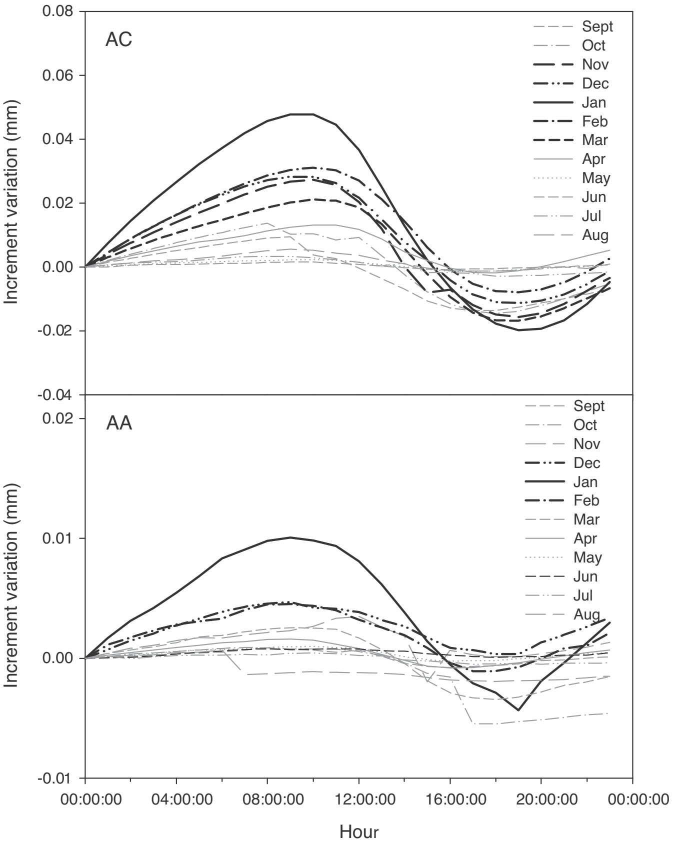

Fig. A1. Daily cycles found for the mean of the stem radius variation in Alerce Costero (AC, top) and Alerce Andino (AA, bottom). Months depicted in bold black, which present more defined and higher amplitude cycles, were the ones selected as the growing season according to K-means cluster analysis.

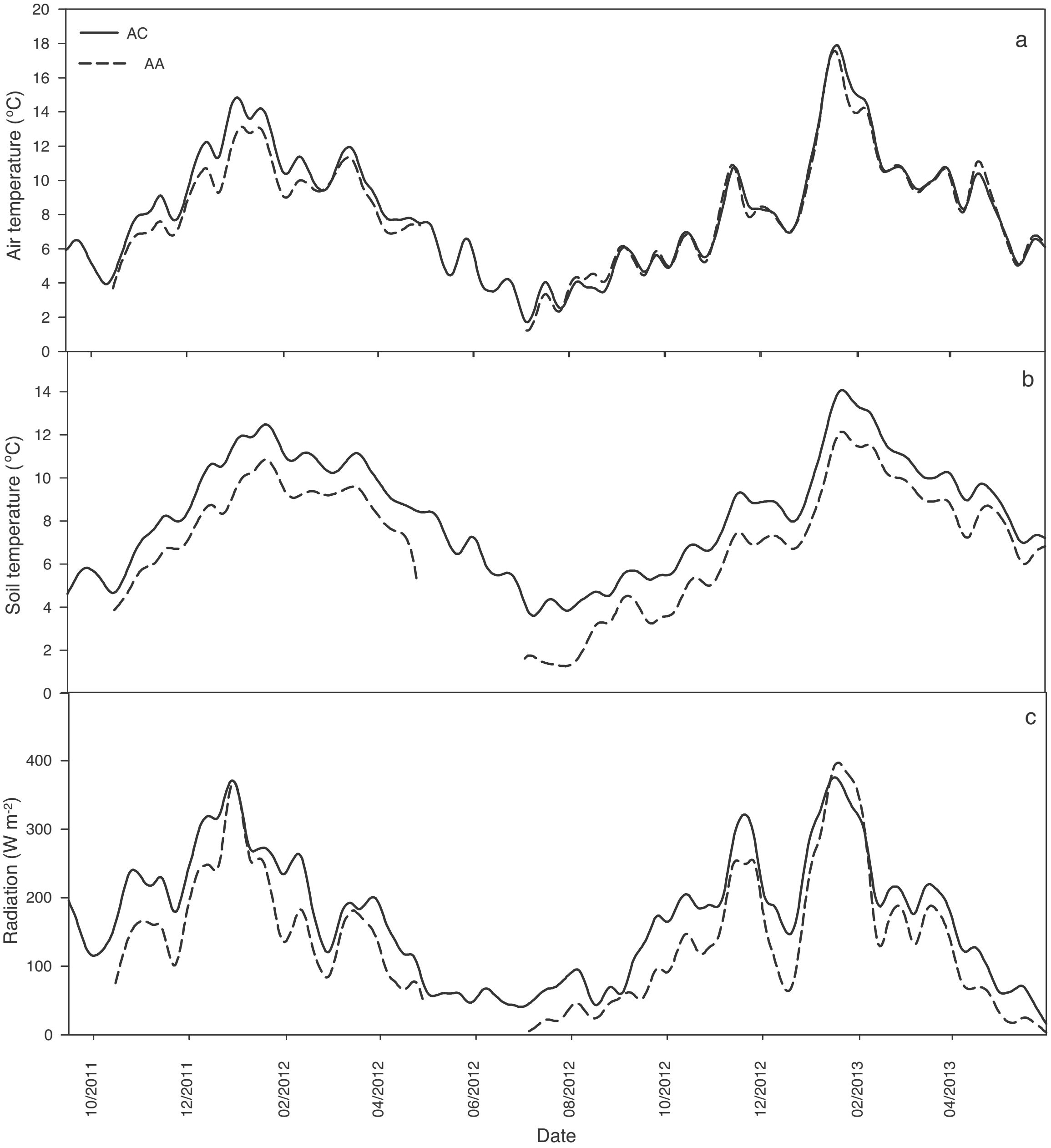

Fig. A2. (a) Daily mean air temperature for the period October 2011 May 2013 in both study sites (Alerce Costero, AC and Alerce Andino, AA), (b) daily mean soil temperature and (c) daily mean total solar radiation. For illustration purposes data were smoothed using a cubic spline designed to reduce 50% of the variance in a sine wave with a periodicity of 25 days. Soil temperature and radiation are clearly higher in Alerce Costero than Alerce Andino throughout the year, but the difference is less clear for air temperature.

Table A1
Kendall-tau b correlations between stem radius increment at a "daily" basis (considering short, regular and long cycles), stem radius change at 7, 21 and 31 days and environmental variables in Alerce Costero (AC) and Alerce Andino (AA). "Daily" refers to the time scale given by the stem cycle. Significant correlations are marked with an asterisk.

Site and time period	Mean temp.	Max. temp.	Min. temp.	Precip.	Humidity	VPD	Radiation	Soil temp.
AC "daily" increment	0.01	0.10	−0.08	0.34*	0.18*	−0.18*	−0.15*	0.01
AC 7 days	−0.21*	−0.32*	0.02	0.25*	0.47*	−0.45*	−0.34*	0.05
AC 21 days	−0.25*	−0.37*	0.01	0.30*	0.49*	−0.46*	−0.42*	0.01
AC 31 days	−0.26*	−0.38*	−0.01	0.28*	0.48*	−0.46*	−0.40*	−0.02
AA "daily" increment	0.03	0.18*	−0.11	0.48*	0.28*	−0.24*	−0.02	0.05
AA 7 days	−0.20*	−0.30*	−0.05	0.33*	0.45*	−0.41*	−0.38*	0.16*
AA 21 days	−0.18*	−0.29*	−0.05	0.30*	0.44*	−0.39*	−0.39*	0.11
AA 31 days	−0.19*	−0.29*	−0.05	0.28*	0.43*	−0.39*	−0.37*	0.07

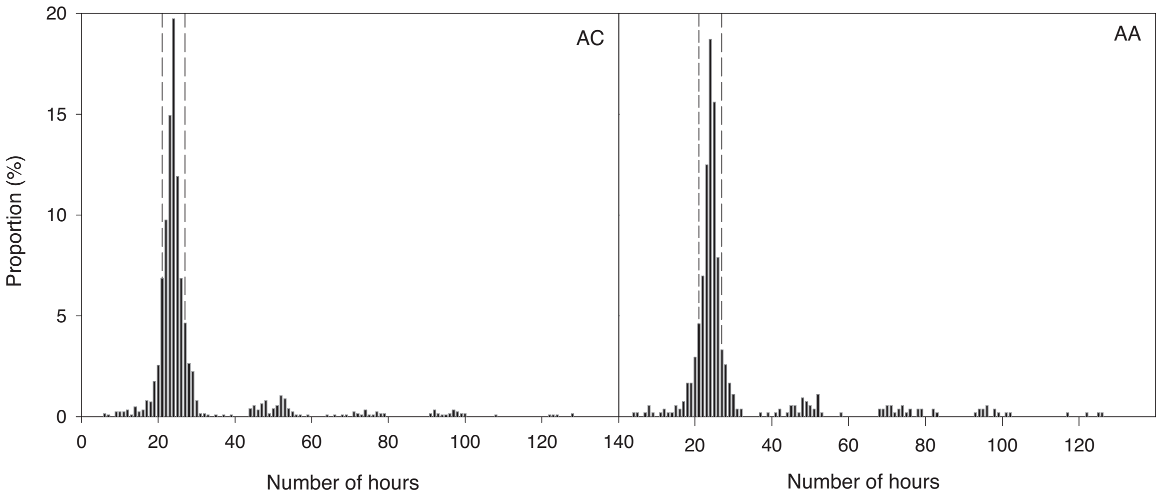

Fig. A3. Distribution (in percentage) of the number of hours of stem circadian cycles in Alerce Costero (AC) and Alerce Andino (AA). Regular cycles (24 ± 3 h), representing the highest proportion of cycles, are delimited by dashed lines.

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Linking statement: Chapter 6 to 7

In Chapter 6 I addressed the environmental variables mostly related with stem radius increment at a daily to monthly timescale. In Chapter 7 I expand my understanding of the environmental variables related with radial growth, but now at an interannual and decadal timescale, and evaluate the growth and physiological changes that the trees have experienced in recent decades. I address the sixth specific objective: **to evaluate changes in *Fitzroya*'s annual radial development in both Ranges during recent decades using tree ring width chronologies in combination with tree-ring stable carbon isotopes data, and determine which climatic and environmental factors are more related to these changes.** Thus, this paper investigates trends in tree-ring width (woody productivity) and carbon isotope chronologies (physiological changes that influence productivity), as well as trends in climate records during recent decades, and the relationship between climate and tree-ring data.

I was the main author and wrote the paper. I organised and conducted the dendrochronological fieldwork with the help of research assistants. Among the co-authors of the manuscript, Emilio Cuq and Carmen Gloria Rodríguez developed the tree-ring chronologies. C.G. Rodríguez did the sampling for the isotopic analyses and Antonio Delgado ran the analyses in Spain. He also provided advice with the sampling and he and Antonio Lara provided guidance with data analyses. Yadvinder Malhi supervised the writing of the manuscript, Jonathan Barichivich provided advice with data analyses and helped with their interpretation and he and Antonio Lara commented on the paper. This chapter was submitted to Global Change Biology in March 2015.

Chapter 7: Increased water use efficiency but contrasting tree-growth patterns in *Fitzroya cupressoides* forests of southern Chile

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Keywords: Alerce forests, tree-ring width chronologies, carbon isotope chronologies, intrinsic water use efficiency, climate change, CO_2 fertilization, carbon sequestration, old-growth forests, temperate rainforests, southern Chile.

Abstract

Little is known about how old-growth and high massive forests are responding to environmental change. We investigated tree-ring growth and carbon isotopes of the long-lived and potentially high biomass *Fitzroya cupressoid*es in two stands growing in contrasting environmental conditions in the Coastal Range (~ 300 years old) and Andean Cordilleras (> 1500 years old) of southern Chile. The annual $\delta^{13}C$ was assessed for the period 1800-2010 and changes in discrimination and intrinsic water use efficiency (iWUE) were evaluated in relation to changes in climate and tree-ring growth during the last century.

^{13}C discrimination has significantly decreased and iWUE has increased since the 1900s in both sites, with similar rates of change. These trends in isotopic composition have been accompanied by a trend for decreasing growth rates in the Coastal Range site in the last 40 years, but by an increasing growth trend in the Andean site since the 1900s. Trees growing in the Coastal Range appear to have become more efficient in their use of water, probably due to reduced stomatal conductance caused by increases in CO_2 and warming, but this increase in iWUE has not produced an increase in carbon sequestration. Trees growing in the Andes have also been more efficient in their use of water, but this has been likely due to increased photosynthetic rates, rather than to a reduction in stomatal conductance. A combination of increasing CO_2 and decreasing cloudiness may be stimulating growth in this cloudy and rainy site, despite the extreme age of these trees. Different site conditions, in particular a more Mediterranean climate influence and more restrictive soil conditions in the Coastal Range site, can influence the different response of these particularly wet forests to environmental changes.

7.1. Introduction

The impacts of atmospheric change on forests vary from being potentially positive, such as benefits resulting from higher atmospheric CO_2 concentrations, to being negative, such as decreased growth and increased mortality due to increases in temperature and in the frequency and intensity of drought events (Allen et al. 2010). The rapid increase in atmospheric CO_2 from ~ 285 ppm in 1850 to 396 ppm in 2013 (Robertson et al. 2001; Keeling et al. 2014), has been one of the most prominent and consistent environmental changes in the last century. This ongoing increase may lead to a reduction in stomatal conductance and transpiration in plants, resulting in an improved water use efficiency, as well as in an enhancement of the rate of photosynthesis and tree growth (Drake et al. 1997; Andreu-Hayles et al. 2011). Despite ecophysiological arguments for CO_2 fertilization, there is still an ongoing debate about the actual and future effects of CO_2 increase on tree growth in natural ecosystems (Jones et al. 2014). Results from FACE (Free-Air CO_2 Enrichment) experiments indicate that growth has not been always stimulated, that it can be constrained by nitrogen limitation in temperate forests, or that responses might be short lived (Norby et al. 2010; Bader et al. 2013; Körner 2013).

Tree rings are natural archives that allow assessment of forest growth responses to environmental changes from seasonal to centennial time scales. Dendrochronological studies in different species have reported divergent growth trends ranging from increases attributed to warming and CO_2 fertilization (Soulé & Knapp 2006; Leal et al. 2008; Salzer et al. 2009) to decreases mainly due to rainfall decline in water-limited systems (Sarris et al. 2007; Andreu-Hayles et al. 2011; Linares et al. 2011).

Stable carbon isotopes in tree rings provide information on important physiological changes that can influence growth. They indicate the balance between stomatal conductance and the rate of photosynthesis, which is mainly determined by atmospheric relative humidity and soil water content in dry sites and by summer radiation and temperature in wet sites (McCarroll & Loader 2004). Stable carbon isotopes ratios ($\delta^{13}C$) provide a metric of discrimination against the heavier isotope (^{13}C) during stomatal diffusion and carboxylation (Farquhar et al. 1982). High $\delta^{13}C$ ratios (=less discrimination) in tree-rings indicate low concentrations of CO_2 in the intercellular air spaces, which can be caused by lower stomatal conductance or high photosynthetic rates (McCarroll & Pawellek 2001).

$\delta^{13}C$ in tree rings is usually sensitive to water status in water-limited environments. Partial stomatal closure may occur during warm and dry conditions, reducing the rate of CO_2 diffusion into the leaf, and therefore decreasing the discrimination against ^{13}C and increasing $\delta^{13}C$. Consequently, there is a general negative relationship between $\delta^{13}C$ and precipitation or humid conditions (Brienen et al. 2011; Johnstone et al. 2013). On the other hand, solar radiation can be the dominant driver of tree-ring $\delta^{13}C$ in non-water limited environments (Gagen et al. 2011; Loader et al. 2013). Higher solar radiation induces higher photosynthetic rates in light-limited environments, resulting in less discrimination and a positive correlation between sunshine and tree-ring $\delta^{13}C$ (Johnstone et al. 2013).

By using $\delta^{13}C$ in tree rings, changes in the discrimination by plants (Δ) through time can be assessed. Furthermore, potential changes in the intrinsic water-use efficiency (iWUE), a proxy for the water used per unit carbon gain at the leaf level, can be detected (Saurer et al. 2004). Changes in iWUE result from changes in carbon assimilation (A) and stomatal conductance of water vapour (g_w). Therefore, an increase in this variable may be due to either an increase in A, a decrease in g_w, or both (Andreu-Hayles et al. 2011).

A number of studies have reported an increase in iWUE in different forests around the world in recent decades (Saurer et al. 2004; Linares et al. 2009; Brienen et al. 2011; Nock et al. 2011; Franks et al. 2013). However, this increase has not always been accompanied by positive changes in tree growth, demonstrating that the growth rates of some species are not responding to higher water-use efficiency (Peñuelas et al. 2008; Andreu-Hayles et al. 2011; Lévesque et al. 2014).

Most of the ecological studies assessing changes in water-use efficiency and generally looking at the responses of forests to climate change have focused on Northern Hemisphere ecosystems and to a lesser extent on tropical ecosystems. There have been very few studies on South American temperate forests (e.g. Srur et al. 2008; Villalba et al. 2012) and it has been 20 years since any study assessed changes in the isotopic composition of the longest-lived tree species that grows in this region (*Fitzroya cupressoides*, Leavitt & Lara 1994). These slow-growing trees have the second oldest recorded age after bristlecone pine (*Pinus longaeva,* Lara & Villalba 1993). Our studies of the Andean *Fitzroya* forests suggest they may be the slowest-growing and longest-lived high biomass forest stands in the world (Urrutia-Jalabert et al. Submitted a).

Due to the endemic character, endangered status and the narrow distribution of *Fitzroya cupressoides* (Alerce) forests, this ecosystem may be highly vulnerable to climate change. The tree-ring growth of *Fitzroya* has been found to be mainly negatively associated with summer temperature and positively related to summer precipitation (Villalba 1990; Lara & Villalba 1993; Neira & Lara 2000; Barichivich 2005). In fact, the strong negative relationship between tree-ring growth and previous summer temperature allowed a 3622-year summer temperature reconstruction for southern South America (Lara & Villalba 1993).

Leavitt & Lara (1994) developed a 290-year $\delta^{13}C$ chronology for *Fitzroya* growing in the Andes Cordillera, finding that the $\delta^{13}C$ trend in the rings mostly followed the $\delta^{13}C$ trend of atmospheric CO_2 and did not seem significantly affected by systematic changes in environmental factors.

Both climate and atmospheric CO_2 concentrations have been changing in recent decades, and new isotopic studies on this species can provide important insights into the response of old-growth systems to ongoing environmental changes. Changes in this millennial species would demonstrate real physiological responses, beyond the intrinsic ontogenic effects that frequently influence isotopic studies in younger forests (Franks et al. 2013).

The aim of this study is to understand how *Fitzroya* forests have and are currently responding to environmental changes in recent decades through the investigation of radial growth and tree-ring carbon isotopes in two different stands growing in the Coastal Range (medium age) and the Andean Cordillera (old-growth) of southern Chile. Environmental conditions differ in these two areas, with a more Mediterranean climate influence (drier summer) and restrictive soils in the Coastal Range, compared with the Andes. Our specific objectives are: 1) to evaluate trends in tree growth and basal area increment (BAI) in *Fitzroya* forests from each site over recent decades, 2) to assess trends in $\delta^{13}C$, isotope discrimination and iWUE in these forests over the same period and 3) to determine tree-ring (width/carbon isotope)- climate relationships and assess whether changes in climate have helped to modulate the observed trends in tree-ring growth and the isotopic composition in *Fitzroya* forests in each site.

The answer to these questions will allow us to determine if *Fitzroya* has passively or actively responded to the increase in CO_2 in these two areas, if the species is responding to changes in climate, and if its response has been associated with an increase or decrease in tree growth during recent decades.

To our knowledge this is the only such study (combining tree growth and isotopic composition) developed so far on long-lived and particularly wet temperate forests, providing important information about the response of these unique ecosystems to global atmospheric change.

7.2. Methods

Study sites

Our study sites are in (a) the Alerce Costero National Park (AC), close to the northern distribution of this species in the Coastal Range at 40° 10’ S- 73° 26’ W, and (b) the Alerce Andino National Park (AA) in the Andean Cordillera at 41° 32’ S- 72° 35’ W.

In Alerce Costero the studied stands (two nearby 0.6 hectare plots with similar structural characteristics) are located at around 850 m a.s.l; the forest is medium-age, dense and dominated by *Fitzroya cupressoides.* Mean tree diameter and height (of trees ≥ 30 cm diameter at breast height, DBH) is ca. 27 cm and ca. 14 m, respectively. According to tree-ring samples most of the trees are at least 240 years old, with the oldest tree reaching a minimum age of 294 years old. Forests in this area have been affected by fires in the last few centuries, and the studied stands are aggrading probably following a stand-devastating fire that occurred in 1681 (Lara et al. 1999; Barichivich 2005; Urrutia-Jalabert et al. Submitted a). Total stand biomass reaches around 113 Mg C ha^{-1}, of which the *Fitzroya* trees account for around 93 Mg C ha^{-1} (82%, Urrutia-Jalabert et al. Submitted a). The studied forest stands present a structure that could be considered typical of the widespread condition of *Fitzroya* forests in this Range where human intervention has been substantial in recent centuries. In Alerce Andino, the studied stands (two nearby 0.6 ha plots with similar characteristics) are located at 760 m a.s.l. The old-growth studied forest has fewer stems than in the Coastal Range and *Fitzroya* trees are much larger and taller, reaching a mean of ca. 137 cm diameter and ca. 31 m height. Most of the *Fitzroya* trees are at least 500 years old (tree cores that do not reach the pith) and the oldest tree recorded is at least 1470 years old. Total stand biomass reaches 447-517 Mg C ha^{-1}, of which *Fitzroya* accounts for 329-401 Mg C ha^{-1} (74-78%, Urrutia-Jalabert et al. Submitted a). The size structure of the forest indicates that the *Fitzroya* trees form a single pioneer cohort that established over 1500 years ago after a disturbance (probably a landslide), but that is not regenerating under current forest conditions, but also showing no mortality. Underneath *Fitzroya* there is a high biomass subcanopy of *Nothofagus* (Nothofagaceae) and other evergreen species that have grown up between *Fitzroya* (Urrutia-Jalabert et al. Submitted a).

Both areas are characterized by high annual precipitation, reaching 4860 mm in the coastal site and ca. 6600 mm in the Andes during 2012 (Urrutia-Jalabert et al. 2015). The northern coastal site has a more Mediterranean climate influence that makes summers drier in this area compared to the Andes (Veblen & Ashton 1982; Urrutia-Jalabert et al. 2015). During 2012, mean annual temperature was 7.26 °C and 6.89 °C in the Coastal Range and Andes sites, respectively. Snowfall is common during winter (from May through September) and persists until spring especially in the Andean site (Donoso et al. 1990). Solar radiation is lower during most of the year in the Andes than in the Coastal Range (24% lower during 2012).

Soils in the Coastal Range are derived from Pre-Cambrian to Paleozoic metamorphic rocks, have a sandy loam texture and are generally shallow (ca. 40-60 cm depth), poor in nutrients and severely podzolized (Veblen & Ashton 1982; Urrutia-Jalabert et al. Submitted a). They have poor drainage due to the presence of schist at shallow depths and can get particularly dry during rainless periods in summer, having a low water retention capacity (Barichivich 2005; Gerding V personal communication). Soils in the Andean site are derived from volcanic parental material, they have a silty loam texture, high C/N ratios and high organic matter content (Peralta et al. 1982; CONAF 1985; Urrutia-Jalabert et al. Submitted a). Soils in this site are deeper (ca. 70-90 cm depth), have better water retention properties and are wetter than those in the Coastal Range all year round (Urrutia-Jalabert et al. Submitted b).

Tree-ring sampling and tree-ring width chronologies development

We sampled 60 adult trees at each site. In the Coastal Range our sampling strategy was based on tree size, so the largest trees (likely being the oldest ones) were chosen. In the Andes, the *Fitzroya* stand is even-aged with most of the trees larger than 100 cm, so no specific sampling rule was followed. At each study site trees were double cored with a 5-mm diameter increment borer at ca. 1.3 m height. Cores were mounted, sanded and tree rings measured to 0.001 mm precision. Tree-ring series were visually dated and cross-dating was verified using the computer program COFECHA (Holmes 1983). As the study site is in the Southern Hemisphere the growth season straddles two calendar years. For dating purposes we employed the Schulman convention (Schulman 1956), which assigns to each tree ring the date of the year in which radial growth started. Ring width measurements of each tree-ring series were standardized to remove variability that is not related to climate (e.g. tree age) with a negative exponential curve or a linear regression using the ARSTAN44h2 program (Cook & Krusic 2013). The quality of the mean standard chronology (mean of standardized series) was evaluated using the expressed population signal (EPS) statistic. EPS is a measure of the similarity between a chronology and a hypothetical one that has been infinitely replicated (Wigley et al. 1984). When the EPS value is above a determined threshold (0.85), the chronology can be considered robust, and it is an indication of temporal stability, good quality, and a strong common signal (Wigley et al. 1984). Chronologies were as old as the length of the increment borer (50-60 cm), diameter of the tree and quality of the samples allowed.

Basal area increments (BAI) for each forest stand were calculated as the mean of the basal area increment of each raw series. The basal area increment for every year in each series was calculated as:

$BAI_t = \pi (SR_t^2 - SR_{t-1}^2)$

Where SR is the stem radius and t is the year of tree ring formation.

BAI is a proxy for above-ground woody biomass accumulation, and trends in unstandardized BAI, unlike tree ring widths, are generally positive and do not decline with age (Silva et al. 2010). Thus, a negative trend in this parameter can be a true indication of a growth decline (Jump et al. 2006; Silva et al. 2010).

Unfortunately, during the first collection in both sites, not all the sampled trees were from within the plots (but from immediate surrounding areas within the stands), so some samples did not have an associated diameter, which is required to calculate BAI. However, 62% and 76% of the series in the Andes and Coastal Range, respectively could still be considered for the basal area increment chronologies and they were of good quality as assessed through the EPS. Trend changes in the standardized tree-ring width and BAI chronologies were assessed for recent decades using the Mann-Kendall non-parametric trend test (mkTrend R routine from the fume package, Santander Meteorology group, 2012, R Development Core Team 2014).

Carbon isotope chronologies

Annual rings from five trees (five cores) for the last 210 years (1800-2010) in each site were considered for isotopic analyses. Purified cellulose is a standard wood component for stable carbon isotope analyses (Taylor et al. 2008). However, since it is very time consuming to extract cellulose, different authors have used or recommended using whole wood or whole wood without extractives, due to strong correlations with the isotopic signal in cellulose (Borella et al. 1998; Warren et al. 2001; Loader et al. 2003; Harlow et al. 2006; Taylor et al. 2008; Granados 2011).

In order to maintain the temporal integrity of the isotopic signal in this study (Loader et al. 2007), wood without extractives was used. Due to the small amount of wood available, resin extraction was performed on core samples (2.5 x 50 mm), as done by Lévesque et al. (2014), following the three-step method proposed by Leavitt & Danzer (1993). Cores were treated under the 2:1 toluene/ethanol, 100% ethanol and boiling water steps for 27 hrs (each phase). Annual rings were subsequently split using a razor blade and pooled for the five trees in each site in order to perform the isotopic analysis. The pooled sample was manually ground to 40 mesh and mixed using a razor blade and a ceramic mortar. This is because we did not have a proper mill to grind such a small amount of samples without contamination. An aliquot of 0.3-0.4 mg of each sample was weighed in a tin capsule and was analyzed using a Carlo Erba NC1500 (Milan, Italy) elemental analyzer on line with a Delta Plus XL (ThermoQuest, Bremen, Germany) mass spectrometer (EA-IRMS). This was performed at the Laboratorio de Biogeoquímica de Isótopos Estables at the Instituto Andaluz de Ciencias de la Tierra (CSIC-University of Granada, Spain). Due to the manual mixing, three replicates of each ring were analyzed with the objective of having a representative sample of the whole ring. To have an estimate of inter-tree variability, isotopic analyses were also performed separately for each tree every ten years. Commercial CO_2 was used as the internal standard for isotopic analyses. Standards were systematically interspersed in analytical batches every ten samples and precision was better than ± 0.1‰ for $\delta^{13}C$.

The carbon isotope ratio ($^{13}C/^{12}C$) was expressed with reference to standard material for which the isotopic ratio is known. The ratio corresponds to $\delta^{13}C$ and is expressed as:

$$\delta^{13}C\ (‰) = (R_{sample}/R_{standard} - 1)1000$$

where R_{sample} and $R_{standard}$ are the carbon ratios of the sample and standard (Vienna Pee Dee Belemnite or VPDB), respectively (McCarroll & Loader 2004).

The isotopic discrimination (Δ) resulting from the preferential use of ^{12}C over ^{13}C during photosynthesis was calculated using the following expression (Farquhar et al. 1982):

$$\Delta^{13}C = \frac{\delta^{13}C_{atm} - \delta^{13}C_{tree}}{1 + \delta^{13}C_{tree}/1000}$$

Where $\delta^{13}C_{atm}$ corresponds to the isotopic value of atmospheric CO_2 and $\delta^{13}C_{tree}$ the measured isotopic ratio in tree rings. Following Farquhar et al. (1982), carbon discrimination was linearly related to the ratio intercellular (c_i) to ambient (c_a) CO_2 concentration through the expression:

$$\Delta^{13}C \cong a + (b-a)\ c_i/c_a$$

where a is the fractionation related to the diffusion of atmospheric CO_2 through stomata (4.4 ‰) and b the fractionation associated with the enzymatic carbon fixation (27 ‰). iWUE, which corresponds to the ratio between the net assimilation rate (A) and stomatal conductance to water vapour (g_w, Ehleringer (1993)) was calculated as follows (McCarroll & Loader 2004):

$$iWUE = c_a(1 - c_i/c_a) \times 0.625$$

$\delta^{13}C_{atm}$ and c_a were obtained from McCarroll & Loader (2004) for the period 1850-1997, values for 1998-2010 were obtained from the Scripps CO_2 website (http://scrippsco2.ucsd.edu/).

In order to establish relationships between the $\delta^{13}C$ chronology and climate variables in each site, $\delta^{13}C$ in tree rings was first corrected for its decreasing trend attributed to the rise of ^{13}C-depleted atmospheric CO_2 (adding the difference between the atmospheric carbon isotope composition (for each year) and a pre-industrial standard value of _6.4% , $\delta^{13}C_c$, McCarroll & Loader, 2004). In addition, the Preindustrial (PIN) correction that uses nonlinear loess regression (McCarroll et al. 2009) was applied on top of the previous one using an R routine especially designed for this purpose (provided by G. Young at Swansea University). This PIN correction attempts to correct for changes in the CO_2 content of the atmosphere and estimate the $\delta^{13}C$ values that would be expected under CO_2 levels present before industrialization (Andreu-Hayles et al. 2011). The magnitude of the correction is restricted by two constraints defined by the physiological response of trees to increases in CO_2: 1) that a unit increase in c_a cannot produce more than a unit increase in c_i (passive response) and 2) that increases in iWUE due to higher c_a are constrained to maintain a constant c_i/c_a ratio (active response). This procedure was mainly applied to maximize the climate signal in the tree-ring $\delta^{13}C$ series, retaining any positive or negative $\delta^{13}C$ trend beyond the effect of CO_2 (McCarroll et al. 2009).

Changes in iWUE together with changes in tree-ring growth/BAI can provide insight into the natural responses of trees in both sites to increasing atmospheric CO_2 concentrations (Waterhouse et al. 2004). Leaving aside positive influences of climate, a positive relationship between changes in growth and iWUE could indicate CO_2 stimulation, while negative relationships would reflect the influence of stressors (Silva & Anand 2013). Since iWUE is a direct function of c_a, its trend may likely be positive over time (Silva &

Horwath 2013); so trends in discrimination and c_i were also evaluated. The Mann-Kendall trend test was used to assess changes in these variables during recent decades.

Trends in climate variables and tree rings-climate relationships

In order to assess changes in climate conditions, stations with the longest and more reliable climate records located close to both study areas were used. Monthly records from the Valdivia station, located at a low altitude ca. 45 km north of the Coastal Range site (9 m a.s.l at 39° 48’ S, 73° 14’ W) and from Puerto Montt located at a low altitude ca. 43 km west of the Andean site (85 m a.s.l at 41° 25’ S, 73° 05’ W) were used as proxies for the local climate of each site. Both meteorological stations recorded precipitation, mean, maximum and minimum temperatures and covered the 1960-2010 period (Universidad Austral de Chile, DMC 2014). There was also a record of cloudiness from Puerto Montt measured in oktas (eighths), which refers to the proportion of the sky covered by clouds (1965-2010, DMC 2014).

Trends in climate records were evaluated for austral summer (December-February) and spring-summer (September-February) using the Mann-Kendall trend test. These seasons have been identified as the most important for tree-growth processes in *Fitzroya* (Villalba 1990; Lara & Villalba 1993).

In order to determine the climate variables related to tree-ring growth, correlation analyses were performed between the standardized chronologies from each site and the associated climate data, both processed using a 20-year high-pass filter to isolate the year-to-year variability (de-trend the correlates). Furthermore, the tree-ring width chronology from AC was also prewhitened due to a strong autocorrelation of first order ($r=0.82$ for 1960-2010 period), even in the high-pass filtered series ($r= 0.43$, $p<0.05$, for 1960-2010). Autocorrelation in the Andean chronology was significant only before detrending ($r=0.48$,

$p<0.05$ for 1960-2010). Therefore, in the case of the Coastal Range chronology, residuals of a lag-1 autoregressive model were used for correlation analysis (Monserud & Marshall 2001). Bootstrapped correlations were calculated between monthly climate variables (ranging from January of the previous summer until March of the current summer) and tree-ring data using the “cor” and the “boot” functions from the “stats” and “boot” R packages, respectively (R Development Core Team 2014). Only correlations significant at 95% confidence level were considered.

Bootstrapped correlation analyses for the isotopic data were performed between the PIN corrected $\delta^{13}C$ chronologies from each site and monthly climate variables (also processed using a 20-year high-pass filter). The autocorrelation in both chronologies before detrending was 0.51 and 0.50 in AC and AA, respectively ($p<0.05$, for the 1960-2010 period).

7.3. Results

Tree-ring width chronologies

The Coastal Range chronology was 296 years and the Andean was 1238 years in length (Table 1). The oldest tree found in the Andes is not within the final chronology due to low correlation. Descriptive statistics of these chronologies obtained from the COFECHA and ARSTAN44h2 programs are presented in Table 1.

Table 1. Characteristics of tree-ring width chronologies from Alerce Costero (AC) and Alerce Andino (AA) forests.

Site	AC	AA
Total number of series	97	52
Time span (years)	1714-2010	772-2010
Mean series intercorrelation	0.464	0.453
Mean sensitivity	0.217	0.292
Period EPS ≥ 0.85	1739-2010	1355-2010

The tree-ring width chronology from the Coastal Range site is characterized by two periods of high ring width values at the beginning of the 1800s and particularly the early 1900s. These events likely correspond to growth releases after fire events (Fig. 1a,b). The Andean chronology seems more stable around the mean and has a lower mean ring width (0.34 ± 0.01 mm) compared with the coastal one (0.75 ± 0.01 mm) during the common period when the coastal chronology has an EPS value ≥ 0.85 (1739-2010, Table 1 and Fig. 1c,d).

The Coastal Range standardized chronology shows a decreasing trend that is especially steep and significant since the 1970s (slope =-0.012 (tree-ring index per year), Mann Kendall p=0.02, Fig. 1e). This trend is also seen in the basal area increment record for the last 40 years (slope=-0.07 (cm^2 per year), p=0.05), indicating that trees in this forest are experiencing lower growth in recent decades (Fig. 2a,c). On the other hand, in the Andean site the standardized tree-ring width (slope=0.0025, $p=1.65x10^{-10}$) and basal area increment chronologies (slope=0.065, $p=1.83x10^{-07}$) present an opposite trend, reflecting a higher radial growth since the 1900s (Figs. 1f and 2b,d). The positive trend in the raw tree-ring width chronology during the last 100 years in the Andes does not have any precedent in the last 600 years (Fig. 1c).

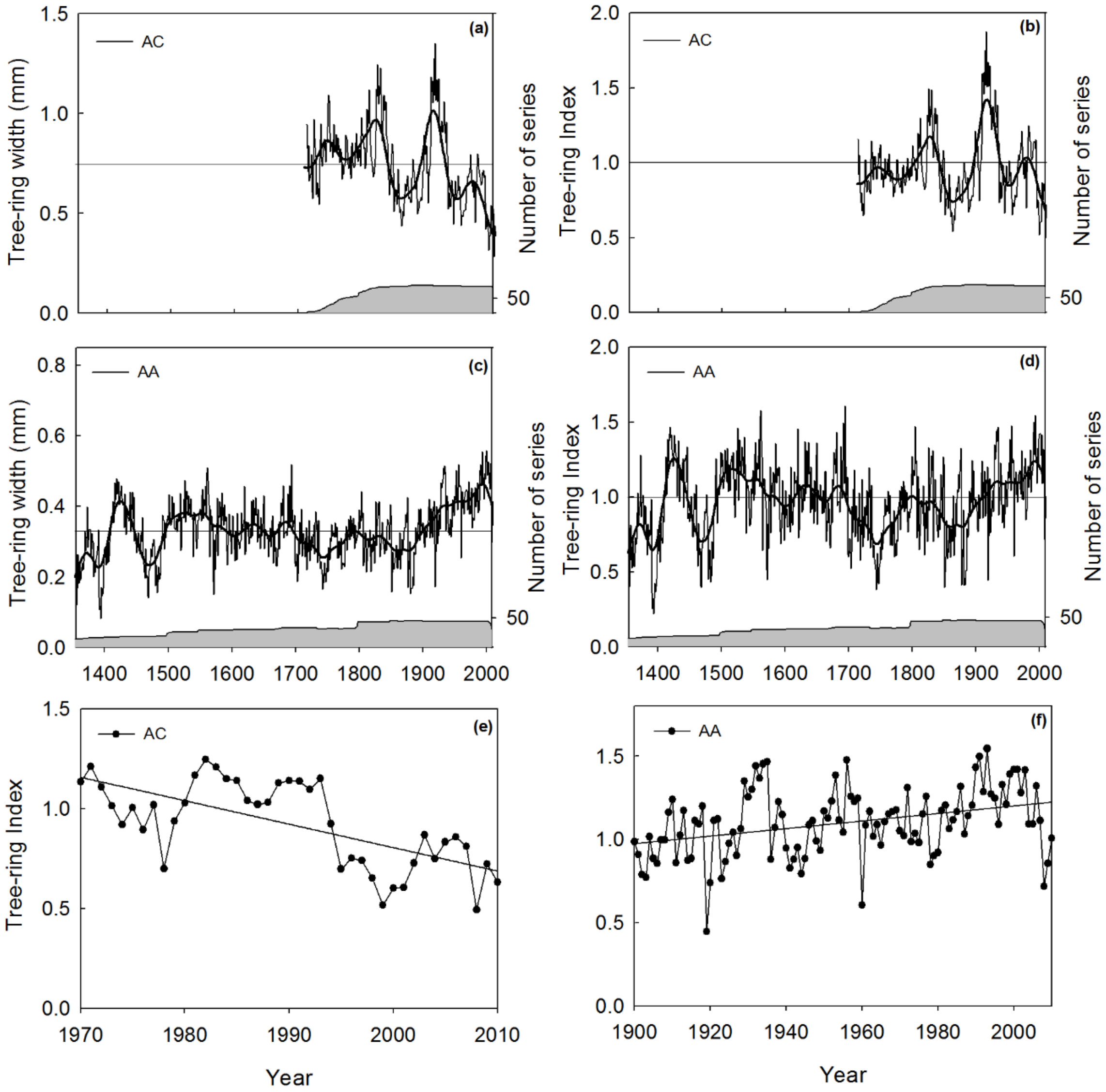

Fig. 1. a) and c) Raw tree-ring width chronologies extending from 1739 to 2010 in Alerce Costero (AC) and from 1355 to 2010 in Alerce Andino (AA), respectively. Only the period with EPS values ≥ 0.85 is shown. The number of series that make up each chronology is also shown. To emphasize the long-term variations, a cubic spline designed to reduce 50% of the variance in a sine wave with a periodicity of 25 years is also shown (Cook & Peters 1981). b) and d) Standardized tree-ring width chronologies during the same period in Alerce Costero and Alerce Andino, respectively. e) and f) standardized tree-ring width chronologies from Alerce Costero (AC) since 1970 (e) and from Alerce Andino (AA) since 1900 (f) showing the significant trends in tree-ring growth in each site.

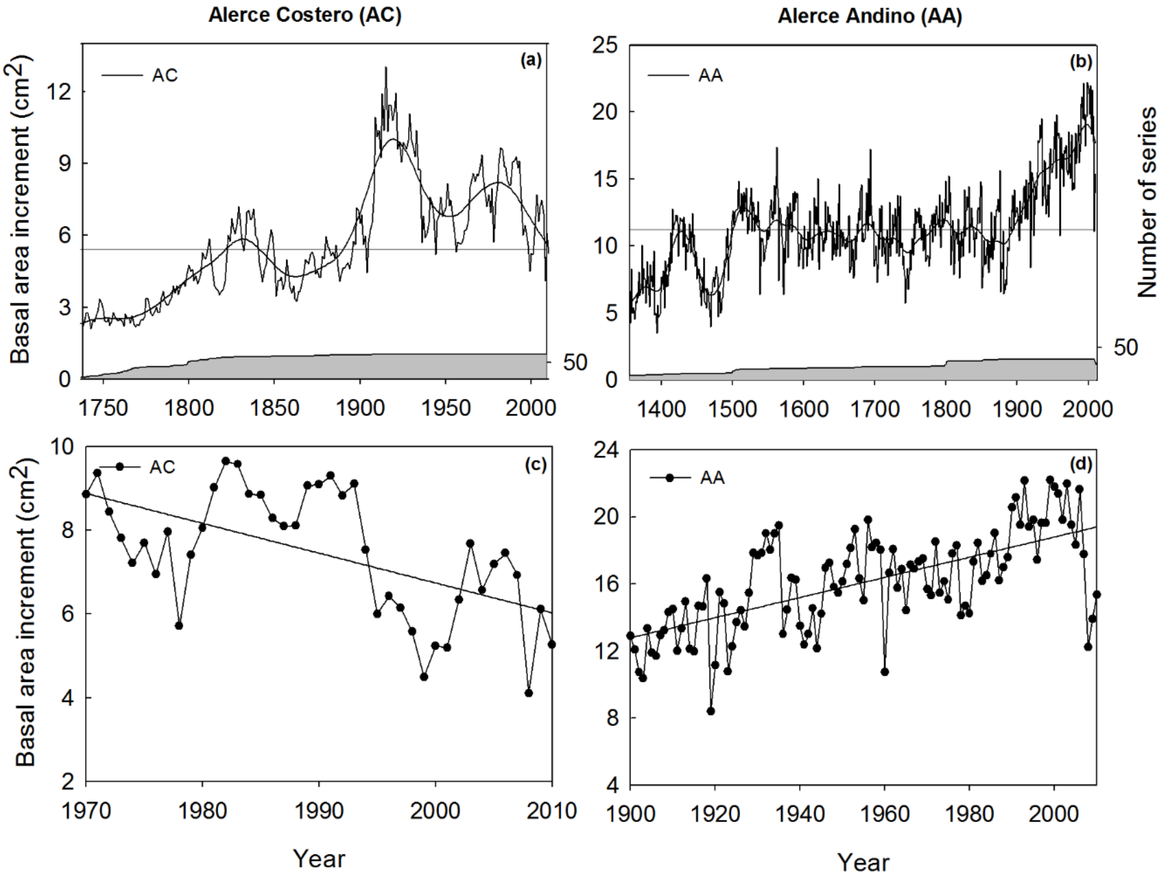

Fig. 2. a) and b): Basal area increment chronologies (in cm^2) for the period 1739-2010 in Alerce Costero (AC) and 1355-2010 in Alerce Andino (AA). A cubic spline version designed to reduce 50% of the variance in a sine wave with a periodicity of 25 years is also shown (Cook & Peters 1981). c) and d) Basal area increment and its significant trend since 1970 in AC and since 1900 in AA.

Carbon isotope chronologies

The raw $\delta^{13}C$ chronologies from both sites show the expected decreasing trend due to the rise of ^{13}C-depleted atmospheric CO_2 caused by the burning of fossil fuels and deforestation since industrialization (Figs. 3, 4 and S1). Raw $\delta^{13}C$ values decreased from -22.52 ‰ to -24.67 ‰ (2.16 ‰) in Alerce Costero and from -23.51 ‰ to -24.89‰ (1.38‰) in Alerce Andino since 1850 (Figs. 3 and S1). The mean standard deviation among trees for the whole period (1800-2010) was 0.47 and 0.59 ‰ in AC and AA, respectively and the mean standard deviation of the three replicates for the total period was 0.31 and 0.23 ‰ in AC and AA, respectively. An important feature is that the δ^{13}C values in the Coastal Range site were slightly lower at the beginning of 1800s (Fig. 3a,b), and since the oldest sampled trees were < 300 years old, the first 50 analyzed years were not considered for further analysis to discard any potential juvenile effect. It has been reported that trees normally do not show any age-related δ^{13}C trend after an initial juvenile phase of ca. 50 years (Gagen et al. 2007).

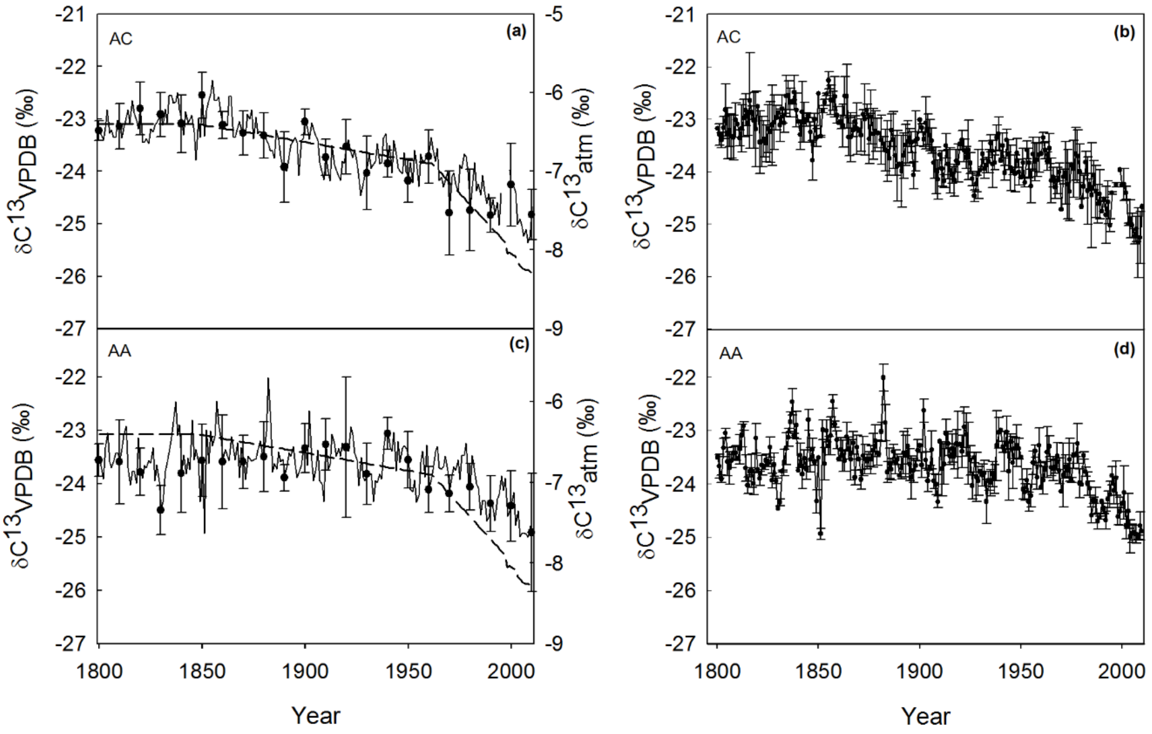

Fig. 3. a) Raw tree-ring δ^{13}C chronology from Alerce Costero (AC) corresponding to the mean of three pooled replicates since 1800. Points and error bars every ten years correspond to the mean and standard deviation of the five tree samples that form the chronology, respectively. This figure also shows the decreasing trend of the $\delta^{13}C_{atm}$ (dashed line, right axis) b) Mean and standard deviation of the three δ^{13}C replicates in AC. c) and d) the same as a) and b), but for Alerce Andino (AA).

In contrast to the raw $\delta^{13}C$, a positive trend was observed in the $\delta^{13}C_c$ (slope= 0.003 ‰, Mann Kendall p= 0.006) and PIN corrected $\delta^{13}C$ chronologies (slope=0.004 ‰, p= 4.2x10^{-7}) in Alerce Costero since the 1900s (Fig. 4a). The discrimination showed a negative trend (slope=-0.003 (‰ per year), p= 0.009) in the same period (Fig. 4b). There is also a positive slope in c_i (slope=0.35 (ppm per year), p=6.68x10^{-5}) and a very steep positive trend in iWUE since the 1900s (slope=0.23 (µmol mol^{-1} per year), p=6.65x10^{-5}), but especially since 1970 (slope=0.95, p=2.13x10^{-7}; slope=0.47, p=1.14x10^{-7}, respectively, Fig. 4c,d). This indicates that trees from the Coastal Range site are being more efficient in the use of water especially in the last few decades.

In the Andes, trends were also significantly positive for $\delta^{13}C_c$ (slope=0.004, Mann Kendall p=4.76x10^{-6}) and PIN corrected chronologies (slope= 0.006, p=1.61x10^{-10}) since the 1900s (Fig. 4e). The trend in discrimination was negative for the same period (slope=-0.004, p=2.89x10^{-6}, Fig. 4f). Positive trends were especially steep for c_i (slope=0.35, p=2.58x10^{-5}) and iWUE (slope= 0.24, p=2.34x10^{-6}) during the last century and even steeper since 1950 (slope=0.66, p=0.0004; slope= 0.43, p=1.85x10^{-17}, respectively, Fig. 4g,h). These trends indicate that trees from the Andes are also being more efficient in their water use, especially during the last few decades.

Comparing both sites, the PIN-corrected chronologies were somewhat similar with a significant correlation on an interannual basis (r=0.30, p<0.05, period 1900-2010). The trend in iWUE was also similar between sites (slopes of 0.23 and 0.24 in AC and AA, respectively). Comparison of raw series are shown in Fig. S2.

Standardized and high-pass filtered tree-ring width chronologies were also significantly correlated between sites, but only when considering the more recent period (r=0.23 and r=0.27, p<0.05, for 1850-2010 and 1900-2010, respectively). Correlations between high-pass filtered tree growth and isotope chronologies were negative, but not significant in AC (r=-0.11 and r=-0.22 for the period 1900-2010 and 1970-2010, respectively). In the Andean site, correlations were only significant and positive when considering the period with the steepest change in iWUE (r= 0.32, $p<0.05$, for 1950-2010).

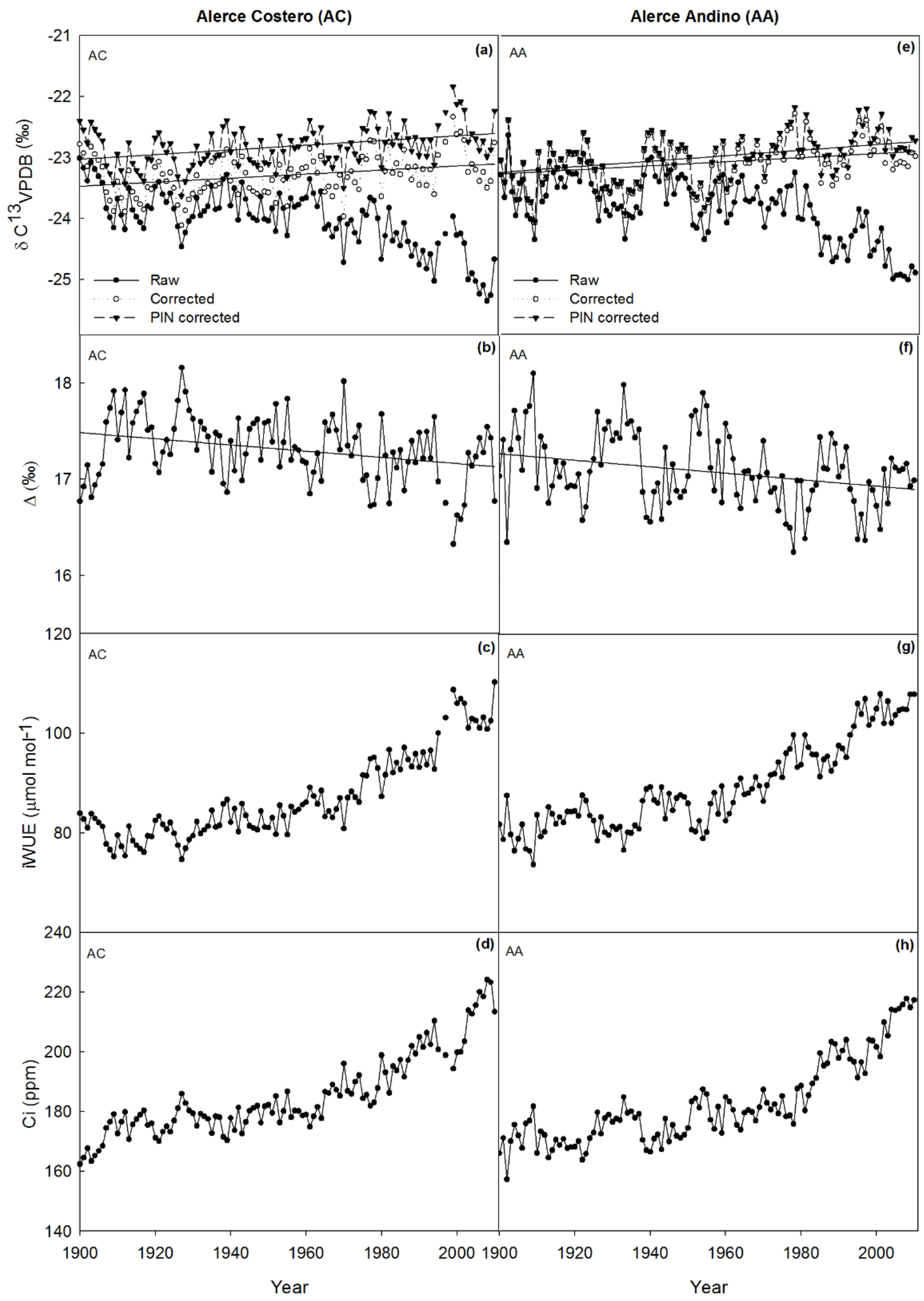

Fig. 4. a) Raw, $\delta^{13}C_c$ and PIN corrected $\delta^{13}C$ for Alerce Costero (AC) since 1900. The raw chronology presents the expected decreasing trend during the last century. b), c) and d) discrimination against ^{13}C, intrinsic water-use efficiency and changes in c_i (intercellular carbon) in Alerce Costero since 1900, respectively. e), f), g) and h) the same as a),b) c), and d), but for Alerce Andino (AA). The trends in corrected $\delta^{13}C$, PIN corrected $\delta^{13}C$ and discrimination are shown.

Trends in climate variables and relationships with tree-ring width and isotope chronologies

The climate records showed that spring-summer and summer precipitation have decreased in Valdivia and Puerto Montt over the period 1960-2010 (Figs. 5a,d and S3a,d). However the precipitation decline has been stronger and significant only in Puerto Montt for both assessed periods (Mann Kendall $p<0.05$). Mean temperature records showed a warming trend during the same seasons, particularly in Valdivia (Mann Kendall $p<0.05$, Figs. 5b,e and S3b,e). This trend is driven only by rising maximum temperatures in both locations (Figs. 5c,f and S3c,f), with no significant trend in minimum temperatures at either site (not shown). Finally, cloudiness from Puerto Montt showed a significant negative trend in spring-summer and summer (1965-2010, Figs. 5g and S3g). The rise in maximum temperatures but not minimum temperatures is consistent with a trend of decreasing cloudiness and increasing insolation.

We next explored correlations between tree ring growth and these meteorological variables. We first performed correlations with individual months to understand broad patterns (Fig. 6). The monthly correlations highlighted distinct relationships with late spring-early summer and mid-late summer conditions, and also possible correlations with preceding summer meteorological conditions. We therefore concentrated our analysis in these broad summer groups: previous mid-late summer (Jan-Mar_p), current late spring-early summer (Nov-Dec), and current mid-late summer (Jan-Mar_c; Fig. 6).

Tree growth in the Coastal Range site was negatively correlated with late-spring-early summer mean and maximum temperatures and positively correlated with precipitation over the same period (November-December, Figs. 6a-c). Partial correlations suggest that maximum temperature seems to have the strongest (negative and significant) influence on growth rate (r = -0.50, p<0.05), with only a moderate positive influence of precipitation (r = 0.12-0.23, p>0.05).

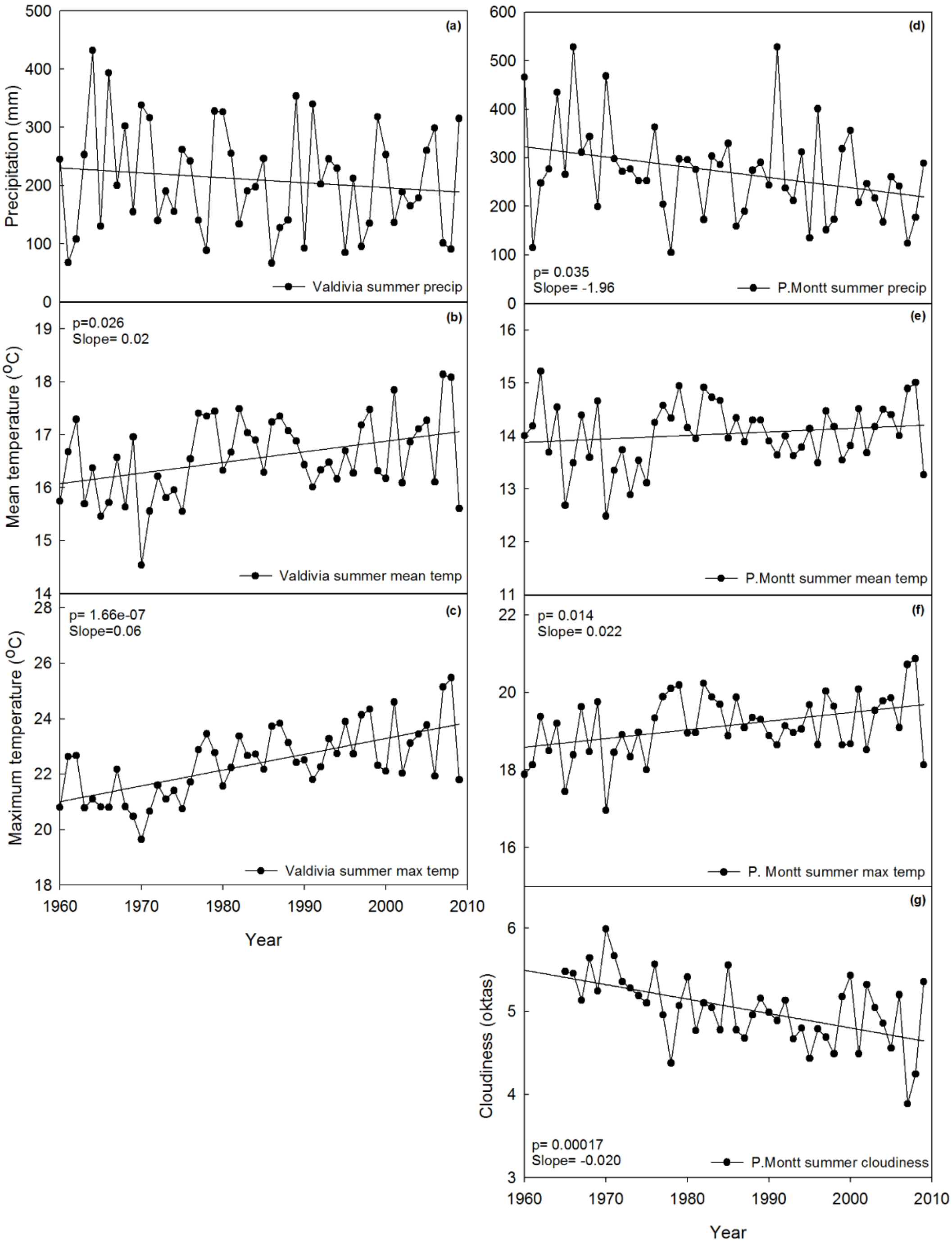

Fig. 5. a), b) and c) Precipitation, mean and maximum temperature during summer (December-February) in Valdivia, respectively. d), e) and f), the same as a), b) and c), but for Puerto Montt. g) Summer cloudiness (in oktas) in Puerto Montt. Only the significant slopes and p-values are shown.

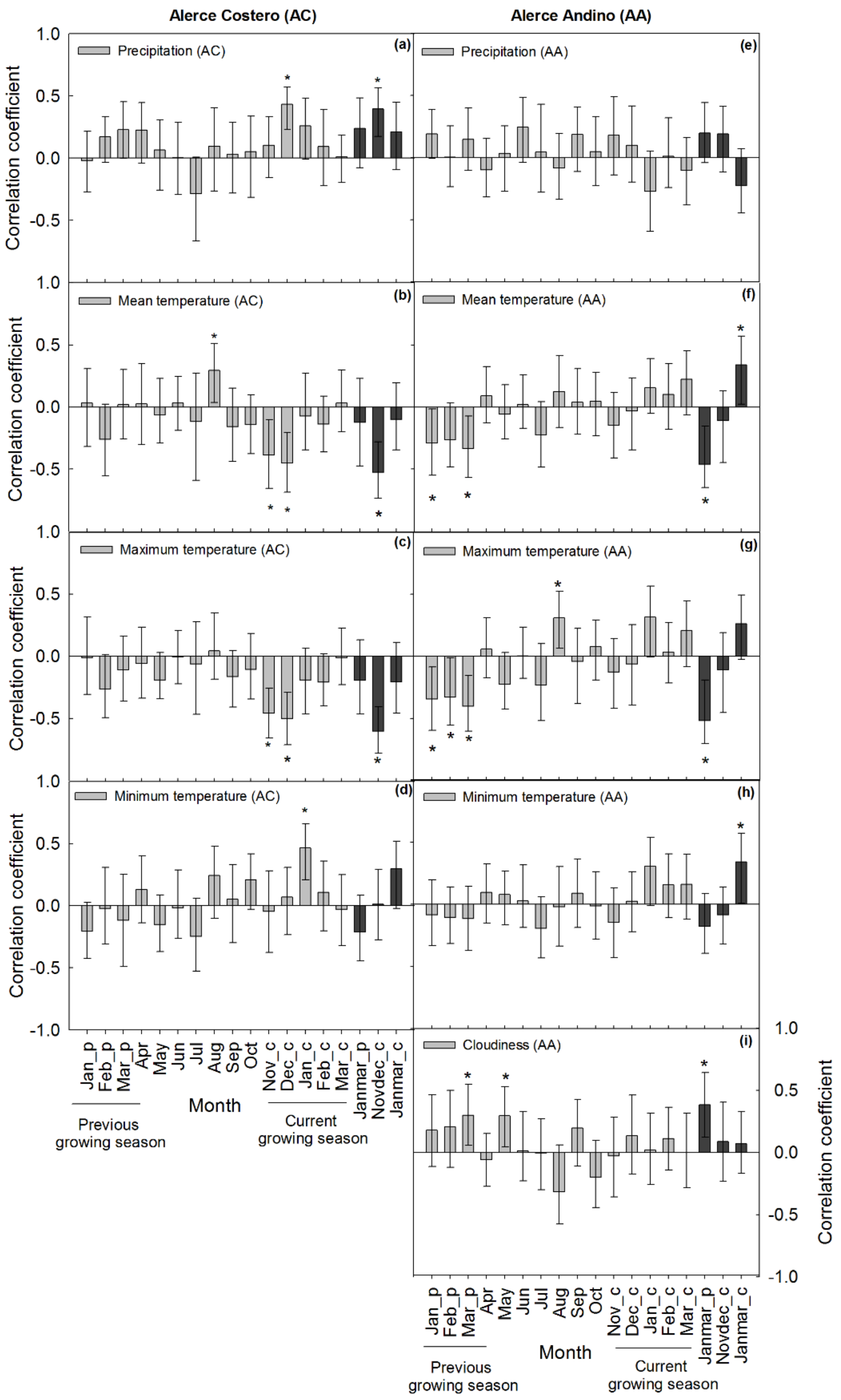

Fig. 6. Bootstrapped correlations between the standardized chronology from the Coastal Range site (AC) and monthly a) precipitation, b) mean temperature c) maximum temperature and d) minimum temperature in Valdivia. e), f),g) and h) as above, but for correlations between the standardized chronology from the Andean site (AA) and monthly

climate variables in Puerto Montt. i) correlations between tree growth in AA and cloudiness in Puerto Montt. In all cases a 20-year high-pass filter was applied to the standardized chronologies and climate data, and prewhitening was additionally applied to the Coastal Range chronology (see the methods section). Correlations with previous and current climate are indicated as “period_p” and “period_c”, respectively. Mean correlations were significant if after 1000 bootstrapped iterations their confidence intervals do not cross zero. Significant correlations are marked with an asterisk (*).

In the Andean site the most significant relationships for growth were negative correlations with the previous summer mean and maximum temperatures and a positive correlation with the previous summer cloudiness (Figs. 6 f,g,i). Partial correlations with previous summer conditions show that when controlling for cloudiness, maximum temperature was the only variable significantly correlated with tree-growth ($r = -0.31$, $p < 0.05$). In contrast to previous year relationships, correlations with current mid-late summer temperatures were positive but only significant for minimum and mean temperatures (Fig. 6 f,g,h). Precipitation did not seem to have a significant effect on tree growth in this particularly rainy site (Figs. 6e). Hence, in the Andean site warm temperatures in preceding years lead to less growth in the current year. A clearer representation of tree growth-climate relationships in both sites is shown in Fig. S4.

For the isotope data (PIN corrected $\delta^{13}C$ chronology), in the Coastal Range site the strongest correlations were a positive correlation with current mid-late summer mean, maximum and minimum temperatures (only significant for mean temperature; Figs. 7 b,c,d). This indicates that higher temperatures during mid- late summer lead to less discrimination and higher $\delta^{13}C$.

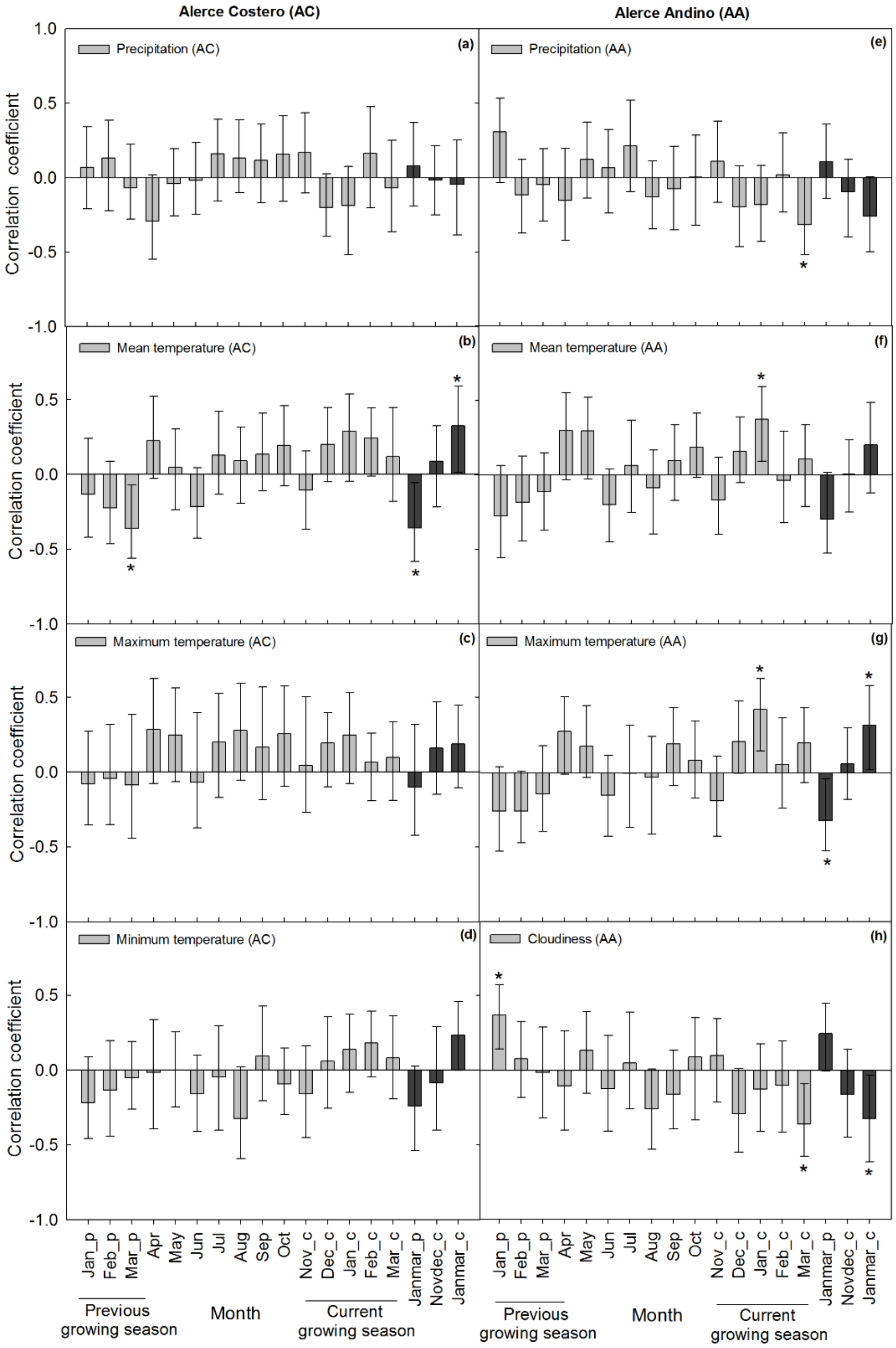

Fig. 7. Bootstrapped correlations between PIN corrected δ^{13}C from the Coastal Range site (AC) and monthly a) precipitation, b) mean temperature, c) maximum temperature and d) minimum temperature in Valdivia. e), f), g) as above, but for correlations between the isotope chronology from Alerce Andino (AA) and monthly climate variables in Puerto Montt. h) correlation between the Andean isotope chronology and cloudiness in Puerto Montt. In all cases a 20-year high-pass filter was applied to the isotope chronologies and

climate data. Correlations with previous and current climate are indicated as “period_p” and “period_c”, respectively. Mean correlations were significant if after 1000 bootstrapped iterations their confidence intervals do not cross zero. Significant correlations are marked with an asterisk (*).

There was also a significant negative correlation with previous mid-summer mean temperature (Fig. 7b). In the Andean site the strongest correlations were a negative correlation with mid-late summer cloudiness and a positive correlation with maximum temperature during the same period (Figs. 7 g,h). Thus, years with low summer cloudiness coincide with low discrimination and high δ^{13}C values. A significant negative correlation was also found with previous mid-late summer maximum temperature (Fig. 7g). There were no significant correlations with summer minimum temperatures in this site (results not shown). Partial correlations between the PIN corrected δ^{13}C and mean and maximum temperatures, controlling for cloudiness, indicate that temperature was the variable that mostly contributed to the relationships found for the previous summer, though no significant partial correlations were found. In contrast, for the current summer, cloudiness was the most important and significant factor when controlling for mean temperature. Examples of the isotopes-climate relationships are shown in Fig. S5.

7.4. Discussion

Trends in tree-ring width and carbon isotope chronologies

The growth trends observed in the tree-ring width chronologies were confirmed when analyzing the basal area increment chronologies in both sites. Since a negative trend in BAI is a strong signal of a genuine decline in tree growth (Jump et al. 2006; Silva et al. 2010), we can be confident that biomass accumulation rates in Alerce Costero have been decreasing since 1970. In the Andes, the sustained positive trend in tree growth is striking in this old stand, suggesting that the giant trees in this forest have been accumulating biomass at a faster rate since the beginning of the century.

The $\delta^{13}C_c$ and PIN corrected $\delta^{13}C$ chronologies showed significant positive trends since the 1900s in both sites. The PIN correction particularly increased the $\delta^{13}C_c$ values for the Coastal Range site, while in the Andean site the PIN correction did not exert much change (Fig. 4a,e). This is in agreement with what has been reported by Andreu-Hayles et al. (2011), that the PIN correction exerts the largest improvements in drier rather than more humid sites, because it is mostly based on the response of stomatal conductance to the rise in c_a. Furthermore, discrimination has significantly decreased since the 1900s in both sites; this is in contrast with what was reported by Leavitt & Lara (1994, for an Andean site) who found a constant c_i/c_a since the 1700s and argued that the *Fitzroya* $\delta^{13}C$ is not being affected by changes in CO_2 or climate conditions. Differences in the trees' response between both studies may be partly caused by the different altitudes of the study sites (450 and 760 m a.s.l in Alerce Andino in Leavitt & Lara (1994) and this study, respectively), which may lead to a different response of the species to climate because of differences in cloudiness and precipitation regimes.

Following patterns in discrimination, iWUE has increased by 29% in Alerce Costero and 32% in Alerce Andino since the 1900s (increase between the period 1900-1910 and 2000-2010), indicating that trees are actively responding to environmental changes. These values compare relatively well with the range of values reported for forests in Europe and are higher than the mean reported just for conifers (25.7%) and for all species (27.8%) in this continent (Saurer et al. 2014). Estimates of 14% and 22% increase in iWUE (without considering the effects of climate) were recently reported for broadleaf and conifer sites in Europe, respectively; while increases of 30% were reported when climate effects were included (Frank et al. 2015).

In order to further explore the evidence for an active response of trees in our study sites to environmental changes, and specifically to increases in CO_2; the observed changes in $\delta^{13}C_c$, discrimination and iWUE can be compared with the expected curves according to the three scenarios reported by Saurer et al. (2004). These scenarios mainly diverge in the degree by which the increase in c_i follows the increase in c_a: 1) c_i constant, so $\delta^{13}C$ increases, c_i/c_a decreases and iWUE increases strongly, 2) c_i increases in proportion to c_a, so c_i/c_a is constant, $\delta^{13}C$ decreases in parallel with $\delta^{13}C_{atm}$ and iWUE increases but not as strongly as in scenario 1, and 3) c_i increases at the same rate as c_a, so c_a-c_i is constant, $\delta^{13}C$ decreases more strongly than $\delta^{13}C_{atm}$ and iWUE is constant (Saurer et al. 2004). Trees from both sites presented an increasing trend in $\delta^{13}C_c$, a decreasing trend in discrimination, and an increasing trend in iWUE since 1900, a response close to scenario 1. Although c_i is not constant as scenario 1 predicts, it increases at a much lower rate than c_a, so c_i/c_a decreases. This demonstrates that trees may not only respond in an active way to CO_2, but also that other environmental factors are influencing their stomatal conductance and photosynthetic rates, producing a decrease in discrimination in these sites. This is further supported by the positive trends in $\delta^{13}C$ maintained after the PIN correction, which indicates that climatic factors are also influencing the response of trees in these two sites (Andreu-Hayles et al. 2011). Changes in iWUE are especially strong since 1970 in the Coastal Range site, and since 1950 in the Andean site, when the decline in raw $\delta^{13}C$ is much less than the decline in $\delta^{13}C_{atm}$ in both sites (Fig. 3a,c).

The most common observed response at changing atmospheric CO_2 recorded in forests worldwide seems to be described by scenario 2, i.e. constant c_i/c_a. Examples include conifers in Eurasia and some species in Mediterranean areas (Saurer et al. 2004; Andreu-Hayles et al. 2011), as well as forests in China (Wang et al. 2012). A recent study in European forests also determined that trees were mostly exerting a moderate control on atmospheric CO_2 increases (Frank et al. 2015). However, other species such as *Pinus sylvestris* in northwestern Spain (Andreu-Hayles et al. 2011), *Abies alba* in the Spanish Pyrenees (Linares & Camarero 2012), and a tropical dry forest species in Mexico (Brienen et al. 2011) have responded in a manner close to scenario 1, with stronger increases in iWUE.

Trends in the isotope series are certainly real responses of trees to environmental changes in the Andean site due to the longevity of the stand. In the Coastal Range, although trees are much younger (< 300 years age), it is unlikely that an age/height effect has played a role in the last 100 years. The steepest increases in height are produced approximately until trees reach 20 cm in diameter, which according to the mean growth rate would correspond to trees of ca. 130 years old (data not shown). Moreover, *Fitzroya* is a shade-intolerant species that tends to grow faster in height than diameter, which is reflected in the small height difference between trees 10-20 cm diameter (9 m) and trees 20-86 cm diameter (13 m) in this site.

Climate relationships with tree-ring width and isotope chronologies

The overall observed trends in climate are in line with climate change projections of warmer and drier summers in southern Chile as summer rain belts migrate poleward in a warmer world (Fuenzalida et al. 2007).

Tree growth was negatively correlated with temperature (especially maximum temperature) in both sites. The higher reliance on previous climate conditions in the Andean site could probably be explained by the shorter growing season, due to lower radiation and temperatures in the Andes compared with the Coastal Range (Urrutia-Jalabert et al. 2015). This shorter growing season may result in the trees being more dependent on carbohydrate reserves from storage to initiate growth. Warmer and drier conditions during summer could cause stomata to close and, combined with higher respiration rates, could reduce the starch reserves that can be utilized in the following growing season (Deslauriers et al. 2014; Urrutia-Jalabert et al. 2015).

In contrast, tree-ring growth in the Coastal Range site is not correlated with the previous year climate, yet presents a high lag-1 autocorrelation, which implies that current's year growth draws on carbon stored in the previous year. We hypothesize that growth at this site might be primary limited by cambial dynamics (i.e. sink activity) rather than by carbon uptake (i.e. source). Thus, hot summers (less humid conditions) will reduce growth, because of their primary effect on cell turgor and expansion. This is consistent with high-precision dendrometers observations showing that prolonged warm and dry conditions lead to strong contractions in the stems and reduce tree growth in this site (Urrutia-Jalabert et al. 2015). It has been reported that carbon investment in storage may help long-lived trees by giving them safety margins to keep hydraulic transport and metabolism under severe stress (Epron et al. 2012; Sala et al. 2012; Palacio et al. 2014). Moreover, it has been argued that use of carbohydrates from storage enables re-sprouting, which seems important for trees exposed to recurrent disturbance (Regier et al. 2010). *Fitzroya* has the ability to re-sprout by root-suckers, strategy that is characteristic in the Coastal Range (Veblen & Ashton 1982; Donoso et al. 2006). The stronger Mediterranean influence and low soil water retention in Alerce Costero would also make the tree-growth relationship with summer precipitation much more likely to be significant at this site, as is observed.

The positive effect of current temperature on tree growth in Alerce Andino might be partly given by the positive effect that night temperatures have on growth in this site (Urrutia-Jalabert et al. 2015) and also by higher radiation levels commonly associated with warmer conditions (this is concluded based on the isotopic response of trees).

The stronger tree-growth relationships with maximum rather than with mean temperature in both sites indicate that vapor pressure deficit (VPD) is the key variable negatively related with *Fitzroya* growth on a monthly-to-seasonal timescale, as also suggested by Urrutia-Jalabert et al. (2015) using high precision dendrometers.

Temperature appears to be the main variable related to δ^{13}C in Alerce Costero. The relationship with temperature is partly indirect, since hot summers are usually drier, so temperature is correlated with vapor pressure deficit and soil water content, which are the variables that directly influence stomatal conductance, and consequently δ^{13}C (McCarroll & Loader 2004). Thus, high temperatures or low humidity reduce stomatal aperture and discrimination against ^{13}C (high δ^{13}C values, Helle & Schleser 2004b), which is consistent with the drier climate and poor soil water retention conditions observed in this site.

In the Andean site there was a negative relationship between δ^{13}C and current summer cloudiness, which can be explained by the direct positive influence that irradiance has on photosynthetic rate, and consequently on a decreased discrimination and increased iWUE (McCarroll & Loader 2004; Johnstone et al. 2013). Although we did not have cloudiness data for the Coastal Range site, using the data from Puerto Montt did not give any significant correlation. The positive influence of radiation in Alerce Andino may be due to the lower radiation and much higher precipitation in this site. As the Andean site is rarely if ever water-limited, the trees are able to take advantage of the extra solar radiation during periods of low rain and cloud cover. In the Coastal Range site, the concurrent water stress prevents trees from taking advantage of higher insolation. A negative correlation between cloudiness and $\delta^{13}C$ has been reported especially in other sites that are usually not water limited, such as coniferous forests in northern Europe (Gagen et al. 2011; Loader et al. 2013) and coast redwoods in northern California (negative relationship with fog, Johnstone et al. 2013). The positive relationship between $\delta^{13}C$ and current temperature in the Andes would be mainly given by its correlation with cloudiness (e.g. less cloudy days are warmer).

The negative correlation between $\delta^{13}C$ and temperature from the previous growing season in both sites is difficult to justify in terms of the isotopic theory. One possible explanation could be related to the strong carryover effect of stored products in this species (significant lag-1 autocorrelation in growth and $\delta^{13}C$). It has been reported, especially for deciduous tree-species, that there is a close correlation between the isotopic signature of the early wood of a year and the latewood of the previous year, or simply that the carbon incorporated in early wood mainly comes from enriched stored starch from the previous season (Helle & Schleser 2004a; Offermann et al. 2011). This effect has also been demonstrated for evergreen and conifer trees (Jäggi et al. 2002; Kagawa et al. 2006; Vaganov et al. 2009).

We hypothesize that increasing summer temperatures would cause higher maintenance respiration rates (Adams et al. 2009), which could reduce the starch reserves that can be used for the following growth period (Deslauriers et al. 2014; Urrutia-Jalabert et al. 2015).

This reduction in storage would cause that less growth during the following year would rely on the enriched starch and then the $\delta^{13}C$ in this ring wood would be more depleted. The opposite pattern would also hold true, if lower temperatures are present. This explanation would be especially supported in the Andean site, with lower tree growth following a warmer preceding summer. In the Coastal Range site this mechanism is less supported; however, if carbohydrates are easily transferred from one year to another (high autocorrelation in growth), the isotopical signal in carbon can be also transferred.

The use of the whole ring in this study is a constraint for deciphering a clear climate signal in carbon isotopes in *Fitzroya* and prevents higher correlation coefficients. The use of stored carbon in early wood and the mixing of carbohydrate pools of different origin and age have been reported to prevent or dampen the relationship between $\delta^{13}C$ and climate (Keel et al. 2007; Offermann et al. 2011; Gessler et al. 2014). Further isotopic studies should aim to separate early and late wood components, although the narrow rings of *Fitzroya* may be a limitation.

Finally, the weak correlation (negative) between tree growth and isotope records in the Coastal Range can be explained because the months with a significant climate effect were different for tree-ring width and $\delta^{13}C$. This can also be due because wood growth is often disconnected from carbon assimilation caused by variable allocation to other tissues and remobilization of carbohydrate reserves (Mölder et al. 2011; Härdtle et al. 2013). In Alerce Andino, positive correlations between tree-growth and carbon isotopes are in agreement with what could be expected, since lower discrimination and high $\delta^{13}C$ values are associated with higher growth in this site.

Changes in tree growth and water-use efficiency

A positive correlation between basal area increment and iWUE changes in the Andean site (Fig. 8), suggests that an increment in the photosynthetic rate, rather than a decrease in stomatal conductance has been taking place (Silva et al. 2010). The increase in tree growth reported for the last century in this site is somewhat unexpected given the climate-growth relationships and the warming trend in southern Chile. We believe that this positive growth trend has been produced by a raise in photosynthesis which has likely been driven by some combination of CO_2 and/or surface radiation increases. A regional rainfall decline in southern Chile has been recorded since the beginning of the 20th century (1901-2005, González-Reyes & Muñoz 2013), so pronounced changes in CO_2 have occurred in parallel with changes in climate, making it difficult to discriminate between both effects. The positive effect of less cloudiness (more radiation) on tree-ring development was inferred looking at the isotope-climate response, because current summer cloudiness is not significantly correlated with tree growth, probably because this variable is strongly correlated with precipitation (positively) and maximum temperature (negatively) and none of these variables are significantly related with growth in the current season. The positive correlation of growth with mean and mainly minimum temperature in the current summer is less likely to be playing a role on the *Fitzroya* increasing growth trend, since minimum temperatures are not increasing in the last decades. Further research is certainly needed to better understand the effects of previous and current climate conditions on the growth of Andean *Fitzroya* forests.

A similar positive growth response under current warming conditions has been reported for *Sequoia sempervirens* during recent decades, which has been attributed to increasing insolation due to decreased summer fog frequency in northern California (Sillett et al. 2015). *Sequoiadendrum giganteum* has also shown an increase in tree level productivity early this century compared with early 20th century, trend that was attributed to different causes such as extended growing seasons, CO_2 fertilization and/or nitrogen deposition (Sillett et al. 2015). The correlation functions between growth and climate in these species are in some respect similar to the ones found in *Fitzroy*a (they like cool and mostly wet conditions); with positive effects of current summer soil moisture availability and minimum temperatures, but also with negative effects of current spring and maximum temperatures in some northern *S.sempervirens* populations (Carroll et al. 2014). In the case of *S.giganteum*, growth is mainly negatively correlated with current summer temperatures and none of both species present correlations with previous summer conditions (Carroll et al. 2014). Positive tree-growth trends in long-lived and old trees emphasize that these trees are indeed able to respond to environmental changes (Sillet et al. 2015).

Growth rates have also been reported to increase in recent decades especially in some other moist temperate sites (Cole et al. 2010; McMahon et al. 2010), but also in Mediterranean species, where the increase was mainly attributed to CO_2 fertilization (Martinez-Vilalta et al. 2008; Koutavas 2013).

A negative relationship between iWUE and BAI in the Coastal Range site reflects an increasingly stressed environment (Fig. 8, Silva & Anand 2013) and suggests that a reduction in stomatal conductance rather than an increase in photosynthetic rates has prevailed (Lévesque et al. 2014). The decreasing trend in growth may be produced by a negative effect of warmer temperatures (higher VPD) on cell growth and enlargement, with the increase in iWUE not being sufficient to counteract this effect.

It is important to point out that the “dual-isotope approach” conceptual model of Scheidegger et al. (2000) may be recommended in the future to better constraint the interpretation of carbon isotopes and separate the effects of carbon assimilation and stomatal conductance on discrimination. This approach uses the oxygen isotope composition ($\delta^{18}O$) in tree rings, which are modified by stomatal conductance and not by assimilation rate, to better interpret changes in iWUE caused by changes in environmental variables (Saurer & Siegwolf, 2007).

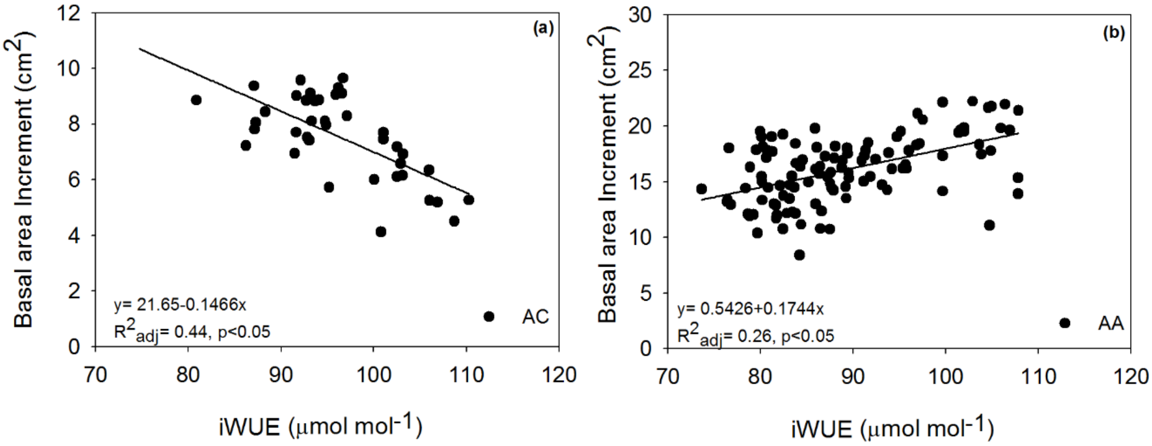

Fig. 8. a) Relationship between the basal area increment chronology from Alerce Costero (AC) and iWUE in trees from this area (period 1970-2010). b) Relationship between the basal area increment chronology from Alerce Andino (AA) and iWUE in trees from this area (period 1900-2010). The linear regression equation and the adjusted R^2 are presented as an approximate indicator of the relationship, but these statistics are not accurate due to temporal autocorrelation.

Numerous studies have reported increases in iWUE, but no increases or even decreases in tree radial growth in Mediterranean (Andreu-Hayles et al. 2011; Linares et al. 2011), high altitude forests (Gómez-Guerrero et al. 2013) and recently in tropical forests (Van der Sleen et al. 2015). Furthermore, no increases in growth have even been reported for mesic sites in Europe, where temperature-induced drought stress has stimulated stomatal closure and consequently reduced carbon uptake and growth (Lévesque et al. 2014). Two global studies concluded that tree growth has not increased as expected due to CO_2 increases during the last 40 years, and that other factors (e.g. warming-induced stress, nutrient limitation, long-term acclimation) have played a role in this pattern (Peñuelas et al. 2011; Silva & Anand 2013). In the case of southern South America, a decreasing trend in tree-growth has been observed in dry-mesic forests species in Patagonia. This decrease in growth has mainly been attributed to drier conditions associated to the positive trend of the Southern Annular Mode (SAM) in recent decades (Villalba et al. 2012). This trend in SAM, especially since the 1950s has been unprecedented in the last 600 years, and it is still uncertain how it will evolve in the future, so its projected effects on forests remain to be understood (Villalba et al. 2012).

Hence, our study finds that the long-term response of one of the longest-lived trees in the world to recent environmental changes has mainly been site dependent, despite the small geographical range of the species. Lower summer (December-February) precipitation in the Coastal Range site (average of 839 mm/year for 2011-2013) compared with the Andes (average of 1413 mm for 2011-2013) may be limiting for growth under current warmer and drier conditions. This sensitivity is amplified because of the shallower and lower water retention capacity soils in the Coastal Range site (Urrutia-Jalabert et al. 2015). Finally, it is also possible that gradual shifts in allocation to other tissues (e.g. roots or canopy) and that the extremely poor nutrient conditions in these soils may also be contributing to the absence of a CO_2-driven woody growth signal in this site (Lapenis et al. 2013; Van der Sleen et al. 2015).

Considering the results from our study, trees from the Coastal Range site or growing under similar conditions are more susceptible to reduce their growth due to climate change, so priority should be given to monitor and protect these populations that have been most strongly affected by fires and human interventions in the past. Despite the current positive trend in *Fitzroya* radial growth in the Andean site, increasing drier and warmer conditions in the future could result in decreasing tree growth at this site too as water supply restrictions dominate over increased sunshine effects (see for example the reduction in growth in 2008 under warmer and drier conditions during 2008 and early 2009, Figure 1e, 5, S3). A recent study reported that conifers growing in mesic sites in Europe are particularly vulnerable to short-term water deficits and may undergo significant growth reductions under drier summer conditions (Lévesque et al. 2013). Our study provides evidence that tree growth even in very wet temperate rainforests can be negatively affected by ongoing and projected changes in climate.

7.5. Acknowledgements

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Chapter 7. Supporting information

Figures

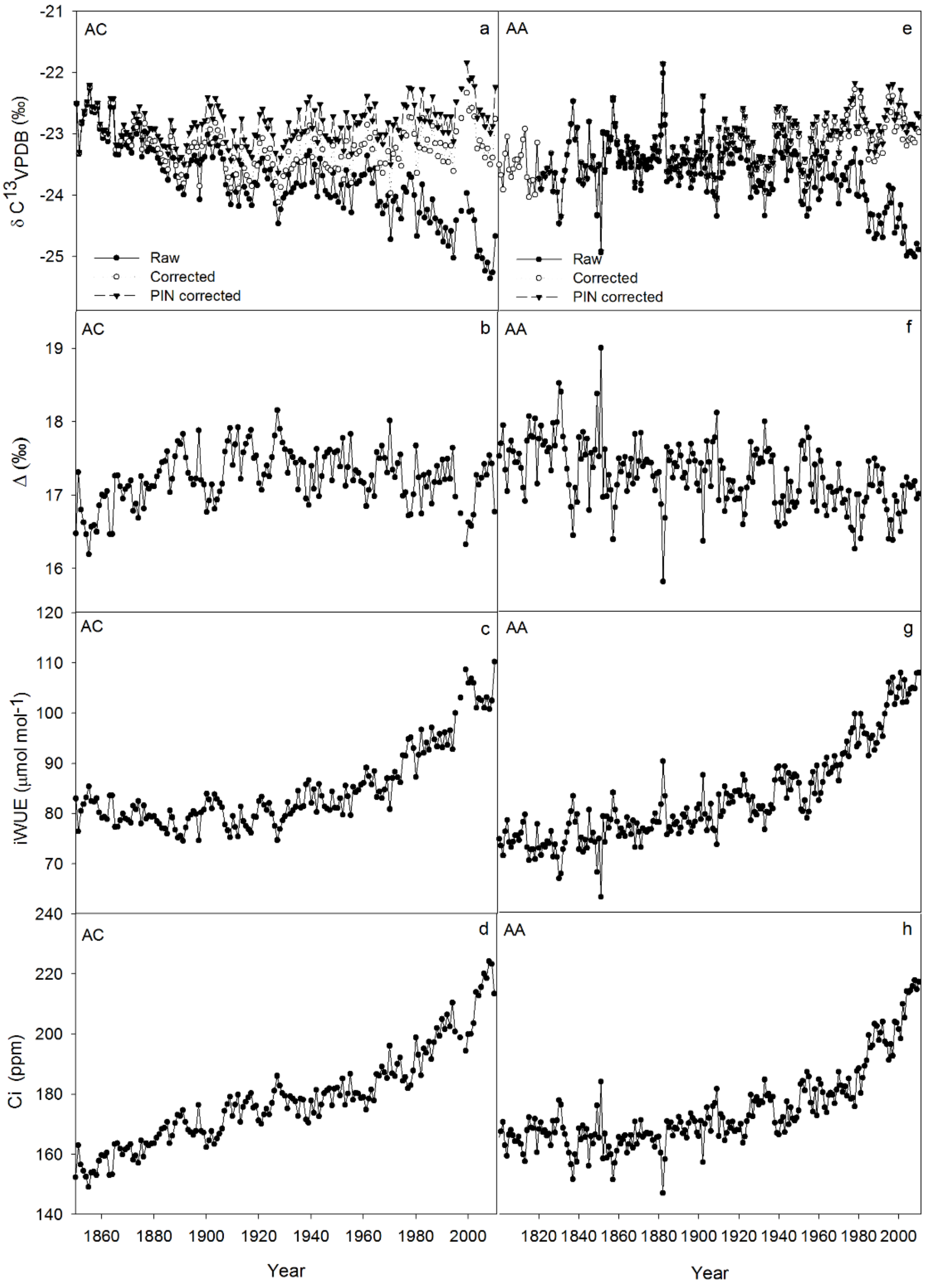


Fig. S1. a) Raw, $\delta^{13}C_c$ and PIN corrected $\delta^{13}C$ for Alerce Costero (AC) since 1850, b), c) and d) discrimination against ^{13}C, intrinsic water-use efficiency and changes in c_i in Alerce Costero since 1850, respectively. e), f), g) and h) the same as a),b) c), and d), but for Alerce Andino (AA) since 1800.

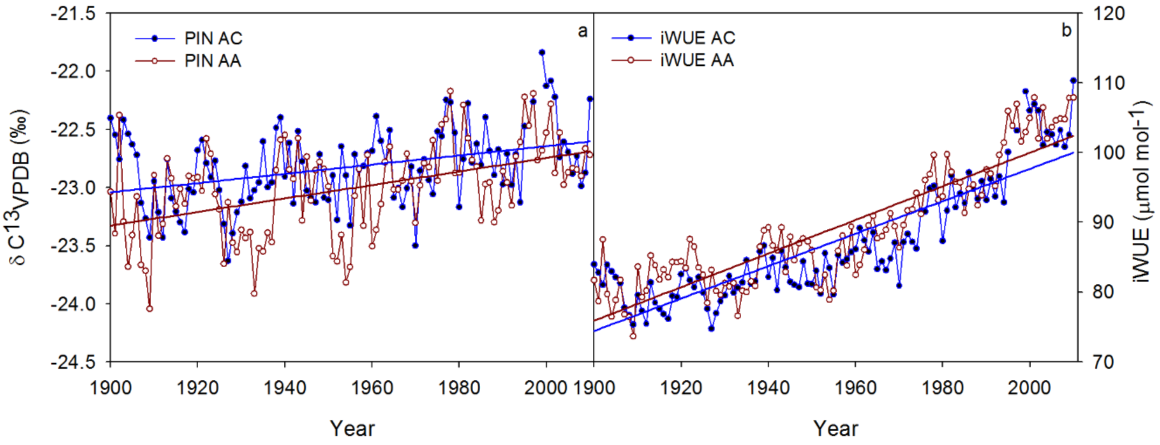


Fig. S2. a) PIN corrected δ^{13}C in Alerce Costero (AC) in blue and Alerce Andino (AA) in dark red, showing a similar trend and years with coincident δ^{13}C values since 1900, b) intrinsic water use efficiency (iWUE) in both sites also demonstrating similarities since 1900.

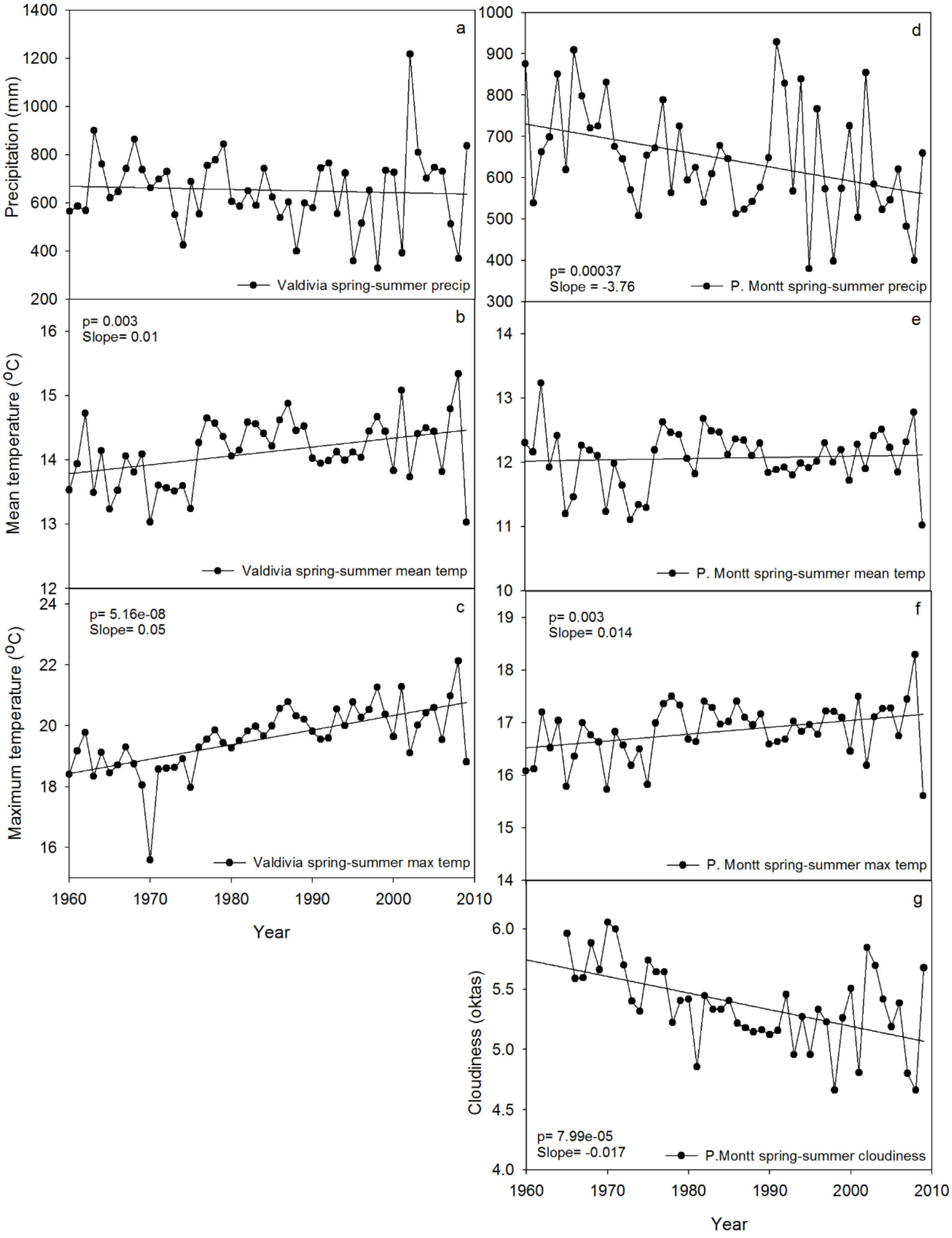


Fig. S3. a), b) and c) Precipitation, mean and maximum temperature during spring-summer in Valdivia, respectively. d), e) and f) the same as a), b) and c), but for Puerto Montt. g) Spring-summer cloudiness in Puerto Montt. Only the significant slopes and p-values are shown.

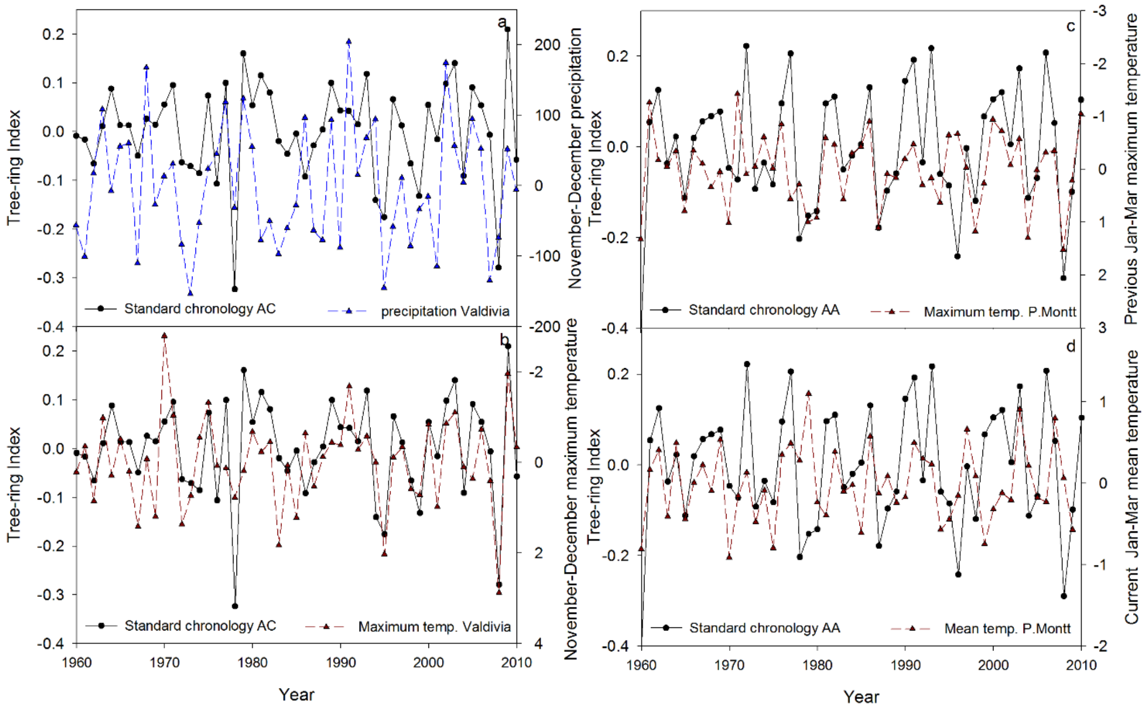


Fig. S4. Relationship between the standard chronology of Alerce Costero (AC) and a) November-December precipitation and b) November-December maximum temperature in Valdivia. Relationship between the standard chronology of Alerce Andino (AA) and c) previous January-March maximum temperature and d) current January-March mean temperature in Puerto Montt. The axes in b) and c) have been inverted for a better portrayal of the relationship. Tree-ring and climatic series have been detrended using a 20-year high-pass filter and the Coastal Range chronology has been additionally prewhitened to remove autocorrelation.

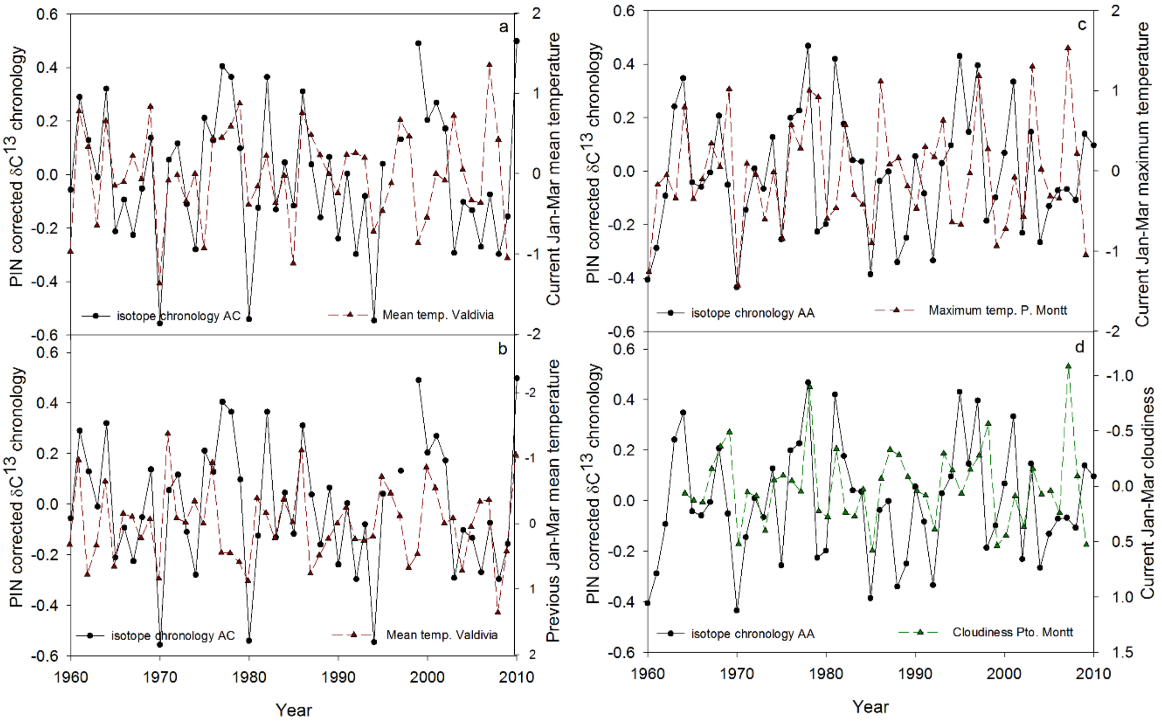
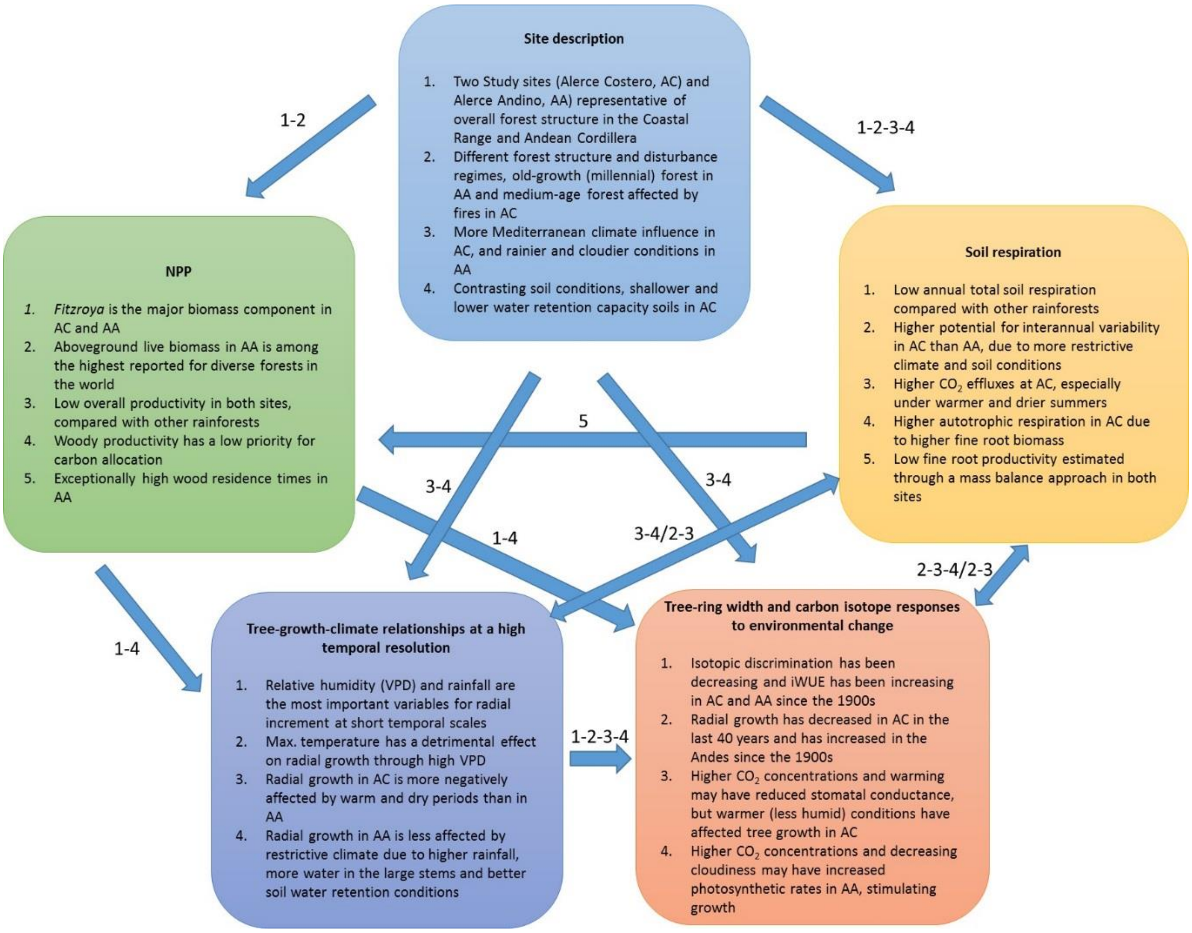


Fig. S5. Relationship between the PIN corrected δ^{13}C of Alerce Costero (AC) and a) current January-March mean temperature and b) previous January-March mean temperature in Valdivia. Relationship between the PIN corrected δ^{13}C of Alerce Andino (AA) and c) current January-March maximum temperature and d) current January-March cloudiness in Puerto Montt. The axes in b) and d) have been inverted for a better portrayal of the relationship. Tree-ring and climatic series have been detrended using a 20-year high-pass filter.

Chapter 8: Discussion

This study assessed the main components of the carbon cycle (represented by primary productivity and soil respiration) and the environmental factors that are most related to them in medium-age *Fitzroya cupressoides* forests growing in the Coastal Range and old-growth forests growing in the Andes Cordillera of southern Chile.

This chapter summarises, highlights and brings together the key findings of the four presented empirical chapters in order to achieve the main goal and specific objectives outlined in the Introduction of this thesis (Chapter 1). To provide a better sense of the interrelationship among chapters, Figure 8.1 portrays the main findings and identifies the key links between them. It also shows the site characteristics (within Chapter 4) as one important connecting topic.



Site description

1. Two Study sites (Alerce Costero, AC) and Alerce Andino, AA) representative of overall forest structure in the Coastal Range and Andean Cordillera
2. Different forest structure and disturbance regimes, old-growth (millennial) forest in AA and medium-age forest affected by fires in AC
3. More Mediterranean climate influence in AC, and rainier and cloudier conditions in AA
4. Contrasting soil conditions, shallower and lower water retention capacity soils in AC

NPP

1. *Fitzroya* is the major biomass component in AC and AA
2. Aboveground live biomass in AA is among the highest reported for diverse forests in the world
3. Low overall productivity in both sites, compared with other rainforests
4. Woody productivity has a low priority for carbon allocation
5. Exceptionally high wood residence times in AA

Soil respiration

1. Low annual total soil respiration compared with other rainforests
2. Higher potential for interannual variability in AC than AA, due to more restrictive climate and soil conditions
3. Higher CO_2 effluxes at AC, especially under warmer and drier summers
4. Higher autotrophic respiration in AC due to higher fine root biomass
5. Low fine root productivity estimated through a mass balance approach in both sites

Tree-growth-climate relationships at a high temporal resolution

1. Relative humidity (VPD) and rainfall are the most important variables for radial increment at short temporal scales
2. Max. temperature has a detrimental effect on radial growth through high VPD
3. Radial growth in AC is more negatively affected by warm and dry periods than in AA
4. Radial growth in AA is less affected by restrictive climate due to higher rainfall, more water in the large stems and better soil water retention conditions

Tree-ring width and carbon isotope responses to environmental change

1. Isotopic discrimination has been decreasing and iWUE has been increasing in AC and AA since the 1900s
2. Radial growth has decreased in AC in the last 40 years and has increased in the Andes since the 1900s
3. Higher CO_2 concentrations and warming may have reduced stomatal conductance, but warmer (less humid) conditions have affected tree growth in AC
4. Higher CO_2 concentrations and decreasing cloudiness may have increased photosynthetic rates in AA, stimulating growth

Figure 8.1. Diagram showing the main findings and interconnections among the overall site description and the four empirical chapters. Numbers next to each arrow indicate the main bullet points that make the connection between two chapters. In the case of the double arrow, the numbers separated by the slash indicate the bullet points on the left and right boxes, respectively.

The key findings from this thesis contribute to answering the following overall questions: i) How do the forest structure, composition and aboveground carbon stocks differ between medium-age *Fitzroya* forests growing in the Coastal Range and old-growth forests growing in the Andean Cordillera?, ii) Can we quantify the above- and belowground carbon processes in these forests (productivity and soil respiration) and how do they compare between the areas?, iii) How do the aboveground biomass and carbon fluxes in these forests compare with other temperate forests worldwide?, iv) What are the main environmental variables associated with the carbon dynamics (woody productivity and soil respiration) in these forests on different timescales?, and based on findings from question iv, v) Can we attribute changes in *Fitzroya* radial growth patterns (carbon accumulation) in the study sites over recent decades to changing climate conditions? The woody productivity component of questions iv and v focuses on *Fitzroya cupressoides* as the dominant species of these forests and the subject of this study. The answers to these questions will help us to understand the ecological status and carbon cycle of these ecosystems, as well as evaluating their place among other temperate forests in the world. They will also help untangling the main climate variables related to *Fitzroya*'s wood production, in order to understand the vulnerability of radial growth and carbon sequestration in these ecosystems to climate change.

This chapter will conclude by discussing the vulnerability of this endangered species to climate change and the implications for its conservation. It will also provide some guidance for future research directed to further understanding the carbon cycle and climate change vulnerability of these uniquely long-lived and high biomass ecosystems. Bullet points are given at the beginning of each section to summarize the most important findings in each of them.

8.1. Key findings

8.1.1. How do the forest structure, composition and aboveground carbon stocks differ between medium-age *Fitzroya* forests growing in the Coastal Range and old-growth forests growing in the Andean Cordillera?

- ***Fitzroya* forests from the Coastal Range have been affected by fires, so trees do not reach very large sizes or old ages. Andean forests appear not to have been affected by major disturbances likely for over a millennium, so they present an old-growth structure and are characterized by large sized trees.**
- ***Fitzroya* forests from the Andean site are more diverse and attain four times greater aboveground biomass than forests from the Coastal Range.**
- **Fine root biomass estimates in both sites, but especially in the Coastal Range, are among the highest reported in the world and are likely due to the poor nutrient conditions of the soils.**

Forest structure is very different between medium-age and old-growth *Fitzroya* forests growing in these two Ranges. The forest in the Coastal Range is much younger and denser, and *Fitzroya* is the dominant species in number and basal area in the different diameter classes above 10 cm DBH (Chapter 4). These forests are recovering from fires that occurred during previous centuries (Lara et al. 1999; Barichivich 2005), with a likely stand-forming fire in 1681 and stand-releasing fires (growth releases in tree rings) occurring probably at the beginning of the 1800s and around 1906 (Chapters 4 and 7, Lara et al. 1999; Barichivich 2005). Due to these disturbances trees do not reach a very large size (only up to 86 cm DBH). The forest in the Andes is much older and trees are much bigger, since it appears not to have been affected by major disturbances in a long time (several centuries). *Fitzroya* is the dominant species in basal area and occupies the upper canopy with large diameter trees, demonstrating that it was the first colonizer after a major disturbance (probably a landslide) over a thousand years ago. Underneath *Fitzroya* there is a younger and higher turnover rate forest mostly characterized by evergreen broadleaf species, particularly *Nothofagus nitida*, which forms the dominant component in this sub-canopy forest (Chapter 4). Such *Nothofagus* dominated forest in other contexts would be regarded as a high biomass and old-growth forest in its own right.

In terms of tree species diversity, there is a larger number of species in the Andean site, probably due to better soil conditions (more nutrients, greater depths, less water stress) and the more advanced development stage of the forests that allows more shade-tolerant species to develop underneath the shade-intolerant *Fitzroya* (Chapter 4). The most abundant species accompanying *Fitzroya* in the Coastal Range site*: Nothofagus spp.* and *Drimys winteri* have been identified as being more tolerant to infertile, poorly-drained and shallow soil conditions compared with others growing on the summit plateau of the Coastal Range (Lusk 1996). It has been reported that traits related to nutrient retention (as opposed to growth) are more relevant for the dominance of certain tree species in low fertility sites in the Coastal Range of southern Chile (Lusk & Matus 2000).

Due to the differences in forest structure, aboveground live biomass was much higher (four times more) in the Andean site (448- 517 Mg C ha $^{-1}$) than in the Coastal Range (113- 114 Mg C ha^{-1}, Chapter 4). This difference in biomass would be even greater if I had measured and considered dead biomass (total carbon stocks) in both sites. Estimates of coarse woody debris (logs and snags) in old-growth temperate rainforests of the Chiloé Island reached up to 189 Mg C ha^{-1} and most of this CWD consisted of snags (92%). Log biomass was within the range of values reported for some old-growth forests of North America (8-37 Mg C ha^{-1}) and snag biomass was much higher than values reported for these northern ecosystems (21-32 Mg C ha^{-1}, Carmona et al. 2002). Since the Andean forest is characterized by large amounts of dead trunks (but not recently dead *Fitzroya*) standing or lying on the ground it is expected that CWD is an important contributor of total biomass in these forests. Given the advanced decomposition of the few *Fitzroya* snags present, they appear to have died several decades or likely centuries ago.

Estimates of belowground biomass are scarce and were not thoroughly assessed in this study. Fine root estimates (< 2 mm diameter) up to 30 cm depth in the Coastal Range site (21.3 -22.2 Mg C ha^{-1}) were very close to the small roots (< 1cm diameter) estimate in Chiloé Island (~19.9 Mg C ha^{-1}, Battles et al. 2002) and much higher than the mean value reported for other temperate coniferous forests (~4.1 Mg C ha^{-1}, Jackson et al. 1997). These estimates were also higher than the range indirectly estimated for old-growth wet forests from the US Pacific Northwest (5.2- 12.6 Mg C ha^{-1}, Smithwick et al. 2002). Fine root biomass estimates in the Andes (10.1-12.1 Mg C ha^{-1}) were also higher than the mean value reported for temperate coniferous forests, but close to the upper range estimated for the old-growth US forests (Chapter 5). Although there are no direct estimates of coarse root biomass in my sites, these high fine root biomass values indicate that these forests can hold large amounts of carbon belowground. This is probably due to the particularly poor nutrient conditions especially in the Coastal Range site and to the high C/N ratios that indicate low nitrogen availability in both areas (Chapter 4).

8.1.1.1. Limitations associated with biomass estimations

There are a number of caveats that need to be considered in the biomass estimations of this study, which mainly arose due to funding, time and data availability restrictions. An evident limitation includes the lack of site developed allometric equations for all tree species. This forced me to develop volume equations for *Fitzroya* and estimate a biomass expansion factor and wood density for the species; this also forced me to use allometric equations developed in other sites for the other species. Another limitation includes the use of biomass equations without height in certain species, due to the impossibility of finding an adequate diameter-height relationship. In any case, the allometric equations used in this study are almost the only ones available and the most widely used in Chile.

A further limitation includes the low number of trees that were used to estimate the biomass expansion factor (BEF) in *Fitzroya* and the lack of an estimate for the Andean site. The BEF should be probably different in the Andean site due to the presence of larger trees that are characterised by fewer but larger branches, and a different and more complex branch pattern compared with smaller trees (Clement et al. 2001). The estimated factor was lower than the values reported for other conifer species in the world (Teobaldelli et al. 2009), probably due to some unaccounted missing branches in the logged trees. It took us about one day and five persons to measure a single tree, so it was not possible to measure more than five trees due to funding restrictions. In any case the standard error was low, so that gives more confidence in the obtained factor. In the Andes there were no recently fallen trees available for this measurement.

A likely underestimation of biomass due to the low biomass expansion factor, could be compensated by an overestimation due to not accounting for the presence of rotten heartwood, especially in the Andean site, but there was no available information on this regard.

Lastly, it is likely that estimates of wood density do not reflect the wide variation that might be present across the radial section and at different heights in the stem, but it was a better option than using the mean published value for the species.

8.1.2. Can we quantify the above- and belowground carbon processes in these forests (productivity and soil respiration) and how do they compare between areas?

- **NPP_{AG} during the studied period was higher in the Coastal Range stands, mainly due to higher canopy productivity. However, there is a high interannual variability in litterfall in this site, so more years of measurements are needed to draw firmer conclusions.**
- **The difference in total NPP between sites is less apparent, due to a somewhat higher belowground productivity in the Andean stands. Large uncertainties remain in these belowground indirect estimates.**
- **Forests in the Coastal Range site are likely to be carbon sinks due to their low mortality rates. In the Andes, the forest underneath *Fitzroya* experienced high mortality during the period studied, but *Fitzroya* may still be experiencing long-term biomass accumulation.**
- ***Fitzroya* forests from the Coastal Range site allocate more carbon to the canopy, than to wood structures and finally to fine roots. Andean forests allocate almost equally to the canopy and wood structures and a little more to fine roots. These allocation schemes partly reflect the development stage of the forests.**
- **Annual soil respiration was a little higher in the Coastal Range site and the autotrophic component was more important in this site than in the Andes.**

The assessment of aboveground primary productivity during one year in both study sites led to the preliminary conclusion that this variable is much larger in the Coastal Range site, mainly due to higher canopy productivity (NPP_{AG}: 3.35 -3.36 Mg C ha^{-1} $year^{-1}$ in Alerce Costero and 2.22- 2.54 Mg C ha^{-1} $year^{-1}$ in Alerce Andino). However, an additional year of litterfall data (that included a warmer and drier summer) demonstrated that there is high interannual variability in this site and that values can be similar between both areas (Chapter 4). This indicates that more years of measurements are needed to draw firmer conclusions.

Coarse wood productivity was similar between sites, demonstrating that old-growth forests can be as productive (in terms of woody growth) as young forests. *A priori* I expected to get a higher woody productivity in the Coastal site, because *Fitzroya* stem growth rates have been reported to be generally higher in the Coastal Range than in the Andes (Lara et al. 2002). However, much larger trees in the Andes and the presence of other species with higher growth rates than *Fitzroya* can shorten the difference between sites. If only *Fitzroya* is compared, woody productivity is larger in the Coastal Range (0.42-0.44 Mg C ha^{-1} yr^{-1}) than in the Andes (0.22-0.28 Mg C ha^{-1} yr^{-1}), but this could be mainly explained by the number of *Fitzroya* trees ($\geq$ 10 cm DBH) in each site (1203-1211 trees ha^{-1} in AC compared to 93 trees ha^{-1} in AA) and by the higher growth rate in AC. It is likely that a lower than expected difference in *Fitzroya* woody productivity is in part due to the different stem growth trends that *Fitzroya* has been showing in the past decades (negative in the Coastal Range and positive in the Andes, Chapter 7). In fact, the mean growth rate obtained from the *Fitzroya* tree-ring width chronologies in each site was much higher in the Coastal site than in the Andes for the common period 1739-2010 (0.75 ± 0.01 mm in AC vs 0.34 ± 0.01 mm in AA), while differences during the decreasing trend period in the coastal site (1970-2010) were much smaller (0.58 ± 0.02 mm in AC vs 0.44 ± 0.01 mm in AA). The occurrence of fires in the Coastal Range site (probably in the early 1800s and particularly 1900s, Chapter 7) might have an influence on the higher growth rates when considering the common period though, due to the release of nutrients that can positively affect *Fitzroya*'s stem growth in this nutrient poor area (Chapter 7). Just to reinforce the difference between sites during periods not affected by growth releases; mean growth rates for the period 1925-1969 were 0.66 ± 0.02 mm and 0.41 ± 0.01 mm in the Coastal Range and Andes, respectively.

Branchfall, on the other hand, was much higher in the Andean site, but it is probably not a good predictor of branch turnover in this site, due to the old state of these forests and the stochastic, intermittent nature of branchfall (Chapter 4).

When adding belowground productivity estimates, both forests appear to be much closer in terms of total productivity (4.21-4.24 Mg C ha^{-1} $year^{-1}$ in Alerce Costero and 3.78-4.10 Mg C ha^{-1} $year^{-1}$ in Alerce Andino). These closer estimates are mainly because the Andean forest presented a higher $NPP_{fine\ roots}$ (although differences are not significant due to uncertainty, Chapter 5). The Coastal Range forest allocated more productivity to the canopy (57%), than to wood structures (24%) and fine roots (19%). The Andean forest allocated equally to wood and canopy (31%) and a little more to fine roots (38%, Chapter 4). It is interesting to point out that in the case of *Fitzroya*, most of aboveground carbon was allocated to the canopy (81% in AC and 68% in AA) rather than to wood (19% in AC and 32% in AA). In the case of non-*Fitzroya* species, most of the carbon was allocated to wood (69-72% in AC and 58-59% in AA) rather than the canopy (28-31% in AC and 41-42% in AA, Chapter 4).

Overall allocation schemes mean that the younger forest tends to allocate much more productivity aboveground, probably because trees are competing more for light in this dense forest. Meanwhile, the older, more stable Andean forest, seems not to be much competing for light (the forest underneath *Fitzroya* is mainly composed of shade-tolerant species), allocating an important proportion to root productivity in order to get the unavailable nitrogen. In the case of aboveground allocation patterns in *Fitzroya* and non-

Fitzroya species, I demonstrate the low priority for wood allocation in alerce. A recently reported positive relationship between wood productivity and allocation to stem growth, although in tropical forests (Galbraith et al. 2013) could explain this finding: more productive forests increase their allocation to wood to increase their growth rates. Thus, species like *Nothofagus* or *Drymis winteri* (in the coastal site), that experience more competition for light (i.e. are semi-tolerant), would allocate more to wood growth to overtop competitors (Chapter 4).

An interesting point is that fine root biomass is much higher in the Coastal Range site than in the Andes, but fine root productivity seems to be a little lower (Chapter 5), indicating that the mean residence time of fine roots in this coastal site is much longer (26-27 years compared to 6-8 years in the Andean site) and that the high biomass of long-lived roots would be responsible for getting the appropriate resources in these very poor soils. Mycorrhizae may certainly help in this task, since a strong association has been found between *Fitzroya* growing in the Coastal Range and vesicular-arbuscular mycorrhizae; this type of symbiosis is also common in other tree and shrub species growing in the area (Godoy & Mayr 1989; Godoy et al. 1994). Root productivity may be low especially in the Coastal Range site due to the high exchangeable aluminium content in soils (Chapters 4 and 5).

Regarding total soil respiration in these forests, annual fluxes were a bit higher in the Coastal Range forest than in the Andes, which could be explained by the high amounts of root biomass (higher autotrophic respiration) in this forest and the more extreme soil conditions that this site experiences under warmer and drier summers (see more on this in section 8.1.4). It is likely that the autotrophic respiration in Alerce Costero mostly corresponds with maintenance, rather than growth respiration, due to the low fine root productivity. The high amounts of coarse woody debris in the Andean site (although just visually assessed), could explain the predominance of the heterotrophic component in this site, although root productivity is a little higher in this area (Chapter 5).

Hence, assuming that the difference in NPP estimates between sites holds for years with similar climate conditions; differences in NPP between medium-age and old-growth forests (not significant, although values could be even lower in the Andean site due to possible branch turnover overestimates), may be the result of different forest developmental stages. However, differences in site conditions may also contribute to lower production values in the Andean forest due to a rainier and cloudier climate. NPP_{AG} has been positively associated with growing season degree days and negatively associated with soil moisture along a climate gradient (with different forest types) in Chiloé Island (Joshi et al. 2006). Moreover, NPP has been reported to decline at sites where annual precipitation exceeds 4000 mm, mainly because forests become radiation limited (Schuur 2003; Del Grosso et al. 2008). An additional explanation for the difference in NPP between sites would be that other factors such as the long distance transport of assimilates in larger trees may limit growth more in the Andean than the Coastal Range site (Sala et al. 2011).

In the case of belowground fluxes, site conditions would have a stronger effect than age on the difference in CO_2 emissions between the two studied forests, since differences between sites are minimal under relatively “normal” climate conditions. Site characteristics would provoke a stronger interannual variability in carbon fluxes in the younger forest than in the Andes.

From the aboveground carbon dynamics it can be concluded that younger forests in the Coastal Range are accumulating carbon since woody productivity is higher than mortality. In the Andes this is also the case for *Fitzroya*, but it is not that clear for the other species, particularly *Nothofagus*, which experienced high mortality (although mortality is a stochastic process). The lack of recently dead *Fitzroya* (and the very long decay times) in the Andes indicates that the absence of *Fitzroya* mortality is real and not an artefact of my relatively short sampling period.

Finally, the high amounts of woody biomass and low productivity in the Andean site determine the exceptionally high mean wood residence times recorded, especially for *Fitzroya*. These high values demonstrate that substantial amounts of carbon can be stored for millennia in these old-growth forests and it could be assumed that the younger forests from the Coastal Range may have the potential to do so. This carbon sequestration can greatly contribute reducing carbon emissions to the atmosphere, especially if adequate protection is given to the species.

8.1.2.1. Limitations associated with carbon fluxes estimation

An important limitation of aboveground carbon fluxes estimates is that they corresponded to only one year. This was mainly due to funding constraints that prevented carrying out a second year census and woody productivity sampling, plus the restriction in the permit to collect tree-ring cores. Additionally, the time restrictions of the DPhil did not allow me to wait until a second year of measurements to start writing my chapter.

Another important limitation in woody productivity estimates, was the lack of relationship between radial growth rates and diameter classes which led to the use of the mean growth rate per species as a proxy of woody growth. The restricted amount of samples possibly played a role on this, but since I worked in National Parks I had to comply with the regulations imposed by the Forest Service in Chile. Nevertheless, it seems likely that in the Andean site differences in growth rates are not perceptible across diameter classes in

Fitzroya trees, since they are mostly over 100 cm DBH. Moreover, it is possible that growth rates are not that different across diameter classes in shade tolerant trees growing underneath *Fitzroya*. Limitations mentioned in the previous section (8.1.1.) also apply to estimates of woody productivity.

Regarding soil respiration, limitations to overcome in the future are the lack of estimates of diurnal variation of total soil CO_2 efflux and the interpolation between monthly estimates in order to calculate annual fluxes. These limitations can be surmounted by installing automatic high resolution sensors in each site, a situation that is currently being addressed in the Coastal Range forest.

In terms of the partitioning experiment, more replicates are needed to reduce the uncertainty in the autotrophic/heterotrophic respiration and fine root productivity estimates, and slight modifications in the set up are also needed to avoid waterlogging (e.g. build some holes in the long collars that allow water flux, but not root growth). In addition, soil water content should be measured inside the partitioning units, so differences between collars can be detected. Finally, fine root productivity needs to be concurrently assessed through direct (e.g. ingrowth cores) and indirect measurements (mass balance approach), to validate current estimates.

8.1.3. How do the aboveground biomass and carbon fluxes in these forests compare with other temperate forests worldwide?

- **Carbon fluxes (above and belowground) in *Fitzroya* forests from both areas, and especially in the Andes, are low compared with other coniferous forests from wet environments. This may be partly due to the longevity and slow growth of *Fitzroya* and the associated species and the poor soils and extremely wet conditions where *Fitzroya* develops.**
- **Wood residence times are exceptionally high in the Andean site, reaching well above 1000 years in the case of *Fitzroya* and constitute the highest values reported for a forest stand anywhere in the world.**

Both *Fitzroya* forests present high aboveground biomass compared to other temperate ecosystems, although the Andean forest is by far more massive, due to the much larger trees found in this site. The Andean forest biomass values are among the highest values reported for conifer species worldwide (present in the US Pacific Northwest and New Zealand, Keith et al. 2009, Chapter 4), and are largely only surpassed by *Sequoia* forests.

In terms of woody productivity in both sites, this was really low compared with other coniferous forests, which mostly allocate the major proportion of productivity to wood. This reflects the slow growth rate, especially of *Fitzroya*, which due to its longevity does not need to grow faster (Chapter 4). Additionally, it has been reported that wood production per unit photosynthesis is lower at low nutrient availability sites, which may also support the lower woody productivity in these nutrient poor sites compared with others (Vicca et al. 2012). According to these authors, a lower biomass production per unit photosynthesis in low fertility sites may be associated with a higher investment of photosynthates in root symbionts (Vicca et al. 2012). This fact needs further research, but at least it merits some consideration in one of my sites, due to the reported association of *Fitzroya* and other species with vesicular-arbuscular mycorrhizae (Godoy & Mayr 1989; Godoy et al. 1994). It seems crucial to quantify the productivity allocated to the "difficult to measure components" not only in this study, but in other ecosystems, in order to refine the theory behind carbon allocation and better understand the effect of environmental conditions on changes in forest productivity. Measuring these unquantified components, would help to understand why root allocation is not in proportion to nutrient limitation in *Fitzroya* forests for example (Chapter 4). The high woody biomass and slow growth rate of *Fitzroya*, especially in the Andes, leads to the highest mean wood residence time values reported in the world (1368-1393 years).

Considering aboveground NPP, values in both sites, and especially in the Andes, are much lower than values reported for coniferous forests growing under somewhat similar conditions (wet sites in the US Pacific Northwest), being more similar to values reported for coniferous forests at higher elevations (Chapter 4). Values of fine root productivity estimated in this study (0.81-1.50 Mg C ha^{-1} yr^{-1}) are much lower than mean values reported for temperate forests worldwide (~2.14 Mg C ha^{-1} yr^{-1}, Finér et al. 2011, Chapter 5). Finally, total NPP in these forests is much lower than values reported for coniferous forests in the US Pacific Northwest.

Annual soil CO_2 effluxes (6.37-8.64 Mg C ha^{-1} yr^{-1} in Alerce Costero and 4.98-6.14 Mg C ha^{-1} yr^{-1} in Alerce Andino during 2011-2013) are generally lower than values reported for other coniferous wet forests in the world and more similar or in the range of values reported for conifers growing in drier environments (Chapter 5). This means that *Fitzroya* forests (especially in the Andes) have a lower NPP that translates into relatively low carbon emissions from the soil, compared with other coniferous forests growing in wet environments in the northern hemisphere. On the other hand, fine root biomass values in

Fitzroya forests are among the highest worldwide, mainly due to the poor soil conditions where this species grows. These high values further influence the slow carbon dynamics of these forests, with long fine root residence times (Chapter 5).

Hence, above- and belowground carbon fluxes in *Fitzroya* forests are among the lowest reported for temperate wet forests in the world. The slow carbon dynamics of these forests are likely induced by the longevity of *Fitzroya* and its slow growth rates (and the slow growth rates of the accompanying species as well). *Fitzroya* is a slow-growing species that inherently develops and dominates in nutrient poor soils. However, other native species that are commonly more dominant in more fertile sites and grow faster (e.g. *Nothofagus nitida*, *Weinmannia trichosperma*), have been reported to slow their height growth rates when growing under poorer soils accompanying *Fitzroya* (Lusk & Matus 2000).

Furthermore, particularly rainy conditions with precipitation amounts, as far as I could review, never reported in other studies in temperate forests, could partly explain the lower productivity and soil emissions in these ecosystems (Chapters 4 and 5).

Lastly, and related to the paragraph above, nutrient limitation may play a role on the slow carbon dynamics in these forests. A strong nutrient limitation was reported for soils in somewhat similar montane conifer forests of southern Chile (Chiloé Island), which show lower nitrogen mineralization rates (<20 kg ha^{-1} yr^{-1}), lower hydrological outputs (< 1 kg ha^{-1} yr^{-1} of inorganic N) and higher nitrogen-use efficiency compared with northern hemisphere ecosystems (Perakis & Hedin 2002; Vann et al. 2002; Pérez et al. 2003a; Pérez et al. 2003b; Pérez et al. 2005; Joshi et al. 2006).

8.1.4. What are the main environmental variables associated with the carbon dynamics (woody productivity and soil respiration) in these forests?

- ***Fitzroya*** **radial growth in both study areas is positively related with humidity and precipitation at a high temporal resolution. Strong stem shrinkage was observed under prolonged warm and dry conditions, especially in the Coastal Range site, implying fewer available days with appropriate conditions for growth to take place.**
- **Tree-ring width chronologies from both sites were negatively correlated with mean temperature, but especially with maximum temperature during the summer months. The Andean tree-ring growth was more strongly related with previous summer weather conditions, rather than current growing season weather. This is most likely due to the trees depending on reserves of stored non-structural carbohydrates to start growing in this colder and cloudier site.**
- **Vapour pressure deficit appeared to be the variable that most likely explains the negative correlation between summer temperatures and tree growth in both sites.**
- **Drier conditions in Alerce Costero seemed to accentuate the effect of higher temperatures on soil respiration in this site that is usually very wet, but can nevertheless dry considerably during summer. Thus, annual soil respiration estimates increased significantly only in the Coastal Range site under warmer and drier summer conditions, indicating the higher sensitivity of this site to future changes in climate.**

Due to the short-term nature of this study, it was not possible to determine if interannual changes in woody productivity effectively corresponded with interannual changes in total productivity, and not only with changes in allocation. However, studying stem radial growth allowed me to assess the main environmental variables that might affect forest growth and carbon sequestration in these forests, and it is the only approach that can provide a long-term understanding of the potential drivers of productivity in these ecosystems.

When stem radial change-climate relationships were analysed at a higher temporal resolution using dendrometers, stem radial growth of trees in both study areas appeared negatively affected by warm and dry conditions during summer. Strong stem shrinkage was observed when these conditions lasted for several days especially in the Coastal Range site, implying fewer available days with appropriate conditions for growth to take place (Chapter 6). Moreover, humidity (lower vapour pressure deficit) and precipitation were positively related with stem radial increment in both sites, mainly due to their effect on turgor and subsequent cell enlargement.

Tree-ring growth from both sites at a year-to-year basis was negatively correlated with mean summer temperature, but especially with maximum summer temperatures (Chapter 7). Tree-radial growth-climate relationships on temporal scales up to a month, indicated that vapour pressure deficit appears to be the variable that most likely explains this negative correlation, since there is a strong positive relationship between VPD and maximum temperature (Chapter 6). An important difference between sites is that the Andean chronology was mostly related with previous rather than current temperature, most likely because these trees need carbohydrates from storage to start growing in this colder and cloudier site. I hypothesise that warmer temperatures in the preceding season could lead to high respiration rates and affect the accumulation of storage available for next year's growth (Chapters 6 and 7). It has been reported that, because of hydraulic constraints, tall trees commonly close stomata and reduce carbon assimilation (Ryan et al. 2006), so this could play a role on the tree growth-previous year climate relationship found in this site.

In the Coastal Range, tree-growth was mainly related with current early summer temperature and precipitation (positive correlation), indicating that trees from this site are mostly affected by climate conditions that directly reduce growth (cellular turgor and expansion) rather than carbon assimilation or storage (Chapter 7). It has been reported that the amount of NSC increases with height in *Pinus ponderosa* trees, particularly in drier compared with moister sites, demonstrating that water availability might have a greater impact on growth (C-sink limitation) than on assimilation activities (C-source limitation, Sala et al. 2011).

Warmer and drier conditions also affect soil respiration, but especially in the Coastal Range site. Warmer and drier summers, such as the one experienced in 2013, seem to greatly increase soil CO_2 effluxes just in this site, probably because soil conditions are especially susceptible to change under more extreme climate. Soils warm and dry out considerably in this site if air conditions are particularly warm and dry for many days. This does not occur in the Andes, as these deeper and wetter soils are more resilient to air changes (Chapter 5). Particularly dry conditions in Alerce Costero seem to accentuate the effect of higher temperatures on soil respiration in this very wet site that can dry considerably during summer. The lowest water content values recorded in both sites during summer (24% in Alerce Costero and 44% in Alerce Andino) are higher than threshold values recorded for other temperate sites below which a positive influence of soil humidity on respiration exists (12-15%). However, it is especially uncertain in the case of the Coastal Range forest if further reductions in soil humidity under climate change (beyond a certain threshold), will provoke soil effluxes to decrease instead of increase in this site.

8.1.5. Can we attribute changes in *Fitzroya* radial growth patterns (carbon accumulation) in the study sites over recent decades to changing climate conditions?

- **Climate has become drier and warmer during at least the last five decades in southern Chile. Increasing maximum temperatures are driving increasing mean temperatures in sites close to both study areas.**
- **Intrinsic water use efficiency has increased in trees from both study sites since the 1900s, most likely due to the increase in CO_2 and changes in climate in both study areas. Slopes of iWUE trends have been more pronounced since 1970 and 1950 in the Coastal Range and the Andes respectively. Warmer conditions have likely contributed to decrease stomatal conductance in the Coastal Range site; while less cloudiness has contributed to increase photosynthetic rates in the Andean site.**
- **Increases in iWUE have been accompanied by different growth trends in both study sites. Tree growth has consistently decreased in the Coastal Range site during the last 40 years, indicating that these forests have been under stress, most likely because of drier and warmer conditions. Tree growth has increased in the Andean site since the 1900s. Different growth responses are a consequence of more restrictive soil conditions and a more Mediterranean climate influence in the Coastal Range site than in the Andes.**

According to the data analysed in Chapter 7, drying and warming trends have characterised the change in climate close to both study areas. Summer and spring-summer precipitation have been decreasing in Valdivia (close to Alerce Costero) and Puerto Montt (close to Alerce Andino, period 1960-2010), although these trends were significant only in

Puerto Montt. Cloudiness has also significantly decreased in Puerto Montt since 1965, and mean and particularly maximum temperature, have significantly increased in both areas.

The tree-ring width and basal area increment chronologies from Alerce Costero present a decreasing trend since 1970 and the ones from Alerce Andino an increasing trend since the 1900s. Carbon isotope analyses shed light on the physiological changes that this species is experiencing in each of my study sites. Water use efficiency is increasing in trees from both areas since the 1900s, but especially since 1970 and 1950 in Alerce Costero and Alerce Andino, respectively. This increase in iWUE has been accompanied by significant decreases in ^{13}C discrimination in both sites, implying that beyond the increase in $CO_{2,}$ other environmental factors are influencing the isotopic composition of *Fitzroya* trees. Specifically, it was hypothesised that warmer temperatures (lower humidity) have been promoting lower stomatal conductance in trees from the Coastal Range site and that decreases in cloudiness have been contributing to the increase of photosynthetic rates in trees from the Andean site (Chapter 7).

It is likely that warmer and drier conditions in recent decades have negatively influenced tree growth in the Coastal Range site due to the direct effect that water has on cell turgor and enlargement (Chapter 7). On the other hand, warmer and drier conditions have not caused tree growth to decrease in the rainy Andean site yet; instead a possible raising of photosynthetic rates (induced either by decreasing cloudiness and/or rising atmospheric CO_2) have caused growth to increase in the old trees from this site.

As can be inferred from the information reported above, the main differences in tree-growth responses to climate change are given by different soil and climate conditions. Soils are considerably shallower and have a lower water retention capacity in the Coastal Range site than in the Andes. Furthermore, the more Mediterranean climate influence with warmer and drier summers in the coastal site can considerably affect trees more in this area than in the Andes.

8.1.5.1. Limitations associated with tree-ring width and carbon isotope studies

A key limitation in the isotopic study was the use of the whole ring instead of latewood, which is the component that likely records the current photoassimilate signal (Kagawa et al. 2006). Carbon from storage was likely used in earlywood formation, so using the whole ring dampened the climate signal and drove a relationship with previous summer temperatures in both sites. This relationship was given by the use of stored starch rather than by a direct effect of climate on the isotopic composition. Future studies should address this limitation (see section 8.3).

Another limitation in the isotopic study is the fact that only whole wood without extractives could be used to analyse the isotope composition in tree-rings and that the samples had to be manually ground. This was mainly due to funding restrictions and the lack of appropriate equipment in Chile to grind the samples and perform cellulose extraction. Additionally, the test run to estimate the difference in the isotopic composition among wood components did not work, so this is a pending task to develop in the future in order to know the carbon isotope offset between whole wood and cellulose in *Fitzroya*. Adequate instrumentation and laboratory techniques are urgently needed to grind and homogenise wood samples, as well as to effectively extract and homogenise cellulose in Chile. Given that the final goal of this study was not to maximize the climate signal in order to reconstruct climate, but to assess the overall trends in isotopic composition and the environmental variables that could play a role on these trends, the final objective is not affected by these limitations. Furthermore, the three replicates performed give me confidence that the results obtained represent the mean isotopic composition of each ring.

Another potential limitation is the relatively low number of samples employed and the fact that I had to pool trees for the isotopic analysis. I partially addressed inter-tree variability by analysing individual trees every ten years, but future studies should consider analysing trees separately in order to obtain statistical parameters for the mean chronology.

Finally, my study mainly focused on statistical relationships between tree-ring variables and climate, which do not allow a full understanding of the physiological drivers of growth and isotopic composition. Therefore, and since tree responses are most of the time multifactorial, future studies should especially focus on physiological aspects and also on modelling to disentangle the effects that CO_2, climate and other environmental factors have on growth in these forests (see more on section 8.3).

8.2. Climate change implications for *Fitzroya cupressoides* forests

- **Climate change, characterised by increases in temperature and decreases in precipitation, is likely causing and might continue to cause negative effects on the growth of *Fitzroya* forests in the Coastal Range. Forests in the Andes seem more resilient, but they could also be affected by climate change in the future, due to the negative effect of warmer temperatures (higher water pressure deficits) on tree growth.**

Climate has been changing and is projected to change even more in southern Chile (Fuenzalida et al. 2007). According to González-Reyes & Muñoz (2013) who joined different rainfall records from Valdivia, annual, seasonal and monthly precipitation has decreased in the period 1901-2005. This trend is especially significant for the whole year and the autumn season. Additionally, droughts have been more frequent since the 1950's (González-Reyes & Muñoz 2013). The increasing trends in temperature (especially maximum temperatures) in the sites close to both study areas were not previously identified by a study over the period 1979-2006 (Falvey & Garreaud 2009, Chapter 7).

Given this current and the projected climate change scenario characterized by a decrease in summer precipitation of up to 50% and an increase in summer temperature of up to 4° C (Fuenzalida et al. 2007), there are a number of potential risks that the growth of *Fitzroya* may face.

Drier and warmer conditions (higher VPD) in the future could have a more negative effect on stem growth and carbon sequestration in *Fitzroya* forests from the Coastal Range site (Chapters 6 and 7). Increasing warming (largely driven by higher maximum temperatures) and consequently higher VPD, has been the main driver of the ongoing drought stress and decrease in productivity observed in different drought-sensitive forests from the southwestern US (Williams et al. 2013). Coastal Range forests seem more affected due to the Mediterranean influence of climate and the restrictive soil conditions that characterise the summits of this Range. This coastal site also appears to be more sensitive to interannual climate variability in terms of litterfall production. Although it is not possible to draw final conclusions about the direct association between climate and canopy production, it was possible to observe that litterfall was lower during the autumn after a drier and warmer summer (Chapter 4). This lower litterfall may indicate that trees do not shed that many leaves, because they are not able to produce as many leaves under these conditions.

A warmer and drier climate could also exacerbate the release of CO_2 from soils in *Fitzroya* forests growing under similar conditions in the Coastal Range, although if soils get dry enough they can start counteracting high emissions due to reduced biological activity. In summary, if patterns observed during this study hold, lower wood and presumably canopy productivity can be expected under future climate scenarios in the Coastal Range. If lower productivity and particularly higher heterotrophic emissions occur due to the high amounts of carbon and potential increased mortality (but see below in this section), this forest could act as a carbon source under extreme summer conditions. Further research is of course needed to draw conclusions (see next section 8.3).

Fitzroya forests in the Andes, on the other hand, seem more resilient to ongoing climate changes, probably due to the much higher precipitation that falls in this site (even during summer) and the generally colder air and better soil conditions. Trees in this area for example, did not experience very strong contractions in the stem when several days without precipitation occurred, probably due to the soil conditions and greater water capacitance of these large trees (Scholz et al. 2011, Chapter 6). Soils are much deeper than in the Coastal Range and the silty-loam texture helps retaining water in the profile. Soil water content never dropped significantly during the monitored period (always above ~44%), seeming more stable during more extreme summer conditions (Chapter 5). Radiation is also lower in the Andes, so trees are exposed to lower transpiration demands. Besides this higher resilience in growth responses, soil respiration was not significantly affected either under a warmer or drier summer in the Andes.

The *Fitzroya* tree ring and basal area chronologies from the Andes show an increasing trend, indicating that trees have been accumulating more carbon during recent decades. This change can be attributed to the decreasing trend in cloudiness in this rainy and cloudy site, but also to a positive effect of CO_2 fertilisation, it being not possible to discriminate between them from the data available. The effect of warming on the availability of nutrients (e.g. higher nitrogen mineralization) and of nitrogen deposition from the burning of firewood and agricultural activities in the Central Depression may also be important factors, and perhaps all the mentioned causes are playing a role.

Although *Fitzroya* tree-growth from the Andean site is positively responding to increases in radiation, it is unlikely that this trend can continue indefinitely in the future, mainly due to the negative relationship that the growth in this species has with temperature and vapour pressure deficit (Chapter 6). Warming was even higher in the Andes than in the Coastal Range site during the summer of 2013, so trees might well start experiencing higher vapour pressure deficits that may affect productivity in the future if conditions get significantly warmer and drier. There are no studies available on the foliar water uptake of *Fitzroya*, but it seems plausible that these trees might have this strategy, as so plants from many ecosystems that experience high water demand at some point and frequent exposure to leaf wetting events (Limm et al. 2009). Therefore, a decrease in cloudiness and its associated cloud-born mist could also affect the growth of *Fitzroya* in the future. Furthermore, given the high amounts of carbon in dead material in these forests, warmer conditions can significantly increase the heterotrophic respiration component and reduce the carbon sink capacity of these ecosystems.

A number of studies have suggested that climate change, through warming and reduced precipitation, is already amplifying a drought-induced forest decline and causing reductions in productivity (Breshears et al. 2005; Zhao & Running 2010; Anderegg et al. 2012; Williams et al. 2013). Furthermore, and although later questioned by Klein et al. (2014, see below), it has been stated that there is a global convergence in the vulnerability of forests to drought events, mainly because most of species operate under narrow hydraulic safety margins independent of the amount of rainfall that they receive. Thus, long-term reductions in productivity and overall drought induced forest decline are not only occurring in arid, but also in wet forests (Choat et al. 2012). Similarly, it was found that the growth of forests from humid areas of the Northern Hemisphere tends to more negatively respond to short-term rather than long-term droughts, being more vulnerable to water deficits than trees growing in drier sites (Vicente-Serrano et al. 2014). Although the vulnerability of forests to drought events cannot be generalised (Klein et al. 2014), these findings certainly raise a cause of concern about what would be the future of *Fitzroya* forests growth under the projected climate change scenario, so further studies are urgently needed to address these threats (see section 8.3).

Figure 8.2 portrays a conceptual diagram about the effects of warming and drying on the radial growth of *Fitzroya* forests and place the two studied sites within this scenario. An important point to remember is that the mentioned growth changes and potential impacts of climate change on *Fitzroya* forests are focused on what is happening and could happen to *Fitzroya* as a species (being dominant in these forests), but they say nothing about the impacts of climate change on the growth of the accompanying species. This would have more importance on the Andean site, which is more species diverse. Since significant inter-specific differences exist in tree-growth changes, mainly driven by diverse physiological responses to climate stress (Granda et al. 2014), future studies should address this point.

Although *Fitzroya* could experience the scenarios portrayed above in terms of tree-growth reductions, the potential occurrence of mortality due to climate stress in this species is a more complex process that is far from being easy to predict. Moreover, due to the diverse responses of different species to stress, and specific mechanisms to cope with drought events (e.g. drought-stress avoidance, recovery from embolism); similarity in the vulnerability of forests to drought cannot be anticipated and specific studies in *Fitzroya* are needed (Klein et al. 2014). Given the importance attributed to stored carbon pools to maintain hydraulic transport and integrity, particularly during episodes of severe stress (Sala et al. 2012), *Fitzroya* trees from the Coastal site may be partially protected from suffering hydraulic failure under stressful conditions. This is mainly due to the apparent adequate pool of carbohydrates in trees from this site that allow stored carbon to be transferred year-to-year (Chapter 7). An external cause of potential mortality, the broad distribution of the aphid *Cinara cupressi* in Chile, which has even been reported to infest *Fitzroya* (Montalva et al. 2010), and its apparent susceptibility to be influenced by climate change (Lara et al. 2010) may constitute an additional threat to the species.

It is finally important to mention that results from this study cannot be extrapolated to other *Fitzroya* forests that grow under different soil conditions. Thus for example, some *Fitzroya* forests growing under better soil conditions in the Coastal Range might not be experiencing reductions in growth and some Andean forests growing under more restrictive soil conditions might not be behaving the same way as in this study. However, this study on representative *Fitzroya* forests (in terms of structure and overall site conditions) provides a good understanding of the main variables that affect *Fitzroya* growth and carbon sequestration and clearly shows important changes that these forests are already experiencing under current climate conditions.

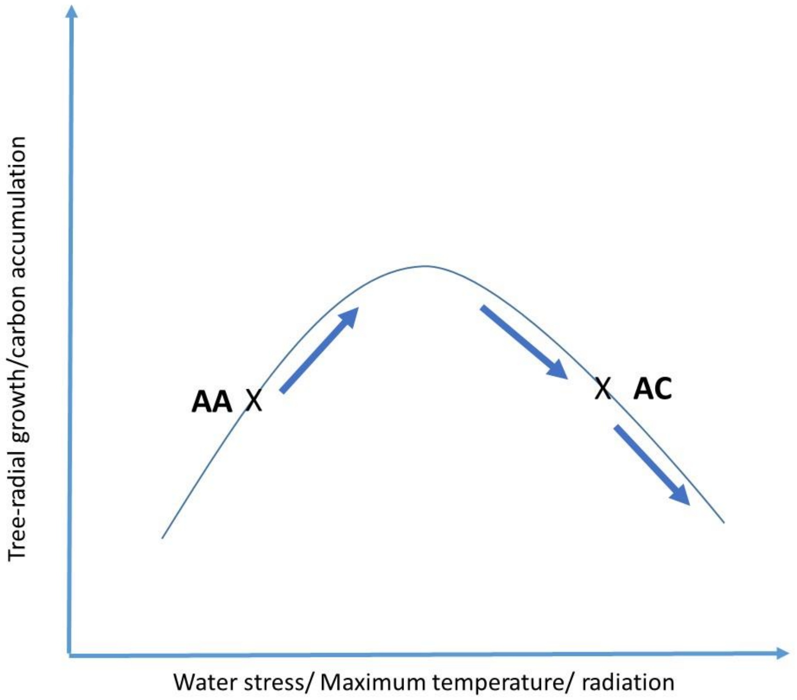


Figure 8.2. Conceptual diagram showing the vulnerability of *Fitzroya* tree-radial growth and/or carbon accumulation to current and projected climate changes in southern Chile. Alerce Costero (AC) and Alerce Andino (AA) forests sit at points marked as X in this conceptual curve, and the direction of their change under likely future climate change are shown as arrows in this conceptual diagram. Hence moderate warming, and increased water stress and insolation are likely to diminish growth in AC, but increase growth in AA, as is observed in tree-ring width chronologies. However, more severe climate change may also cause growth rates to decline in AA.

8.3. Future research

- **Further research is needed to have a complete understanding of the carbon cycle in *Fitzroya* forests and to understand the links between changes in wood production and total productivity.**
- **Future studies on physiological aspects of *Fitzroya* are needed to better understand its functioning and overall responses to climate change.**

This study leads to further questions that need to be answered in order to have a better picture of these forests carbon dynamics and their environmental drivers. Additionally, further research is needed to better understand the response of *Fitzroya* forests and their vulnerability to climate change. Since plots have already been established in my study sites and there is a baseline in terms of carbon measurements, further research is especially encouraged to continue in these study sites. Longer term studies are needed to assess interannual variations in productivity and determine the effects of climate on total net primary production and allocation changes. Furthermore, aboveground autotrophic respiration measurements (from wood and canopy) are needed to have an estimate of GPP and aboveground heterotrophic respiration measurements (from coarse woody debris) are needed to have an estimate of NEP in each site. These last measurements will especially make a difference in the Andes, where coarse woody debris is an important forest component.

An eddy covariance flux tower was recently installed in the Coastal Range site, being a great opportunity to monitor changes in carbon fluxes associated with interannual climate variability. This tower would therefore be a great tool to determine if these forests still act as a carbon sink under extreme summer conditions. It also offers the opportunity to validate both approaches (bottom-up and top-down) if biometric measurements continue to take place.

An important step to improve future estimates of biomass and productivity in this protected species is to quantify tree structure and develop allometric equations to predict aboveground attributes from ground-based measurements as done by Sillet et al. (2015) for redwoods.

In order to better understand the potential effects of warmer and drier conditions on *Fitzroya* trees beyond reductions in radial growth, physiological studies on water and carbon relations in this species are urgently needed and priority should be given to them (for conservation purposes). Physiological studies that especially look at the tree water potential and photosynthetic capacity are needed not only in mature trees, but also in seedlings to assess the future of these forests. Thus, pressure-volume curves and subsequent measurements of the water potential at midday during particularly warm/dry periods are needed to determine if the hydraulic integrity of the trees can be affected under these conditions. Furthermore, since no studies are available on the topic, it is important to quantify the contribution of fog to the water balance in each area and determine its potential effect on alleviating water stress in this species.

The cavitation resistance of a number of species from the Cupressaceae family, including *Fitzroya*, was assessed using trees growing in botanical gardens and arboreta from Central California (Pittermann et al. 2010). This study found that the xylem pressure at which 50% of hydraulic conductivity was lost in *Fitzroya* (the most widely used indicator of embolism resistance) was ca -8 MPa, closer to the lowest value reported for the studied species (range of -2.8 to -11.3 MPa across the 15 studied species). Despite this finding that ranks *Fitzroya* as a relatively cavitation resistant species, vulnerability curves are needed under field conditions to confirm or reject this statement. Furthermore, photosynthetic rates should be measured under relatively normal and particularly warm/dry periods to determine the degree to which photosynthesis is affected under these stressful conditions and assess the difference in the response between both sites.

A question that remains open and that needs to be addressed is the real importance of stored carbohydrates for tree growth in both areas. For this purpose it would be recommended to sample non-structural carbon (NSC) in stem sapwood, branches, leaves and roots at different times of the year to analyse its seasonal variation, or at least at the end of the growing season to quantify the balance between carbon demand and supply (Hoch et al. 2003; Sala et al. 2011). The difference in the NSC pool available in trees from both sites seems very interesting *per se*: is it larger on the big trees from the Andes or on the smaller trees from the drier Coastal Range site?

Furthermore, the study and comparison of intra-annual variation patterns in $\delta^{13}C$ between trees from both study sites, could help to better understand the signal present within the whole-ring carbon isotope analyses (Helle & Schleser 2004; Roden et al. 2009; Vaganov et al. 2009). This proposed study for a multi-year period would allow an understanding of the relative contribution of storage and the effect of seasonal and interannual climate on the tree-ring carbon isotopic composition in each site. If this study is not possible, at least the use of latewood in future isotopic analyses would improve correlations with climate.

In this study I am mainly assessing the long-term response of *Fitzroya* growth to an ongoing and steady climate change, but in the case of the Coastal Range site for example, I cannot say anything about the specific and long-term response of *Fitzroya* growth to strong drought events. It has been stated that the response of trees within a population varies much more under an extreme event (Zang et al. 2014), so future studies should consider studying the specific response of woody growth (multi-year response) to droughts, which are being more common in recent decades.

Finally, it is of course recommended to assess the climate change response and vulnerability of the other species within these forests and to expand the here presented and proposed measurements to other areas in somewhat different site conditions in both Ranges, and in the Central Depression. The overall proposed studies will allow a better and broader understanding of the carbon cycle dynamics and climate change vulnerabilities of these forests.

8.4. Implications for conservation

- **Adequate resources need to be placed especially for the conservation and restoration of Coastal Range forests. Forest fires are a deadly threat not only in the Coastal Range, so strong environmental education programs, as well as ample resources for monitoring and firefighting, are needed to effectively protect this endangered species.**

Results in this thesis highlight the importance that *Fitzroya* forests have for carbon sequestration and the enormous capacity of old-growth forests for sustaining exceptional amounts of biomass in the long term. This information should be taken into account and these forests considered under current national and international carbon initiatives such as the one entitled “Emissions reduction with emphasis in the degradation of temperate forests” led by the National Forest Service in Chile. This is in order to decrease the pressure on these forests, to avoid their degradation, especially through illegal cuttings and fires, and promote their restoration in areas affected by these activities.

The higher vulnerability of *Fitzroya* tree growth to climate change in the Coastal Range site gives an extra reason to strengthen the protection of forests in this Range by assigning additional resources for fire and illegal cuttings prevention and fighting. Special attention should be given to the monitoring, prevention and control of any potential *Cinara cupressi* aphid attack to *Fitzroya* populations under changing climate conditions. A governmental effort is also needed to promote these forests’ restoration in areas historically inhabited by this species (such as the Central Depression) and to incorporate more *Fitzroya* forests into the national protected area system (18% of its area is under the State protection) or promote their inclusion into private protected areas. These forests’ protection would not only benefit *Fitzroya*, but also all the associated endemic species of flora and fauna that live in these forests.

Finally, but no less important, is the educational component especially directed to children and communities living close to *Fitzroya* forests, so they can learn to appreciate and value these ecosystems beyond their value for wood. To contribute to the educational component, while doing my thesis I led a project directed at educating school children from rural areas about the historical exploitation of *Fitzroya*, and the value and importance of the species (Rufford Small Grant, http://www.rufford.org/rsg/projects/rocio_urrutia_jalabert, Figure 8.3).

Figure 8.3. Kids from the Mashue rural school (close to the Alerce Costero site) showing the brochures on alerce after a workshop (left), and planting a tree in the school backyard (right, Photos: Jorge Silva).

This thesis has been conducted with the underlying purpose of providing crucial information on unknown aspects of *Fitzroya cupressoides* and therefore contribute to understand their actual ecological condition and potential vulnerabilities to climate change. I hope I have addressed these aims as best as possible in order to contribute to the adequate protection and long-term preservation of these unique ecosystems.

8.5. References

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