



# Improvement of isotope-based climate reconstructions in Patagonia through a better understanding of climate influences on isotopic fractionation in tree rings



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## ABSTRACT

Very few studies of stable isotopes exist across the Andes in South America. This study is the first presenting annually resolved chronologies of both  $\delta^{18}\text{O}$  and  $\delta^{13}\text{C}$  in *Nothofagus pumilio* and *Fitzroya cupressoides* trees from Northern Patagonia. Interannual variability in  $\delta^{18}\text{O}$  and  $\delta^{13}\text{C}$  was assessed over the period 1952–2011. Based on these chronologies, we determined the primary climatic controls on stable isotopes and tree physiological responses to changes in atmospheric  $\text{CO}_2$  concentrations ( $c_a$ ), temperature and humidity. Changes in specific intrinsic water use efficiency (iWUE) were inferred from variations in  $\delta^{13}\text{C}$  whereas the effects of  $\text{CO}_2$  increase on stomatal conductance were explored using  $\delta^{18}\text{O}$ . Over the 60-year period, iWUE increased significantly (by ca. 25%) in coincidence with the rise of  $c_a$ . The two species appear to have different strategies of gas-exchange. Whereas iWUE variations were likely driven by both stomatal conductance and photosynthetic assimilation rates in *F. cupressoides*, they were largely related to stomatal conductance in *N. pumilio*. After removing the low-frequency trends related to increasing  $c_a$ , significant relationships between  $\delta^{13}\text{C}$  and summer temperatures were recorded for both species. However,  $\delta^{13}\text{C}$  variations in *F. cupressoides* were more strongly influenced by summer temperatures than in *N. pumilio*. Our results advocate for an indirect effect of summer temperatures on stable isotope ratios, which is mostly influenced by sunlight radiation in *F. cupressoides* and relative humidity/soil moisture in *N. pumilio*.  $\delta^{13}\text{C}$  variations in *F. cupressoides* were spatially correlated to a large area south of  $35^\circ\text{S}$  in southern South America. These promising results encourage the use of  $\delta^{13}\text{C}$  variations in *F. cupressoides* for reconstructing past variations in temperature and large-scale circulation indexes such as the Southern Annular Mode (SAM) in the Southern Hemisphere.

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## 1. Introduction

Several attempts to document past climate variability in subtropical and temperate regions of South America have been conducted over the last decades. Long-term, climate sensitive dendrochronological records from the outer tropics to southern South America (e.g. Boninsegna et al., 2009) provide unique opportunities to reconstruct climate and large-scale atmospheric circulation patterns (e.g. Villalba et al., 2012; Morales et al., 2015). The use of tree-ring width (TRW) variations as tracers of past climate

fluctuations has significantly expanded our understanding of climate variability over the South American continent during the past centuries. However, instabilities in the relationships between climate and TRW have been identified in some records, with changes mostly coincident with major shifts in climate regime (Álvarez et al., 2015; Lavergne et al., 2015; Suarez et al., 2015). These decouplings have been observed in regions strongly influenced by both the Southern Annular Mode (SAM) and the El Niño Southern Oscillation (ENSO) variability patterns (Villalba, 2007), which questions the use of TRW for reconstructing long-term climatic variations across southern South America.

A recent study has highlighted the potential of the isotopic composition of oxygen in cellulose extracted from *Nothofagus pumilio* tree-rings to reflect variations in December–May temperatures ( $T_{\text{Dec-May}}$ ) over a large region in the Patagonian Andes

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south of 38°S ( $r = 0.57$ ,  $p < 0.05$ ; Lavergne et al., 2016). It has also shown that the relationship between  $\delta^{18}\text{O}$  in *Fitzroya cupressoides* and  $T_{\text{Dec-May}}$ , thought significant, was weaker than in *N. pumilio* ( $r = 0.30$ ,  $p < 0.05$ ). Due to the poor performance of  $\delta^{18}\text{O}$  in *F. cupressoides* as a climate proxy, the use of  $\delta^{18}\text{O}$  does not seem to increase the dendrochronological potential of this multi-millennium species with individuals exceeding 3600 years in age (Lara and Villalba, 1993). However, previous studies have shown that temperature and precipitation variations have a strong imprint on the carbon isotopic composition ( $\delta^{13}\text{C}$ ) of the tree-rings in both *N. pumilio* and *F. cupressoides* species (Leavitt and Lara, 1994; Srur et al., 2008; Urrutia-Jalabert et al., 2015).  $\delta^{13}\text{C}$  in tree-rings depend on factors affecting the photosynthetic uptake of  $\text{CO}_2$  and are mainly controlled by stomatal conductance ( $g$ ) and the rate of carboxylation during photosynthesis ( $A$ ) (Farquhar et al., 1989). These factors influencing isotopic fractionation are closely related to meteorological conditions suggesting that  $\delta^{13}\text{C}$  in *F. cupressoides* and *N. pumilio* could be considered targeted climate proxies in Patagonia. These studies have also shown that the climatic information encoded in  $\delta^{13}\text{C}$  ratio is specific and, in some case, site dependent. Consequently, an improved understanding of the specific physiological responses to local and regional environmental conditions is, therefore, a prerequisite for an accurate interpretation of  $\delta^{13}\text{C}$  in tree rings.

Variations in the intrinsic water-use efficiency (i.e. the ratio of carbon gain to water loss;  $i\text{WUE} = A/g$ ) calculated using tree-ring  $\delta^{13}\text{C}$  provide insights on tree's physiological responses to climate (e.g. Frank et al., 2015; Wieser et al., 2016). This isotopic approach has been widely used in several regions, showing a consistent enhancement in  $i\text{WUE}$  over the 20th century at most sites (e.g. Lévesque et al., 2014; Saurer et al., 2014). However the specific nature of changes in  $i\text{WUE}$  (due to variations in water availability, in the efficiency of  $A$  or in  $g$ ) cannot be precisely determined using  $\delta^{13}\text{C}$  alone (e.g. Scheidegger et al., 2000). Since  $\delta^{18}\text{O}$  is not modulated by  $A$ , the information on evaporative enrichment recorded in  $\delta^{18}\text{O}$  can be useful to discriminate the effects of  $A$  and  $g$  on  $i\text{WUE}$  (Scheidegger et al., 2000; Roden and Siegwolf, 2012). Therefore, the joint analysis of  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  in tree-rings can be used to distinguish the effects of  $g$  and  $A$  on  $i\text{WUE}$  variations, and therefore, to disentangle the different gas-exchange strategies of tree species under changing ambient  $\text{CO}_2$  and environmental conditions (e.g. Nock et al., 2011).

The main goals of our study were twofold: 1) to characterize the physiological responses of *Fitzroya cupressoides* and *Nothofagus pumilio* to environmental changes for improving our interpretation of  $\delta^{13}\text{C}$  in tree rings, and 2) to evaluate the performance of the carbon isotopic composition of the cellulose in these two species as a paleoclimate proxy. For reaching these goals, we developed five annually resolved  $\delta^{13}\text{C}$  chronologies covering the last 60 years (1952–2011) from sites at different elevations in Northern Patagonia (41°S), and combined them with  $\delta^{18}\text{O}$  chronologies from Lavergne et al. (2016). The main novelty of our approach lies in the simultaneous examination of both isotopes of two species in southern South America. Only by improving our knowledge about the forcings of the isotopic variability in tree-rings and the eco-physiological information recorded therein, we will be finally able to improve stable-isotope based climate reconstructions from tree-rings.

## 2. Materials and methods

### 2.1. Regional setting and climate

The regional climate along the Patagonian Andes is mainly driven by the interactions between the circum-Antarctic cyclonic belt to the south and the subtropical Pacific high-pressure cell

to the northwest. The strong Westerlies coming across the Pacific Ocean permanently interact with the north–south mountain range of the Andes (e.g. Garreaud et al., 2013). The air masses from the Pacific lose moisture during the ascent on the western slope of the Andes and descend drier on the eastern foothills. Therefore, the mountain range induces a dramatic decline in precipitation from total annuals of about 4000 mm near the continental divide to less than 500 mm toward the Patagonian forest-steppe ecotone (Villalba et al., 2003). At Bariloche, in the eastern side of the gradient (41°12'S–71°12'W, 840 m asl), the mean annual temperature is 8.5 °C, with relatively cold winters (mean JJA temperature ~3 °C) and mild summers (mean DJF temperature ~14.2 °C). Seasonal variations in the Pacific anticyclone positions induce strong precipitation seasonality. Precipitation is largely concentrated from late fall to early spring followed by a drier period during summer and early fall (López Bernal et al., 2012).

### 2.2. Sampling and tree-ring processing

Sampling was conducted in March 2013 in five sites in northern Patagonia. Two sites for *F. cupressoides* (950–1050 m elevation) and three sites for *N. pumilio* (1270–1610 m) were selected in Parque Nacional Nahuel Huapi, near Bariloche (at 41°05'S–71°21'W, see Lavergne et al., 2016 for details). Cores were taken at breast height (1.3 m above the ground) using a 5 mm diameter Pressler increment borer. The core samples were dated to the calendar year of their formation following the techniques described by Stokes and Smiley (1968) and ring widths were measured at an accuracy of 0.001 mm. Cross-dating of growth rings to detect any error in the measurements was based on more than 60 cores from 30 or more trees at each site. Quality-control and accuracy of measurements and cross-dating were conducted with the COFECHA program, which allows the matching of ring-width among radii within a tree and among different trees (Holmes, 1983).

According to the procedure described in Lavergne et al. (2016), 6 trees for *F. cupressoides* and 4 trees for *N. pumilio* were selected for building the isotopic chronologies at each sampling site. All trees from each site were pooled to produce an average  $\delta^{13}\text{C}$  series at each site. Annual tree rings were split using a scalpel and pooled year by year. The wood samples were then chipped and grounded in a ball mill for homogenization.  $\alpha$ -cellulose was extracted from the wood according to the SOXHLET chemical method derived from Leavitt and Danzer (1993).  $\alpha$ -cellulose was homogenized ultrasonically with a sonotrode apparatus and freeze-dried. Cellulose samples of around 0.10 mg were loaded in tin-foil capsules. The  $\delta^{13}\text{C}$  was determined with an elemental analyser (EA NC2500, Carlo Erba) coupled with a Finnigan MAT252 mass spectrometer. An internal laboratory reference of cellulose (Whatmann® CC31) was used to correct for instrument drift and to normalize the data to internationally accepted standards. Along the sequence analyses, the isotopic composition of CC31 was measured every three samples. The standard deviation (SD) obtained from the measurement of the isotopic composition of 10 consecutive CC31 standards was typically  $\pm 0.1\text{‰}$ . The analytical procedure for  $\delta^{18}\text{O}$  measurements is described in Lavergne et al. (2016). The analyses of both stable isotopes on each sample were repeated at least once and up to three times. This methodology allows rejecting outlier measurements, which do not fit with the maximum accepted range.

### 2.3. Correction of non-climatic trends

The measured  $\delta^{13}\text{C}$  chronologies ( $\delta^{13}\text{C}_{\text{raw}}$ ) show a decreasing trend due to the rise of  $^{13}\text{C}$ -depleted atmospheric  $\text{CO}_2$  induced by fossil fuel burning and deforestation since industrialization (the Suess effect; Keeling, 1979). Therefore, the  $\delta^{13}\text{C}_{\text{raw}}$  series were corrected for the anthropogenic influence by removing the preindus-

trial isotope signature (e.g.,  $-6.4\%$ , AD 1850; Saurer et al., 1997) from the actual atmospheric  $\delta^{13}\text{C}_{\text{atm}}$  (for each year), such as:

$$\delta^{13}\text{C}_{\text{cor}} = \delta^{13}\text{C}_{\text{raw}} - (\delta^{13}\text{C}_{\text{atm}} + 6.4) \quad (1)$$

Records of  $\delta^{13}\text{C}_{\text{atm}}$  over the 1952–1999 period were obtained from interpolated data from direct ice-core measurements tabulated in McCarroll and Loader (2004), while the ones for the period 2000–2011 were extracted from the South Pole station (Scripps data) (Keeling et al., 2005). When both data were available (from 1978 to 2005), we calculated and used the mean  $\delta^{13}\text{C}_{\text{atm}}$  for the correction. Furthermore, to account for any increase of leaf internal discrimination against  $^{13}\text{C}$  caused by higher  $c_a$ , we also applied the ‘preindustrial’ or PIN correction described in McCarroll et al. (2009) and calculated  $\delta^{13}\text{C}_{\text{pin}}$  using R package ‘pin’ (McCarroll et al., 2009).

After that, we calculated composite specific  $\delta^{13}\text{C}_{\text{cor}}$  and  $\delta^{13}\text{C}_{\text{pin}}$  chronologies as the average of individual  $\delta^{13}\text{C}_{\text{cor}}$  and  $\delta^{13}\text{C}_{\text{pin}}$  series of *F. cupressoides* or *N. pumilio*. For assessing the common signal recorded in the isotopic ( $\delta^{13}\text{C}_{\text{cor}}$  and  $\delta^{13}\text{C}_{\text{pin}}$ ) chronologies between sites and species, we computed inter-correlations between all chronologies and between the two specific  $\delta^{13}\text{C}_{\text{cor}}$  and  $\delta^{13}\text{C}_{\text{pin}}$  chronologies over the common period (1952–2011). We also used a Mann–Kendall trend test to assess cumulative changes in specific  $\delta^{13}\text{C}_{\text{cor}}$  and  $\delta^{13}\text{C}_{\text{pin}}$  over the past six decades. The associated regression slopes were used as rates of change through time.

#### 2.4. Estimation of physiological responses

To evaluate the physiological changes over the period 1952–2011, different parameters were estimated using  $\delta^{13}\text{C}_{\text{raw}}$ . The isotope discrimination  $\Delta^{13}\text{C}$ , resulting from the preferential use of  $^{12}\text{C}$  over  $^{13}\text{C}$  during photosynthesis, is defined following Farquhar et al. (1982):

$$\Delta^{13}\text{C} = (\delta^{13}\text{C}_{\text{atm}} - \delta^{13}\text{C}_{\text{raw}}) / (1 + \delta^{13}\text{C}_{\text{raw}}/1000) \sim \delta^{13}\text{C}_{\text{atm}} - \delta^{13}\text{C}_{\text{raw}} \quad (2)$$

where  $\delta^{13}\text{C}_{\text{atm}}$  is the isotopic value of atmospheric  $\text{CO}_2$  and  $\delta^{13}\text{C}_{\text{raw}}$  the measured isotope ratio of the cellulose in tree rings.

For C3 plants, Farquhar et al. (1982) described the dependence of  $\Delta^{13}\text{C}$  on plant physiology by linearly relating the ratio of inter-cellular ( $c_i$ ) to ambient ( $c_a$ )  $\text{CO}_2$  concentrations through:

$$\Delta^{13}\text{C} = a + (b - a)c_i/c_a \quad (3)$$

where  $a$  is the fractionation associated with the diffusion of  $\text{CO}_2$  through the stomata ( $4.4\%$ ), and  $b$  the fractionation resulting from the enzymatic carbon fixation ( $27\%$ ). The  $\delta^{13}\text{C}_{\text{atm}}$  data were taken from McCarroll and Loader (2004) and Keeling et al. (2005) as indicated in section 2.3.

The intrinsic water-use efficiency (iWUE) was estimated as follows:

$$\text{iWUE} = A/g = (c_a - c_i) \times 1/1.6 = (c_a - c_i) \times 0.625 \quad (4)$$

where  $A$  is the photosynthetic assimilation rate and  $g$  the stomatal conductance.

Trend significances in specific  $\Delta^{13}\text{C}$ ,  $c_i$  and iWUE were evaluated using the Mann–Kendall test. Finally, pairwise correlations between specific  $\delta^{13}\text{C}_{\text{cor}}/\delta^{13}\text{C}_{\text{pin}}$  and  $\delta^{18}\text{O}$  series were used for characterizing the gas-exchange strategy for each species.

#### 2.5. Climate trends and $\delta^{13}\text{C}$ –climate relationships

Climate variations during the austral summer (December–February) were evaluated over the period 1952–2011. We used

**Table 1**

Temporal trends in  $\delta^{13}\text{C}_{\text{cor}}$ ,  $\delta^{13}\text{C}_{\text{pin}}$ ,  $\Delta^{13}\text{C}$ ,  $c_i$  and iWUE evaluated using Mann–Kendall trend test<sup>a</sup>.

Species	$\delta^{13}\text{C}_{\text{cor}}$	$\delta^{13}\text{C}_{\text{pin}}$	$\Delta^{13}\text{C}$	$c_i$	iWUE
Fc	0.007*	0.010**	$-0.007^*$	0.52**	0.49**
Np	0.006	0.015*	$-0.006$	0.65**	0.42**

<sup>a</sup>  $\delta^{13}\text{C}_{\text{cor}}$  in  $\%$  per year,  $\Delta^{13}\text{C}$  in  $\%$  per year,  $c_i$  in part per million (ppm) per year and iWUE in  $\mu\text{mol mol}^{-1}$  per year. Correlation coefficients are significant at 95% (\*) and at 99% (\*\*). Np: *Nothofagus pumilio*, Fc: *Fitzroya cupressoides*.

monthly records of temperature and precipitation from Bariloche, the nearest meteorological station to our sampling sites ( $41^\circ 12'S$ – $71^\circ 12'W$ , 840 m asl; Servicio Meteorológico Nacional, Argentina). Vapor pressure deficit (VPD; hPa) was calculated as the difference between the saturation vapor pressure ( $e_s$ ) and the actual vapor pressure. Actual vapor pressure was extracted from the CRUTS 3.2 gridded dataset (Harris et al., 2014), and  $e_s$  (hPa) at a given air temperature  $T$  ( $^\circ\text{C}$ ) was obtained following Tetens (1930):

$$e_s = [6.112 \times e^{(17.27 \times T/237.3 + T)}] \quad (5)$$

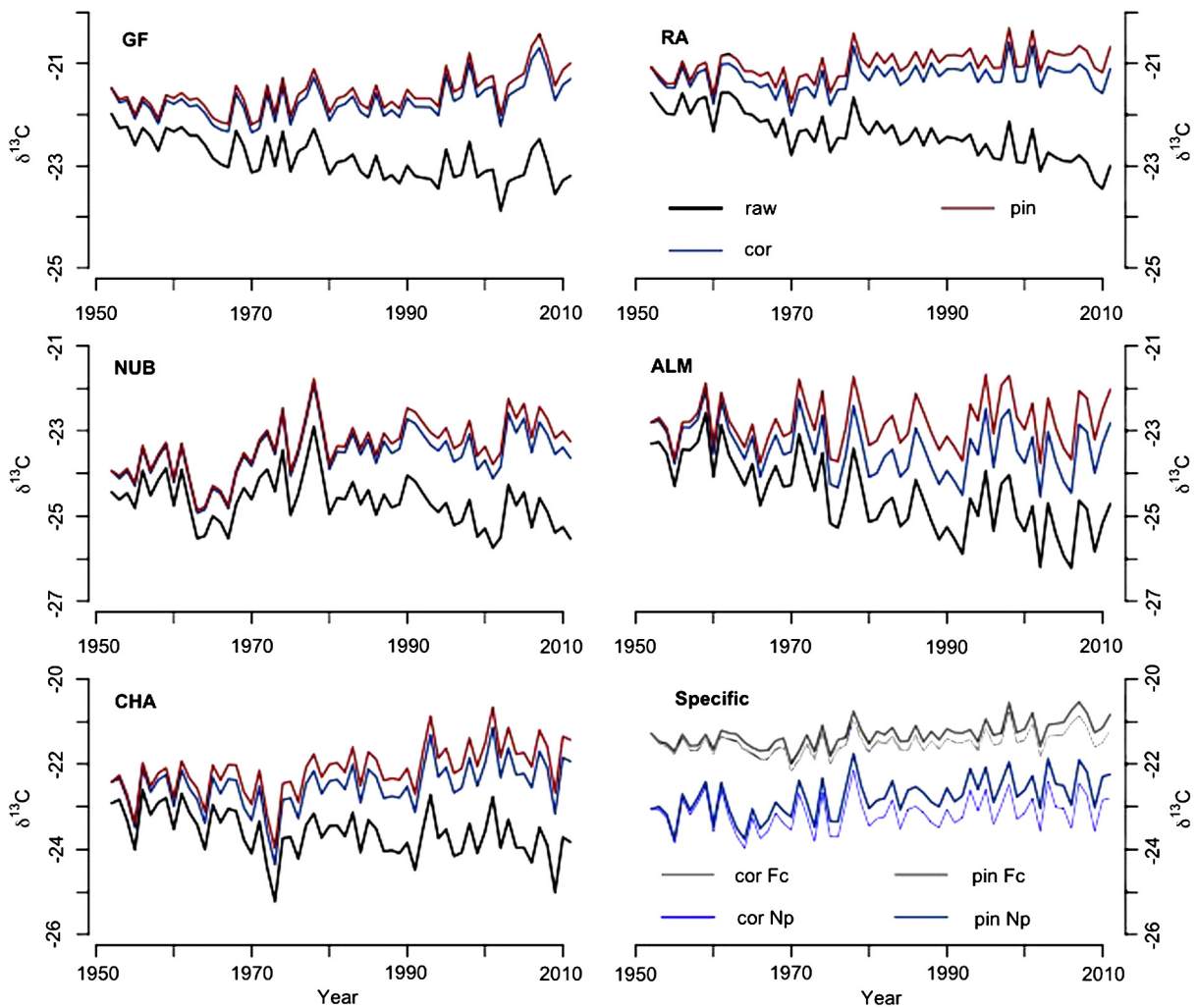
The monthly mean  $e_s$  was calculated as the average of monthly maximum  $e_s$  calculated using monthly maximum air temperatures ( $T_{\text{max}}$ ), and monthly minimum  $e_s$  derived from monthly minimum air temperatures ( $T_{\text{min}}$ ). Monthly values of cloud cover, also extracted from the CRUTS 3.2 gridded dataset, were used to estimate the photosynthetically active radiation or PAR (following: cloud cover  $\approx$  –PAR). All meteorological records are available over the period 1952–2011.

To determine the relationships between the specific isotopic chronologies and climate, we computed simple correlation functions between  $\delta^{13}\text{C}_{\text{cor}}$  and  $\delta^{13}\text{C}_{\text{pin}}$  from each species and monthly temperature, precipitation, VPD and cloud cover. Comparisons between  $\delta^{13}\text{C}$  series and climate variables were conducted over the austral growing season overlapping two calendar years from September to May and the correlation significance were estimated from bootstrapping with 1000 Monte Carlo simulations (Ebisuzaki, 1997). These analyses were intended to determine those climatic variables with the strongest imprint on  $\delta^{13}\text{C}$  ratios. Based on these relationships, we conducted the calibration-verification tests using half of the selected climatic series (identified as potential climatic target) for calibration and half for assessing the reconstruction quality (verification). In order to assess the spatial representativeness of the targeted climatic signal recorded in the carbon isotopes chronologies, we conducted a spatial correlation analysis between specific chronologies and gridded climate data from the CRUTS 3.2 dataset (Harris et al., 2014) over the area extending from  $30^\circ\text{S}$  to  $56^\circ\text{S}$  and from  $55^\circ\text{W}$  to  $80^\circ\text{W}$ .

### 3. Results

#### 3.1. Corrected $\delta^{13}\text{C}$ : temporal variations and inter-series correlations

Trends for the  $\delta^{13}\text{C}_{\text{cor}}$  chronologies of *F. cupressoides* and *N. pumilio* are both positives (0.007 and 0.006 $\%$  per year) although only significant at 95% confidence level for *F. cupressoides* (Table 1 and Fig. 1). The  $\delta^{13}\text{C}_{\text{pin}}$  for the two species exhibit significant positive trends, slightly larger for *N. pumilio* than for *F. cupressoides* (0.015 $\%$  and 0.010 $\%$  and per year,  $p < 0.05$ , respectively). The two  $\delta^{13}\text{C}_{\text{cor}}$  chronologies of *F. cupressoides* are strongly inter-correlated ( $r = 0.55$ ,  $p < 0.05$ ; Table 2), while those of *N. pumilio* are not ( $p > 0.05$ ). For both species, the  $\delta^{13}\text{C}_{\text{pin}}$  chronologies show stronger inter-correlations than the  $\delta^{13}\text{C}_{\text{cor}}$  chronologies ( $r = 0.61$  for *F. cupressoides* and  $r = 0.41$  for *N. pumilio*,  $p < 0.05$ ). Finally,



**Fig. 1.** Temporal variations over the past 60 years of tree-ring  $\delta^{13}\text{C}_{\text{raw}}$ ,  $\delta^{13}\text{C}_{\text{cor}}$  and  $\delta^{13}\text{C}_{\text{pin}}$  at each site and for each species. *Fitzroya cupressoides* (Fc): Glaciar Frias (GF) and Rio Alerce (RA). *Nothofagus pumilio* (Np): Paso de las Nubes (NUB), La Almohadilla (ALM) and Challhuaco (CHA). Black curve: raw  $\delta^{13}\text{C}$ ; Blue:  $\delta^{13}\text{C}$  data corrected from the Suess effect; Red: pre-industrial (pin) corrected  $\delta^{13}\text{C}$ . (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

**Table 2**

Pearson's correlation coefficients between  $\delta^{13}\text{C}_{\text{cor}}$  and  $\delta^{13}\text{C}_{\text{pin}}$  chronologies over the period 1952–2011<sup>a</sup>.

	RA	NUB	ALM	CHA	Fc	RA	NUB	ALM	CHA	Fc
	$\delta^{13}\text{C}_{\text{cor}}$					$\delta^{13}\text{C}_{\text{pin}}$				
GF	0.55*	0.47*	0.32	0.43*		0.61*	0.55*	0.46*	0.49*	
RA		0.38*	0.33*	0.58*			0.52*	0.45*	0.65*	
NUB			0.15	0.24				0.39*	0.39*	
ALM				0.30					0.43*	
Np					0.67**					0.74**

<sup>a</sup> Correlation coefficients are significant at 95% (\*) and 99% (\*\*). Np: *Nothofagus pumilio* (NUB: Paso de las Nubes, ALM: La Almohadilla, CHA: Challhuaco). Fc: *Fitzroya cupressoides* (GF: Glaciar Frias, RA: Rio Alerce).

the two specific carbon isotopic chronologies are strongly correlated to one another ( $r = 0.67$  for  $\delta^{13}\text{C}_{\text{cor}}$  and  $r = 0.74$  for  $\delta^{13}\text{C}_{\text{pin}}$ ,  $p < 0.01$ ; Table 2).

### 3.2. Physiological responses through dual isotope analyses

The two specific iWUE chronologies exhibit similar positive slopes over the whole 1952–2011 period (ca.  $0.45 \mu\text{mol mol}^{-1}$  per year,  $p < 0.001$ ; Table 1, Fig. 2). *F. cupressoides* exhibits higher values of iWUE (from 95 to  $130 \mu\text{mol mol}^{-1}$ ) than *N. pumilio* (from 70–80 to  $110\text{--}120 \mu\text{mol mol}^{-1}$ ). The  $\Delta^{13}\text{C}$  values for *N. pumilio* are almost constant through time, whereas those for *F. cupressoides*

slightly decreased (Table 1, Fig. 2). The  $c_i$  values, which increase over time for both records, are larger in *N. pumilio* than in *F. cupressoides* (Table 1, Fig. 2). Notably, for *F. cupressoides* the  $\delta^{18}\text{O}$  composite record is not significantly related to  $\delta^{13}\text{C}_{\text{cor}}$  ( $r = 0.26$ ,  $p > 0.05$ ; Fig. 3) and only slightly to  $\delta^{13}\text{C}_{\text{pin}}$  ( $r = 0.30$ ,  $p < 0.05$ ; Fig. S1). In contrast, highly significant relationships are found for *N. pumilio* with both corrections ( $r = 0.59$  and  $0.64$ ,  $p < 0.01$ ; Fig. 3 and S1).

### 3.3. Trends in climate and relationships between $\delta^{13}\text{C}$ and climate

A significant warming is recorded in Bariloche during summer over the period 1952–2011 ( $+0.2^\circ\text{C}$  per decade,  $p < 0.05$ ; Fig. 4).



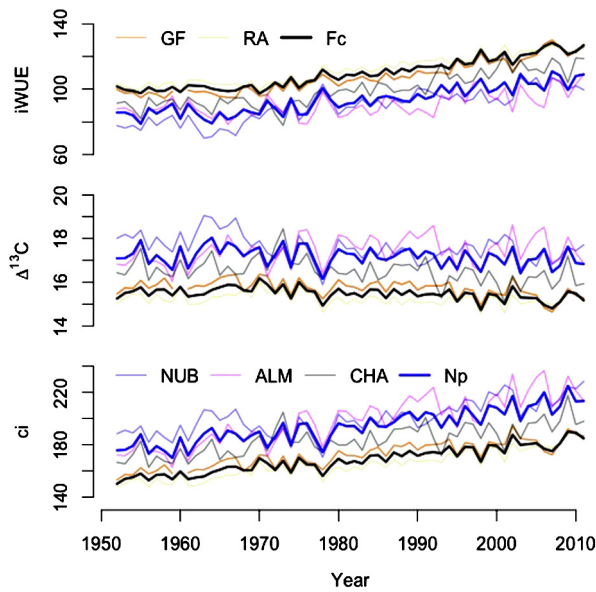


Fig. 2. Temporal evolutions of specific iWUE,  $\Delta^{13}\text{C}$  and  $c_i$  over the 1952–2011 period. In bold lines are the specific chronologies during the whole period.

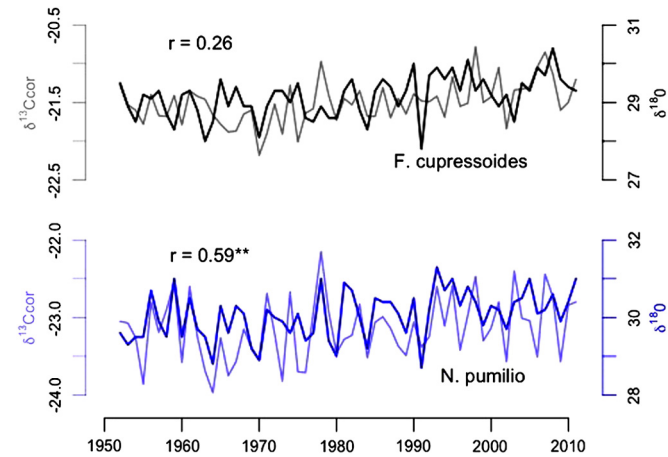


Fig. 3. Specific  $\delta^{13}\text{C}_{\text{cor}}$  variations versus  $\delta^{18}\text{O}$  over 1952–2011. Correlation coefficients are significant at 99% (\*\*).

A large contribution to this positive trend is related to the regional temperature jump in 1976–1977 associated with the negative-to-positive phase shift in the Pacific Decadal Oscillation (Villalba et al., 1997). Over the same interval, no significant trends were recorded in precipitation, vapor pressure deficit (VPD) and cloud cover during summer. However, from mid-1990s onwards, cloud cover started to decrease significantly ( $-0.4\%$  per year,  $p < 0.05$ ; Fig. 4).

The composite specific  $\delta^{13}\text{C}_{\text{cor}}$  and  $\delta^{13}\text{C}_{\text{pin}}$  chronologies from *F. cupressoides* and *N. pumilio* show similar correlation patterns with climate (Fig. 5 and S2):

- Strong positive relationships between  $\delta^{13}\text{C}_{\text{cor}}$  and  $\delta^{13}\text{C}_{\text{pin}}$  chronologies and mean monthly temperatures from December to March, highly significant over the three summer months (DJF,  $r = 0.78$  and  $r = 0.75$ ,  $p < 0.001$  for *F. cupressoides* and  $r = 0.69$  and  $r = 0.68$ ,  $p < 0.001$  for *N. pumilio*, respectively).
- Negative relationships with precipitation for both  $\delta^{13}\text{C}_{\text{cor}}$  and  $\delta^{13}\text{C}_{\text{pin}}$  in December ( $r = -0.28$ ,  $p < 0.05$  for both species) and in January ( $r = -0.26$ ,  $p < 0.05$  for *N. pumilio*).
- Positive relationships with December–February VPD for both species ( $r = 0.67$  for *F. cupressoides* and  $r = 0.68$  for *N. pumilio*

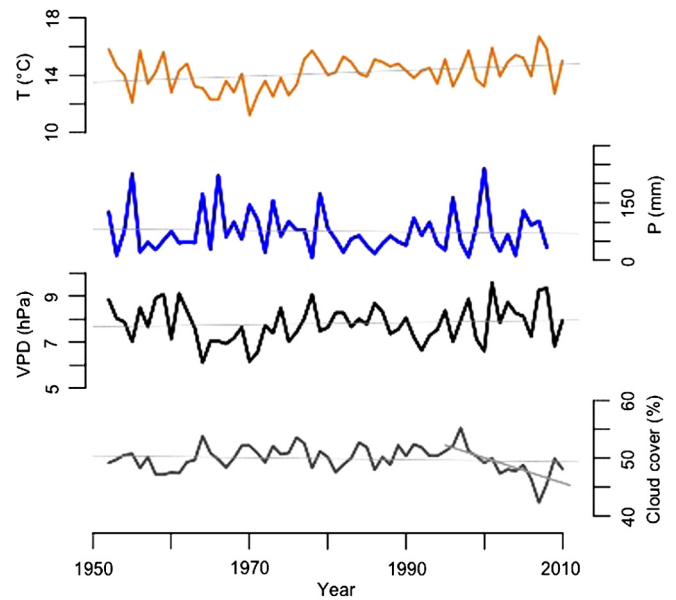


Fig. 4. Temporal variations in summer (December–February) mean temperature, total precipitation, mean vapor pressure deficit (VPD) and mean cloud cover over the period 1952–2011. Temperature and precipitation data are derived from Bariloche meteorological station. VPD and cloud cover are extracted from CRUTS 3.2 gridded dataset (Harris et al., 2014). Data were fit by linear regression analysis over the 1952–2011 period:  $T: y = 0.020x - 26.39$ ,  $r^2 = 0.097$ ,  $p < 0.05$ ;  $P: y = -0.2026x + 477.15$ ,  $r^2 = 0.004$ ,  $p > 0.05$ ;  $\text{VPD}: y = 0.005x - 1.2835$ ,  $r^2 = 0.009$ ,  $p > 0.05$ ; cloud cover:  $y = -0.0178x + 85.144$ ,  $r^2 = 0.019$ ,  $p > 0.05$ ; over the 1995–2011 period: cloud cover:  $y = -0.4373x + 924.697$ ,  $r^2 = 0.49$ ,  $p < 0.05$ .

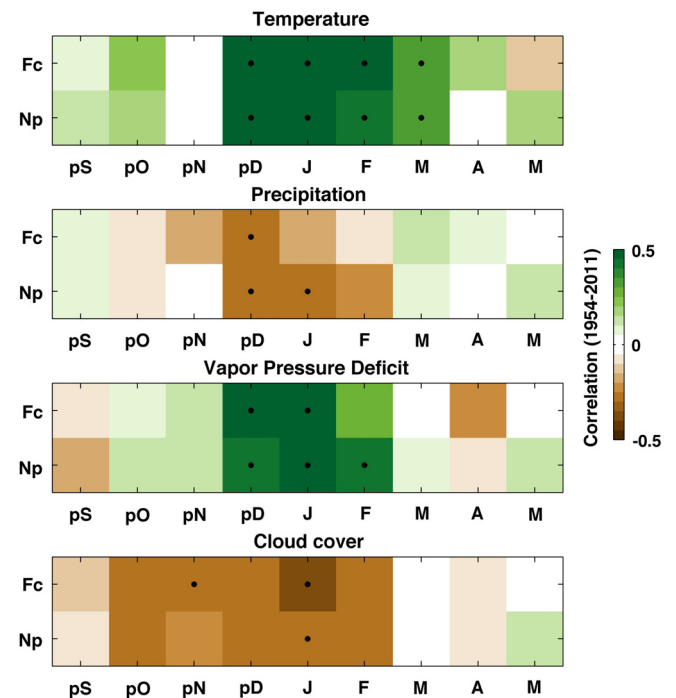
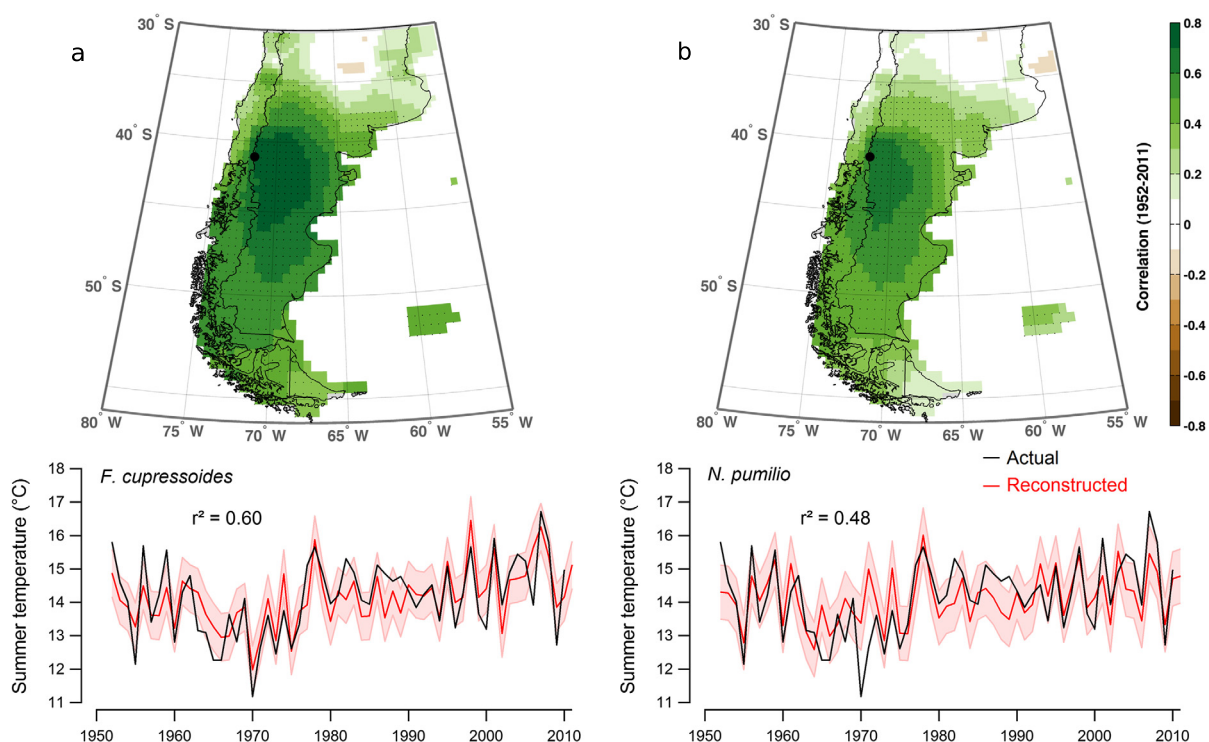


Fig. 5. Correlations between the  $\delta^{13}\text{C}_{\text{cor}}$  chronologies from *F. cupressoides* (Fc) and *N. pumilio* (Np) and monthly temperature and precipitation from Bariloche meteorological station, vapor pressure deficit and cloud fraction derived from CRUTS 3.2 gridded dataset (Harris et al., 2014). Correlations are given for a 9-month window over the 1954–2011 period from September to May corresponding to the growing season, which overlaps two calendar years ( $p$  = previous calendar year). The stippling points indicate months with significant correlations ( $p < 0.05$ ).

using  $\delta^{13}\text{C}_{\text{cor}}$  composite chronologies,  $p < 0.001$ ;  $r = 0.61$ ,  $p < 0.001$  for both species using  $\delta^{13}\text{C}_{\text{pin}}$  composite chronologies).



**Fig. 6.** Top: Correlation fields across southern South America between December–February temperature (CRUTS 3.2 gridded dataset; Harris et al., 2014) and the two specific  $\delta^{13}\text{C}_{\text{cor}}$  chronologies over 1952–2011: (a) for *F. cupressoides* and (b) for *N. pumilio*. The stippling points indicate grid points with significant correlations ( $p < 0.05$ ). The black points situate the sampling sites. Below: Actual and reconstructed December–February mean temperature variations inferred from  $\delta^{13}\text{C}_{\text{cor}}$  *F. cupressoides* (left) and *N. pumilio* (right) chronologies. The pink color band around the reconstruction indicates the root mean squared error (RMSE). The explained variance ( $r^2$ ) is also indicated. Data were fit by linear regression analysis over the 1952–2011 period: Fc:  $y = 3.247x + 83.99$ ,  $r^2 = 0.60$ ,  $p < 0.05$ ; Np:  $y = 1.90x + 58.14$ ,  $r^2 = 0.48$ ,  $p > 0.05$ .

**Table 3**

Calibration and verification statistics of the two specific  $\delta^{13}\text{C}_{\text{cor}}$  chronologies against December–February temperature using simple linear regression<sup>a</sup>.

Species	Calibration				Verification				
	Period	$r^2$	RMSE	DW	Period	RMSE	$r^2$	RE	CE
<i>F. cupressoides</i>	1952–1980	0.67	0.86	2.14	1981–2010	0.68	0.42	0.423	0.721
	1981–2010	0.42	0.68	2.39	1952–1980	0.76	0.67	0.648	0.786
<i>N. pumilio</i>	1952–1980	0.46	0.73	1.74	1981–2010	0.46	0.46	0.460	0.739
	1981–2010	0.46	0.63	1.79	1952–1980	0.62	0.46	0.460	0.671

<sup>a</sup>  $r^2$ : Squared correlation coefficient, RE: reduction of error statistic, CE: coefficient of efficiency, DW: Durbin–Watson statistic, and RMSE: root mean squared error of the estimate for two independent periods. Overall period (1952–2010): for *F. cupressoides*,  $r^2 = 0.60$ ; DW = 2.033, RMSE = 0.71; for *N. pumilio*,  $r^2 = 0.48$ ; DW = 1.55, RMSE = 0.81.

– Negative correlation coefficients between cloud cover from October to January and  $\delta^{13}\text{C}_{\text{cor}}$  composite chronologies, statistically significant in November and January for *F. cupressoides* ( $r = -0.29$  and  $r = -0.38$ ,  $p < 0.05$ , respectively) and in January for *N. pumilio* ( $r = -0.28$ ,  $p < 0.05$ ). Negative and significant relationships with monthly cloud cover were registered from October to February using the  $\delta^{13}\text{C}_{\text{pin}}$  composite chronology of *F. cupressoides* (range from  $r = -0.29$  to  $-0.39$ ,  $p < 0.05$ ).

The  $\delta^{13}\text{C}_{\text{cor}}$  composite chronologies, which exhibit slightly stronger relationships with climate than  $\delta^{13}\text{C}_{\text{pin}}$  records were used for calibrating summer (Dec to Feb) temperature variations against the isotopic ratios. Split-period (1952–1980 and 1981–2010) verifications of the calibrated relationships for *F. cupressoides* and *N. pumilio* (Table 3) indicate that the linear regression models of specific  $\delta^{13}\text{C}_{\text{cor}}$  records on summer temperature are reliable and have strong predictive skills (Residual Error > 0, Fritts, 1976, Confidence Interval > 0, Durbin–Watson statistic  $\sim 2$ , Durbin

and Watson, 1971 and Root Mean Squared Error  $< 0.9^\circ\text{C}$ ). However, the predictive skills for the calibration model for *N. pumilio* are comparatively lower ( $r^2 = 0.48$ ; DW = 1.55, RE = 0.46 and RMSE =  $0.81^\circ\text{C}$ ) than those recorded for *F. cupressoides* ( $r^2 = 0.60$ , DW = 2.03, RE = 0.60 and RMSE =  $0.71^\circ\text{C}$ ). The  $\delta^{13}\text{C}_{\text{cor}}$  composite chronology from *F. cupressoides* accounts for 60% of the actual summer temperature variance over the common period (1952–2010) between the temperature and isotopic records (Table 3, Fig. 6). The strong imprint of summer temperature recorded in the two specific  $\delta^{13}\text{C}_{\text{cor}}$  chronologies extend over a large geographical area across Patagonia and suggest that the regional  $\delta^{13}\text{C}_{\text{cor}}$  chronologies accurately capture summer temperature variations in southern South America south of  $35^\circ\text{S}$  (Fig. 6).

## 4. Discussion

### 4.1. Physiological responses under elevated $\text{CO}_2$

The intrinsic water-use efficiencies of both *F. cupressoides* and *N. pumilio* species have increased by ca. 25% over the past 60 years.

Since iWUE is an indirect measure of the ratio between carbon gained and water loss, the recorded changes may be ascribed to an increase in the photosynthetic assimilation rate ( $A$ ) or a decrease in the stomatal conductance ( $g$ ), or a combination of both (Feng, 1999). Both physiological processes are strongly related to changes in atmospheric  $\text{CO}_2$  concentrations. Indeed, larger  $c_a$  values are expected to increase the rate of carboxylation by Rubisco, which translates into an increase of  $A$ , while  $g$  tends to reduce to backward adjust to a value that optimizes carbon gain to water loss (e.g. Ainsworth and Rogers, 2007; Franks et al., 2013). As expected, over the period 1952–2011,  $c_a$  has risen from ca. 312 to 392 ppm, an increase of ca. 26%, which is similar to the iWUE increase recorded in both species. The enhancement of iWUE we observe in *F. cupressoides* and *N. pumilio* is in the range of values for European conifers (by 25.7%) and deciduous trees (by 32.4% for oak) (Saurer et al., 2014) and in South America (by 31% for *F. cupressoides*) (Urrutia-Jalabert et al., 2015).

As suggested by theory and empirical evidences, increase in  $c_a$  should result in plants regulating gas exchanges through different response patterns of  $c_i$  (e.g. Frank et al., 2015; Voelker et al., 2015). Saurer et al. (2004) proposed three different gas-exchange scenarios to characterize the physiological responses of trees: (1) plant maintenance of a constant  $c_i$ , (2) constant  $c_i/c_a$  ratio, and (3) constant difference between  $c_a$  and  $c_i$ . Here, for *N. pumilio*,  $\Delta^{13}\text{C}$  (or  $c_i/c_a$ ) is constant and iWUE increases (scenario 2; regulative adaptation of trees to rising  $\text{CO}_2$ ). Therefore, *N. pumilio* is probably responding actively to  $c_a$  increase. For *F. cupressoides*,  $\Delta^{13}\text{C}$  decreases slightly (slight decrease in  $c_i/c_a$ ) concurrent with  $c_i$  and iWUE increases, suggesting an intermediate scenario on the continuum between scenarios 1 and 2. This particular response of *F. cupressoides* is in agreement with Urrutia-Jalabert et al. (2015), who show similar increases in  $c_i$  and iWUE and a decrease in discrimination over the last decades for the same species in southern Chile. These results suggest that *F. cupressoides* is actively responding to  $\text{CO}_2$  increase, but that other factors are also influencing  $g$  and/or  $A$ , leading to a decrease of  $\Delta^{13}\text{C}$ .

Although both species likely have an ‘active response’ to  $\text{CO}_2$  increases (McCarroll et al., 2009; Loader et al., 2011) through reduction in  $g$ , increase in  $A$ , or both, our results illustrate the specific gas-exchange strategies to current increase in  $c_a$ . As mentioned above, changes in photosynthetic assimilation rate ( $A$ ) should not be associated with changes in  $\delta^{18}\text{O}$ , whereas changes in  $g$  should affect variations in both  $\delta^{18}\text{O}$  and  $\delta^{13}\text{C}$  in tree rings (Scheidegger et al., 2000). Under elevated  $c_a$ , stomata remain closed for longer periods,  $g$  decreases, the evaporative enrichment in  $^{18}\text{O}$  increases, leading to an enhancement of  $\delta^{18}\text{O}$  in leaf water, in sugars and finally in tree rings (Scheidegger et al., 2000). Therefore, when variations in  $g$  are the main driver of changes in iWUE, both  $\delta^{18}\text{O}$  and  $\delta^{13}\text{C}$  ratios should be significantly and positively correlated. In this study, both species show increasing trends for  $\delta^{18}\text{O}$  of about 0.9‰ from the 1960s to the mid-1990s followed by a relative leveling off during the last decade (Lavergne et al., 2016). Whereas the *N. pumilio*  $\delta^{13}\text{C}_{\text{cor}}$  and  $\delta^{13}\text{C}_{\text{pin}}$  records are strongly positively related to  $\delta^{18}\text{O}$  ( $r = 0.59$  and  $r = 0.64$ ,  $p < 0.001$ , respectively), they are not, or only weakly significantly inter-correlated in *F. cupressoides* chronologies ( $r = 0.26$ ,  $p > 0.05$  and  $r = 0.30$ ,  $p < 0.05$ , respectively). The  $\delta^{18}\text{O}$  increase observed in the two species may reflect an enhancement of the evaporative enrichment at least until the mid-1990s, which would advocate for a decrease of  $g$  in both species. Moreover, the strong relation between  $\delta^{18}\text{O}$  and  $\delta^{13}\text{C}$  in *N. pumilio* suggest that the mechanism for reducing water loss and keeping  $c_i/c_a$  constant has been the reduction of  $g$  due to higher  $\text{CO}_2$  levels rather than an increased efficiency in  $\text{CO}_2$  assimilation ( $A$ ). This gas-exchange strategy has been observed in most trees in both hemispheres (e.g. Saurer et al., 2004; Andreu-Hayles et al., 2011). In contrast, although some decrease in

$g$  may have occurred in *F. cupressoides*, the decoupling between  $\delta^{18}\text{O}$  and  $\delta^{13}\text{C}$  suggests that additional physiological processes (probably related to  $A$ ) may have also operated. According to Urrutia-Jalabert et al. (2015), an increment in  $A$  rather than a decrease in  $g$  may be responsible for the increase of iWUE recorded for *F. cupressoides* in Chile. In our Patagonian sites, a concurrent reduction of both the stomatal conductance and the photosynthetic capacity in response to higher levels of  $\text{CO}_2$  could be advocated. Indeed, it would produce the observed patterns in iWUE,  $c_i$  and discrimination, and would be consistent with a low correlation between  $\delta^{18}\text{O}$  and  $\delta^{13}\text{C}$ . However, a note of caution should be added here. As indicated in Lavergne et al. (2016), the  $\delta^{18}\text{O}$  positive trends in *F. cupressoides* and *N. pumilio* cannot be unequivocally ascribed to evaporative fractionation. They might also reflect changes in the isotopic composition of precipitation. Additional work on the evolution of the isotopic composition of precipitation, which should help clarifying this issue, is in progress. Independent of the  $\delta^{18}\text{O}$  trends in precipitation, our results show that although different mechanisms of response to  $c_a$  increase may be involved, *F. cupressoides* and *N. pumilio* actively control their carbon uptakes.

#### 4.2. Implications of physiological responses for past climate reconstructions

Differences in tree physiological responses to environmental changes are relevant for tree-ring based climate reconstructions. In addition to increased  $c_a$ , variations in summer temperature may significantly affect  $\delta^{13}\text{C}$  in tree rings. The indirect link between temperature and carbon isotope ratios has been widely recognized (e.g. Young et al., 2010). For instance, at the leaf level  $g$  is strongly influenced by VPD, which modulates transpiration (e.g. Barbour, 2007). Thus, an increase in VPD due to warmer temperatures and/or below-average precipitation induced a reduction in  $g$ , but an increase in  $\delta^{13}\text{C}$  ratio and in iWUE. This is consistent with the precipitation, temperature and VPD patterns recorded at our sites, and suggests an indirect influence of temperature and/or precipitation on  $g$  through VPD variations. However, a decrease in  $g$ , induced by reduced soil water content and high VPD, may also constrains photosynthesis and plant productivity (Barbour et al., 2002). The reduction in cloud coverage since mid-1990s could have also stimulated photosynthesis with a concurrent increase in  $A$  and consequently in iWUE. In this case, photon flux, instead of temperature, is the major driver of variations in  $\delta^{13}\text{C}$  ratios. Therefore, changes in VPD, solar radiation or both, could explain the observed relationships between  $\delta^{13}\text{C}$  and temperature.

Our results suggest that the responses of *F. cupressoides* and *N. pumilio* to environmental changes are a little different. Indeed, the water use efficiency appears to be modulated mainly by  $g$  in *N. pumilio*, while both  $g$  and  $A$  may influence iWUE variations in *F. cupressoides*. The larger dependence of  $\delta^{13}\text{C}$  on summer VPD and precipitation in *N. pumilio* than in *F. cupressoides*, and conversely the stronger relationship between  $\delta^{13}\text{C}$  and spring–summer cloud cover in *F. cupressoides* than in *N. pumilio* are consistent with this hypothesis. In summary, our results advocate for an indirect effect of summer temperature on stable isotope ratios, which is mostly influenced by relative humidity/soil moisture for *N. pumilio* and by both relative humidity and sunlight radiation for *F. cupressoides*.

#### 4.3. Tree-ring $\delta^{13}\text{C}$ as climate proxy record

After removing the low-frequency trends due to tree physiological responses to  $c_a$  using the PIN correction (McCarroll et al., 2009), the strength of the relationships between sites and species increases. In contrast, the climatic signal recorded in the  $\delta^{13}\text{C}_{\text{pin}}$  chronologies weakens in comparison to the  $\delta^{13}\text{C}_{\text{cor}}$  chronologies.



Because both species show an active response to  $c_a$  increase, attested by the steady to slight decrease in  $\Delta^{13}\text{C}$ , removing the Suess effect from the  $\delta^{13}\text{C}_{\text{raw}}$  chronologies appear to be sufficient to compensate for the decline in  $\delta^{13}\text{C}_{\text{atm}}$ . Actually, none of the  $\delta^{13}\text{C}_{\text{cor}}$  chronologies shows a declining trend. The PIN correction is known to be more suited to trees showing a passive response to  $c_a$  increase (declining trend in  $\delta^{13}\text{C}_{\text{cor}}$ ) and which are not altering their stomatal conductance or photosynthetic rate (McCarroll et al., 2009; Andreu-Hayles et al., 2011). Therefore,  $\delta^{13}\text{C}_{\text{cor}}$  series were preferentially chosen as climate predictors.

Inter-annual variations in Dec–Feb temperature in northern Patagonia are significantly captured by changes in  $\delta^{13}\text{C}_{\text{cor}}$  in annual rings from *F. cupressoides* ( $r = 0.78$ ,  $p < 0.001$ ) and to a lesser extent from *N. pumilio* ( $r = 0.69$ ,  $p < 0.001$ ). The  $\delta^{13}\text{C}_{\text{cor}}$  variations are consistent with summer temperature variations over a large area in South America south of ca.  $35^\circ\text{S}$ . In this extended region, temperature is mainly influenced by the Southern Annular Mode (SAM), the leading mode of climate variability at higher latitudes in the Southern Hemisphere (e.g., Garreaud et al., 2013). The SAM describes the north–south movement of the Westerlies around Antarctica and is related to sea level pressure anomalies between mid and high latitudes in the Southern Hemisphere. As expected, correlation coefficients between *F. cupressoides* and *N. pumilio*  $\delta^{13}\text{C}_{\text{cor}}$  and Dec–Feb SAM (Marshall, 2003) over the 1957–2010 period are statistically significant ( $r = 0.40$  and  $r = 0.37$ ,  $p < 0.05$ , respectively), but weaker than those reported with temperature.

Lavergne et al. (2016) showed that  $\delta^{18}\text{O}$  variations in *N. pumilio* are valid predictors for reconstructing past Dec–May temperature in South America south of  $40^\circ\text{S}$ . Here, our results suggest that the  $\delta^{13}\text{C}_{\text{cor}}$  variations from the slow-growing *F. cupressoides* are much more sensitive to past summer temperature variations or interrelated parameters such as solar radiation or VPD (humidity). *F. cupressoides* is the second longest living tree in the world, and a 3600-year reconstruction of summer temperature based in ring width has already been produced (Lara and Villalba, 1993). Over the period 1952–2010, variations in  $\delta^{13}\text{C}_{\text{cor}}$  accounts for 60% of the actual summer temperature variance, comparatively larger than those variance reported by Villalba (1990) and Lara and Villalba (1993) based in ring width. Our results show that  $\delta^{13}\text{C}_{\text{cor}}$  is a reliable proxy with significant predictive skills. Therefore, long-term records of  $\delta^{13}\text{C}_{\text{cor}}$  from *F. cupressoides* will provide solid reconstruction of past summer temperature variations across southern South America over the last centuries to millennia.

## 5. Conclusion

Despite likely different strategies in gas-exchanges between *Fitzroya cupressoides* and *Nothofagus pumilio*, a topic that requires further investigations, our study suggests that iWUE has increased in both species during the last six decades. This increase in *F. cupressoides* appears to be mainly driven by both a decrease in stomatal conductance and a strong increase in photosynthetic rate, whereas variations in *N. pumilio* iWUE are more closely tied to changes in stomatal conductance. In addition, variations in growing season temperature modulate the  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  isotopes in similar manner in both species; above-mean temperatures in summer increase the isotopic values. Our results highlight the large potential of  $\delta^{13}\text{C}$  in both species for reconstructing past summer temperature variations over southern South America and their relationships with major hemispheric climatic forcings such as SAM. Certainly, our results encourage the development of multi-centennial  $\delta^{13}\text{C}$  chronologies to increase the reduced number of high-resolution records in Patagonia. Long-term carbon isotopic chronologies are currently been developed in South America to

help documenting past climate variations in the Southern Hemisphere.

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## Appendix A. Supplementary material

Supplementary material related to this article can be found online at <http://dx.doi.org/10.1016/j.epsl.2016.11.045>.

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