



Research paper

Single-provenance mature conifers show higher non-structural carbohydrate storage and reduced growth in a drier location

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Since growth is more sensitive to drought than photosynthesis, trees inhabiting dry regions are expected to exhibit higher carbohydrate storage and less growth than their conspecifics from more humid regions. However, the same pattern can be the result of different genotypes inhabiting contrasting humidity conditions. To test if reduced growth and high carbohydrate storage are environmentally driven by drought, we examined the growth and non-structural carbohydrate (NSC) concentrations in single-provenance stands of mature trees of *Pinus contorta* Douglas and *Pinus ponderosa* Douglas ex C. Lawson planted at contrasting humidity conditions (900 versus 300 mm of annual precipitation) in Patagonia, Chile. Individual tree growth was measured for each species and at each location as mean basal area increment of the last 10 years (BAI_{10}), annual shoot elongation for the period 2011–14, and needle length for 2013 and 2014 cohorts. Additionally, needle, branch, stem sapwood and roots were collected from each sampled tree to determine soluble sugars, starch and total NSC concentrations. The two species showed lower mean BAI_{10} and 2013 needle length in the dry site; *P. ponderosa* also had lower annual shoot extension for 2011 and 2014, and lower 2014 needle length, in the dry than in the mesic site. By contrast, NSC concentrations of all woody tissues for both species were either similar or higher in the dry site when compared with the mesic site. Patterns of starch and sugars were substantially different: starch concentrations were similar between sites except for roots of *P. ponderosa*, which were higher in the dry site, while sugar concentrations of all woody tissues in both species were higher in the dry site. Overall, our study provides evidence that reduced growth along with carbon (C) accumulation is an environmentally driven response to drought. Furthermore, the significant accumulation of low-molecular weight sugars in the dry site is compatible with a prioritized C allocation for osmoregulation. However, since this accumulation did not come at the expense of reduced starch, it is unlikely that growth was limited by C supply in the dry site.

Keywords: carbon dynamics, climate change, conifers, drought, osmoregulation, Patagonia.

Introduction

There is increasing concern regarding how climate change is impacting, and will impact, forests. Recent worldwide tree mortality events that have been related to increasing temperature and decreasing precipitation have caused a growing interest in understanding the physiological responses to these factors at the whole-tree level (Allen et al. 2010, McDowell 2011, Palacio et al. 2014, Hoch 2015). A long-standing belief is that forests are the world's lungs that will buffer atmospheric CO₂ increases, and that such CO₂ increases could compensate for drought-induced stomatal limitations

on photosynthesis. This classical view assumes that biomass production under drought is carbon (C) limited, which is a highly controversial issue (Körner 2006, Leuzinger and Hättenschwiler 2013). Combining drought and different levels of atmospheric CO₂, it has been found that in some species, higher CO₂ prevented seedlings from a fast decline in growth under drought (Guehl et al. 1994). This result is consistent with the hypothesis that drought limits growth via insufficient C availability, as well as with an indirect water-saving effect under high atmospheric CO₂ due to higher net photosynthesis at reduced stomata openness. However, many studies have found

that growth reductions in response to either natural or experimental drought are not accompanied by carbohydrate storage reductions (Regier et al. 2009, Sala and Hoch 2009, Piper 2011, Klein et al. 2014, Piper and Fajardo 2016), which argues against C limitation. These contrasting evidences have led to an ongoing debate about the ultimate mechanism determining growth decline under drought (Wiley and Helliker 2012, Dietze et al. 2014, Palacio et al. 2014). It is well established that growth processes (i.e., cell elongation and differentiation) are more sensitive to drought than photosynthesis (Boyer 1970, Muller et al. 2011). Accordingly, it has been argued that reduced tissue formation at drought occurs independent from C availability (Palacio et al. 2014). This explanation predicts that, in the short term, drought induces a reduction in growth and a concomitant increase in carbohydrate storage (McDowell 2011, McDowell et al. 2011, Muller et al. 2011, Hoch 2015). Alternatively, it has been proposed that C limitation might occur along with C accumulation (Dietze et al. 2014), as a result of a precautionary strategy to supply C for use in the future (Wiley and Helliker 2012), or due to an increasing need of osmoregulation (Sala et al. 2012). Probably, both hypotheses are not exclusive and higher C availability occurs simultaneously with higher active allocation of photoassimilates to storage under drought (Dietze et al. 2014).

Most foundational evidence on C accumulation and growth decline in response to drought comes from experiments with tree seedlings (Galvez et al. 2011, O'Brien et al. 2014, Maguire and Kobe 2015, Zhang et al. 2015, Piper and Fajardo 2016). However, drought responses in terms of growth and C balance may be strongly variable along tree ontogeny (Cavender-Bares and Bazzaz 2000), casting some doubts on whether seedlings do reflect adult trees' trends. The few studies that have investigated the role of drought on growth–storage relations in adult trees under in situ conditions have led to mixed support for the C dynamic model under drought. For example, trees of *Pinus ponderosa* that were growing in sites with contrasting soil humidity exhibited similar non-structural carbohydrate (NSC) levels in spite of lower tree growth at the drier site (Sala and Hoch 2009). Studies carried out in Mediterranean regions have reported concomitant growth and carbohydrate storage reductions after severe summer drought (Galiano et al. 2011), or growth reductions along with increasing carbohydrate storage (Körner 2003). An inferential problem with these studies is that drought usually does not occur at the period of maximal growth demand (spring and early summer) but several weeks later (Körner 2003, Galiano et al. 2011), probably limiting the effect of drought on growth. Also, the examination of carbohydrate storage by the end of the growing season (e.g., Sala and Hoch 2009, Galiano et al. 2011) may be problematic when studying C limitation by drought, because at this time trees are phenologically set to refill their stores (Körner 2003, Klein et al. 2016). It seems then imperative that an adequate test of C limitation must

be done when growth demands are maximal. However, investigations of NSC tissue concentrations at the peak of the growing season have also led to mixed results. For example, Klein et al. (2014) found a clear negative relationship between radial growth and the level of drought stress of mature *Pinus halepensis* trees in a dry forest of Israel, with moderately drought-stressed trees having lower C reserve concentrations than healthy trees during spring and early summer. By contrast, one out of three species of angiosperms subjected to a long-term drought simulation experiment showed growth reduction and concomitant C accumulation (in only one tissue), while neither growth nor carbohydrate changes were observed in the other two species (Rosas et al. 2013).

Although comparative studies of tree C dynamic relationships under contrasting climates are a potentially useful approach to examine drought effects on tree physiology (e.g., Sala and Hoch 2009), local adaptation to drought by tree populations could undermine the inference of this approach. The issue is particularly critical because important intraspecific differences among populations can occur in some physiological traits related to their C balance, which obviously will lead to a diversity of plant responses to drought. For instance, three populations of *P. ponderosa* with contrasting drought tolerances differed in their growth sensitivity to drought, while their gas exchange sensitivity was not altered under the same experimental conditions (Zhang et al. 1997). Similarly, 11 *Fagus sylvatica* ecotypes differed in their drought sensitivity and accordingly their leaf C reserve concentrations changed differently in response to drought (Peuke et al. 2002). Under common garden conditions, Rehfeldt et al. (1999) found that water requirements for growth differed more than twofold across 101 populations of *Pinus contorta*. In this respect, some authors have proposed that increasing NSC concentrations along with decreasing growth rates could reflect an adaptive plant strategy that prioritizes C allocation to storage at the expense of growth (Wiley and Helliker 2012). Such a strategy could have evolved in species or ecotypes inhabiting dry regions (i.e., local adaptation) to face unpredictable periods of very low C gain or tissue damage (Wiley and Helliker 2012), or to meet high osmoregulation demands for the vascular functioning and integrity of the plant (Sala et al. 2012). This view implies that storage 'competes' with growth for C and hence that growth may still be C limited in trees showing carbohydrate reserves accumulation when exposed to drought. Sala et al. (2012) indicated that carbohydrate storage prioritization could be genetically determined in some species. The assessment of growth and carbohydrate storage in trees of a common seed source (i.e., common genotype), growing under contrasting precipitation regimes, could clarify if, and to what extent, the C dynamic of mature trees under natural drought is under environmental control.

In this study, we examined the patterns of growth and carbohydrate storage in single-provenance mature conifer trees growing under contrasting precipitation regimes. We investigated afforestations of *Pinus contorta* Douglas and *Pinus ponderosa*,

Douglas ex Lawson each deriving from a single provenance. We hypothesized that drought will evoke growth limitation and C accumulation in trees during periods of maximal growth demand, and that the concomitant C accumulation will be attributable to a phenotypic response of the trees' C balance to drought. Our study species are well known to show marked growth reductions during dry years in their native distribution ranges in North America (Adams et al. 2014), and thus they constitute an adequate model to study C allocation under drought. In particular, we expect that if a decrease of precipitation is having a direct effect on the C balance of trees, then there will be a variation in the C reserve concentrations between locations of contrasting precipitation, measured as NSC. More specifically, we expect higher NSC concentrations in the drier location.

Materials and methods

Study sites description

We selected two locations that differ in their annual and growing season precipitation, but that are otherwise climatically similar (Figure 1). The mesic study location was within the Coyhaique National Reserve (45° 59' S and 71° 52' W, 650 m above sea level (a.s.l.)), Coyhaique Province, Patagonia (Chile). Here, the mean annual and growing season (October–March) precipitation are 921 mm and 347 mm, respectively (Figure 1), and the mean annual and growing season temperatures are 8.0 °C and 11.2 °C, respectively (Coyhaique National Reserve weather station, Dirección General de Aguas, 2002–15, 400 m a.s.l.). The dry study location was in the area of Coyhaique Alto (45° 33' S,

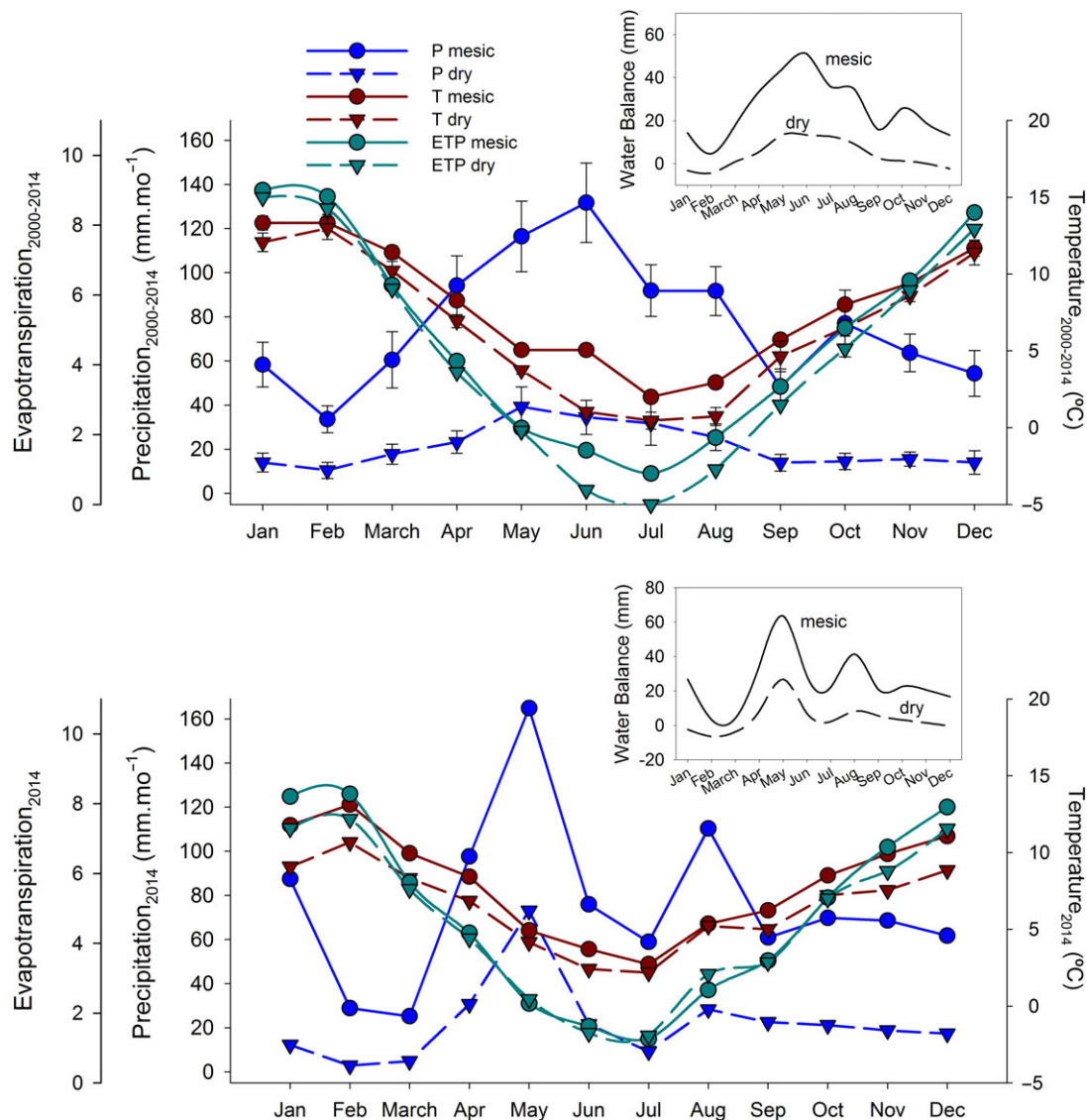


Figure 1. Mean monthly precipitation (P), temperature (T), evapotranspiration (ETP) and water balance (inset) for the period 2000–14 (upper panel) and for the year previous to sampling (2014) (lower panel) in the mesic (Coyhaique National Reserve) and the dry (Coyhaique Alto) study locations in Patagonia, Chile (data from Dirección General de Aguas, Servicio Meteorológico Nacional de Chile). Error bars indicate standard error. For the 2000–14 period, standard error was calculated from the different years.

72° 04' W, 730 m a.s.l.), 35 km east of the Coyhaique National Reserve. This area has been described as an eco-region of cold steppe formation (Luebert and Plischoff 2006). The mean annual precipitation (P) is 284 mm, the mean growing season precipitation (October–March) is 86 mm, the mean annual temperature is 6.6 °C and the mean temperature for the growing season is 10.3 °C (Dirección General de Aguas) (Figure 1). Soil texture, structure and profundity are similar in both locations (Hepp 2014), suggesting that soil water storage capacity is also similar. At the basin of Simpson River, which includes the two study sites, soil water storage capacity has been found to remain relatively constant throughout the year while runoff (Q) has been estimated as ~60% of precipitation (Vargas et al. 2012). According to small differences in monthly temperatures between locations, potential evapotranspiration (ETP) is in general similar (Thornthwaite 1948). Water balance, estimated as $WB = P - ETP - Q$, is overall higher in Coyhaique National Reserve than in Coyhaique Alto (Figure 1).

Afforestations of *P. contorta* and *P. ponderosa* in the Chilean Patagonia were initiated in the early 1970s (Löewe and Murillo 2001). In particular, the afforestations at the Reserva Coyhaique, our mesic location, were established in 1971 for *P. contorta* and 1982 for *P. ponderosa* (CONAF 2009), while in Coyhaique Alto, our dry location, both species were established in 1981 (Fajardo and McIntire 2007, Bravo-Monasterio et al. 2016). Initial bare-root seedlings used in all the afforestations considered in this study were produced in the *Las Lengas* nursery (Corporación Nacional Forestal, Coyhaique), located in the Coyhaique National Reserve, and seeds came from one provenance, the Pacific Northwest (USA), particularly the southeast side of the Cascade Mountains in Oregon, where, at comparable latitude, there is a similar climate and soils of volcanic origin (Gundale et al. 2016). According to the difference in precipitation, both locations also differ in mean basal area per stand and tree, diameter at breast height (DBH) and total height (Table 1).

Field sampling and initial sample processing

Sampling was conducted by mid-summer (ca 19–24 January 2015) for both locations, when needle expansion and shoot growth were still ongoing, but close to finish. At each location,

we selected three trees of each species at six sites (18 trees in total per species). Single-species stands of both species were adjacent to each other at both locations. The sites at each location were at least 80 m from each other, while within a single site each individual was separated from the next by a minimum of 10 m. We restricted our sampling to adult, un-shaded, dominant trees without browsing or other damage. In all stands, stem density had been reduced in the last 5 years by thinning, thus it was relatively easy to find dominant, un-shaded trees. At each site, we used a relaskop (Relaskop-Technik, Salzburg, Austria) to estimate the basal area per site. For each individual sampled tree, we measured the DBH (1.35 m) and the bark thickness using a diameter tape and a bark thickness gauge, respectively (Table 1). We also estimated the total height of each sampled tree using a clinometer (Suunto, Vantaa, Finland). We then identified and cut one terminal, ~1 m long, fully expanded sun-exposed branch using (when necessary) a 5.6 m telescoping pole (ARS Corporation, Sakai, Japan). From each of these branches, we collected sun-exposed needles of the two last cohorts (current and previous growing season) and 4-year-old branch segments (with bark and phloem removed in the field with a knife) for NSC determination. Likewise, we sampled a piece of a coarse root per tree. For this, we dug and identified the root of the target tree and selected and cut a root piece of ~1 cm diameter and removed the bark in the field with a knife. Finally, two to-the-pith long stem cores were taken at a height of ~30 cm for NSC analyses and tree growth determination. For this purpose, we used a 5.15 mm increment bore (Haglöf, Långsele, Sweden). Needles, branches, stem cores and roots were all bagged, labeled and stored in a cooler for transportation, while increment cores for tree growth determination were placed in labeled straws and kept in a map tube. Tissue collection was conducted between 10:00 and 16:00 h. In the laboratory, all of the woody tissues and needles were placed to dry in a forced-air stove (Memmert GmbH, Schwabach, Germany) at 70 °C for 72 h, and then were ground into a fine powder and stored over silica gel at 4 °C until chemical analyses were conducted.

In parallel to tissue collection, we measured the needle and terminal shoots length to assess primary growth in addition to radial stem growth. For this, we measured the length of five

Table 1. Mean basal area per hectare (BA_{stand}) and per tree (BA_{tree}), diameter at breast height (1.35 m, DBH), and tree height with standard errors in parenthesis ($\pm SE$) of stands of *Pinus contorta* and *Pinus ponderosa* at two locations with contrasting precipitation in Patagonia, Chile. *N* stands for the number of trees considered for BA_{tree} , DBH and height (sample sizes).

	BA_{stand} ($m^2 ha^{-1}$)	BA_{tree} (cm^2)	DBH (cm)	Height (m)	<i>N</i>
Mesic location					
<i>P. contorta</i>	30.67 (2.47)	1021.25 (51.66)	31.27 (0.87)	14.65 (0.27)	18
<i>P. ponderosa</i>	33.88 (1.42)	1124.69 (65.13)	35.53 (0.69)	10.53 (0.24)	18
Dry location					
<i>P. contorta</i>	24.00 (1.69)	500.96 (27.19)	24.25 (0.72)	8.67 (0.28)	18
<i>P. ponderosa</i>	29.33 (1.12)	635.91 (51.75)	27.33 (0.28)	7.30 (0.15)	19

needles randomly selected from the two most recent leaf cohorts (displayed in 2013 and 2014 springs) and calculated an average needle length per cohort. Similarly, we measured the length of the last four annual shoot extensions, which were easily recognizable in both species by the presence of annual scars. As in the southern hemisphere the growing season spans two calendar years (ca September–December of 1 year and January–March of the following year), a needle cohort or a given shoot starts to extend in the spring of one year and stops growing in the autumn of the following year. Therefore, much alike with dendrochronology standards, we will refer to each growing season by the calendar year of spring (i.e., when the growing season starts). Thus, our denomination of 2013 includes 2013 spring growth and 2014 summer growth.

Tree growth determination

Cores were prepared following standard dendrochronological techniques (Stokes and Smiley 1996). Cores were dried, mounted, glued firmly on grooved wooden sticks and sanded with successively finer grades of sandpaper until optimal surface resolution allowed the annual rings to be distinguished under magnification (10-fold). Inside-bark bole radius and annual radial increments from the last 10 years were measured to the nearest 0.01 mm using a microscope mounted on a dendrochronometer with a Velmex sliding stage and Accurite measuring system (Bloomfield, NY, USA). Cross-dating accuracy was checked visually since rings in these planted conifers were easily identified. We estimated basal area increment (BAI_{10}) for the last 10 years and basal area increment of the last year (BAI_{01}), which represent relative measures of gains in growth, as

$$BAI_{10} = \pi(R_t^2 - R_{t-10}^2),$$

where R_t is the radius of the stem, and R_{t-10} (or R_{t-1}) is the radius of the stem without the 10 (or 1) outermost years. The radius of the stem, R_t , was computed by considering the diameter at coring height, bark depth and the distance between pith and the last year's ring in the core. We used BAI_{01} to match NSC results of the last year. One stand (*P. contorta* in the mesic location) was 10 years older than the rest, which may confound results, assuming that older, bigger trees would grow more than younger, smaller trees. Considering this ontogenetic variation, we computed a mean annual increment (MAI) metric, as the quotient between individual tree basal area and the tree age.

Chemical analyses

Our assessment of C reserves was based on the determination of NSC concentrations, as the sum of the three most abundant low-molecular weight soluble sugars (glucose, fructose and sucrose) and starch. The NSC concentrations were analyzed following the procedure of Hoch et al. (2002). About 13 mg of

dried powder were extracted with 1.6 ml of distilled water at 100 °C for 60 min. For cores, we only used the last 10 years (i.e., outermost and youngest rings). An aliquot of the extract was used to determine low-molecular carbohydrates after enzymatic conversion (invertase and phosphoglucose isomerase from *Saccharomyces cerevisiae*, Sigma Aldrich I4504 and P5381, respectively, St Louis, MO, USA) of sucrose and fructose to glucose. The concentration of free glucose was determined photometrically after the enzymatic conversion of glucose to gluconate-6-phosphate (Glucose Assay Reagent, G3293 Sigma Aldrich) on a 96-well multi-plate reader. Following the degradation of starch to glucose using a purified fungal amylase ('amiloglucosydase' from *Aspegillus niger*, Sigma Aldrich 10115) at 45 °C overnight, NSC was determined in a separate analysis. The starch concentration was calculated as NSC minus the sum of free sugars. Total soluble sugar, starch and NSC concentrations are presented on a percent of dry matter basis.

Data analyses

The influence of location (as a surrogate of climate), species, and the interaction of them on the BAI_{10} , BAI_{01} , MAI, needle length for the two last cohorts, shoot lengths of the four last cohorts and NSC concentrations for each tissue were analyzed fitting linear mixed-effects models, using the *nlme* package (Pinheiro et al. 2009) in R (R Development Core Team 2016). In the modeling, we considered location (mesic and dry) and species (*P. contorta* and *P. ponderosa*) as fixed factors, with random effects for the sites, to take into account the among-site variation. We tested the fixed effects of location, species and their interaction using likelihood ratio tests (χ^2) in nested models fitted using maximum likelihood (Bolker et al. 2009). In cases where location and species proved to have a significant effect on a variable, we conducted post hoc multiple comparisons (Tukey's procedure) among all combinations of location-species using the 'multcomp' package (Hothorn et al. 2008). Since growth depends also on tree size, models for needle length, shoot length and BAI were also run using DBH as a covariable. This factor resulted not significant for all the response variables, likely reflecting the typical size homogeneity of an even-aged plantation. Thereafter, we excluded DBH from the models.

Results

Growth

Shoot length of the last 4 years before sampling (2011–14) was similar between the two locations and always significantly higher in *P. ponderosa* than in *P. contorta* (Figure 2). However, for the 4 years examined, species-specific growth patterns differed between the two locations (significant effect of location × species' interaction, Figure 2). *Pinus contorta* exhibited significantly lower shoot length in the mesic than in the dry location for every

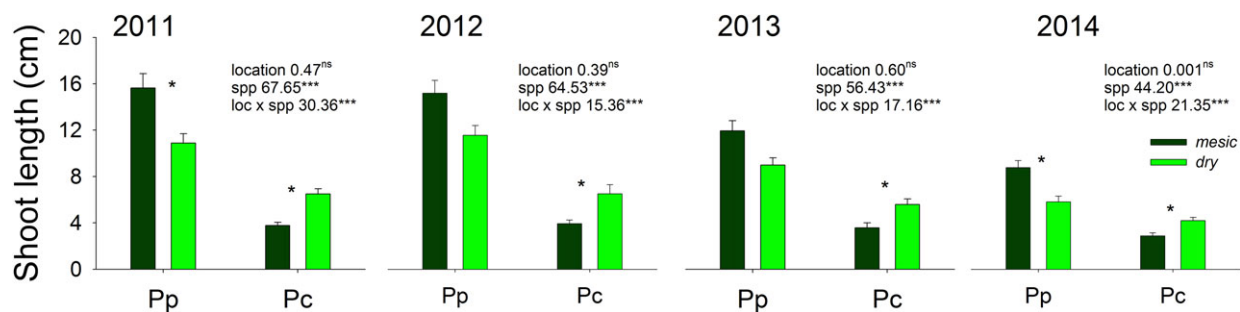


Figure 2. Mean shoot length in 2011, 2012, 2013 and 2014 of *Pinus ponderosa* (Pp) and *Pinus contorta* (Pc) trees from single seed source afforestations in a mesic and a dry location in the Aysén Region, Patagonia, Chile. For 2011, 2012 and 2013, these lengths represent the growth of the whole growing season (ca September of a given year to March of the following year). For 2014, the shoot length reflects the growth from the beginning of the growing season until sampling (i.e., January 2015). Error bars represent standard errors. Asterisks between bars indicate significant differences ($P < 0.05$) between locations for a given species. Insets show statistical results (χ^2 values of likelihood ratio tests) for the effects of location, species and their interaction; ns refers to non-significant effect and *** indicates a significant effect at $P < 0.001$.

year. By contrast, *P. ponderosa* grew less in the dry than in the mesic location in 2011 and 2014, and similarly between the two locations in 2012 and 2013 (Figure 2). In 2013, both species had longer needles in the mesic than in the dry location, although the difference between locations was higher in *P. ponderosa*. Although in 2014, the location had no effect on needle length across both species, *P. ponderosa* appeared to have shorter needles at the dry location (Figure 3). Needle length was significantly lower in *P. contorta* than in *P. ponderosa* in both investigated years (Figure 3). The mean basal area increment of the last 10 years (BAI_{10}), the last year (BAI_{01}) and the MAI was always significantly higher in the mesic than in the dry location for both species and overall significantly higher in *P. ponderosa* than in *P. contorta* (Figure 4). However, the reduction in growth from the mesic to the dry location was greater in *P. ponderosa* than in *P. contorta* (Figure 4).

Non-structural carbohydrate

In general, *P. ponderosa* and *P. contorta* tended to have higher NSC concentrations in woody tissues in the dry than in the mesic location (Figure 5). This effect, however, was only significant for roots (in both species) and stems (in *P. contorta* only) (Figure 5). By contrast, NSC concentrations in needles were significantly higher in the mesic than in the dry location. This pattern was mainly driven by *P. contorta*. Independent of the location, *P. ponderosa* had higher NSC concentrations than *P. contorta* in branch wood and stems, but they were similar between species in roots and needles. In none of the investigated tissues, the interaction term between species and location was statistically significant (Figure 5).

The location had a significant effect on low-molecular weight sugar concentrations of all tissues (Table 2). Woody tissue sugar concentrations were higher in the dry than in the mesic location, whereas needles' concentrations were higher in the mesic than in the dry location. By contrast, only root starch concentrations differed between locations, driven by higher concentrations of *P. ponderosa* in the dry than in the mesic location (Table 2). The

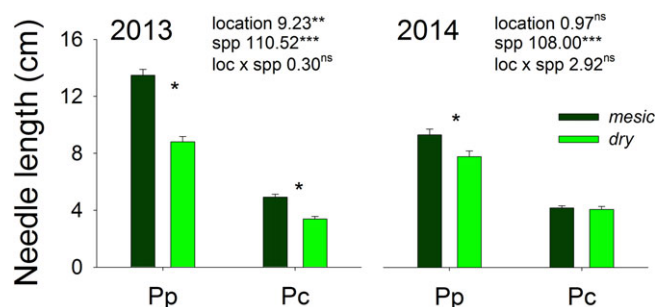


Figure 3. Mean needle length of 2013 and 2014s cohorts in *Pinus ponderosa* (Pp) and *Pinus contorta* (Pc) trees from single seed source afforestations in a mesic and a dry location in the Aysén Region, Patagonia, Chile. For 2013, the length represents the whole growing season (ca September of a given year to March of the following year). For 2014, the length reflects the growth from the beginning of the growing season until sampling (i.e., January 2015). Error bars represent standard error. Asterisks between bars indicate significant differences ($P < 0.05$) between locations for a given species. Insets show statistical results (χ^2 values of likelihood ratio tests) for the effects of location, species and their interaction; ns refers to non-significant effect and asterisks indicate a significant effect (** $P < 0.01$ and *** $P < 0.001$).

effect of location on sugar and starch concentrations of the different tissues was similar in both species (i.e., non-significant effects of location \times spp interactions, Table 2). Sugar concentrations of branches, needles and stems were higher in *P. ponderosa* than in *P. contorta*; for roots they were similar between species (Table 2). Similarly, starch concentrations of branches, stems and roots were higher in *P. ponderosa* than in *P. contorta*, while needle starch concentrations were similar between species (Table 2).

Discussion

We found that in the two investigated conifer species tree growth decreased in most of the variables examined and NSC concentrations remained similar or increased (depending on the tissue under consideration) with a reduction in precipitation.

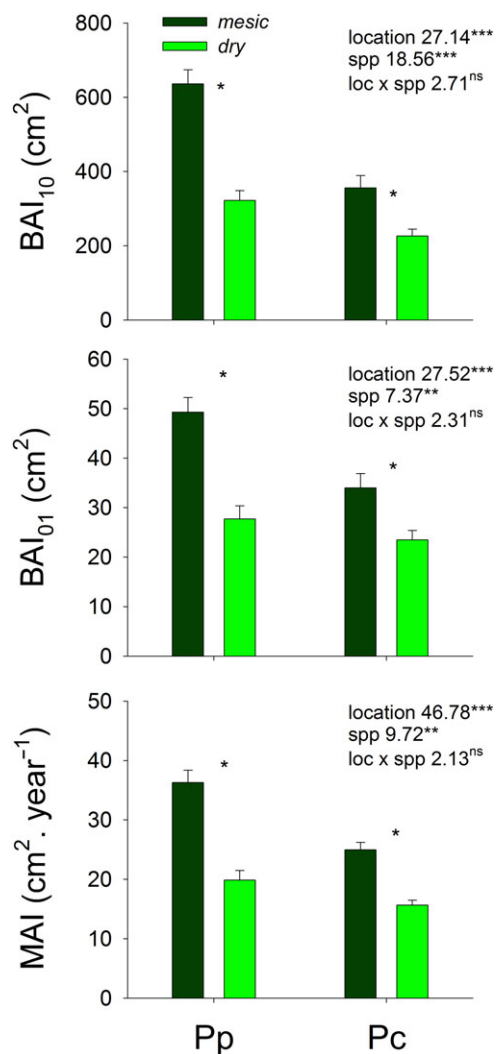


Figure 4. Mean growth rate as basal area increment for the last 10 years (BAI_{10}), basal area increment of the last year (BAI_{01}), and MAI of *P. ponderosa* (Pp) and *P. contorta* (Pc) planted at two locations with contrasting precipitation (mesic and dry) in the Aysén Region, Patagonia, Chile. Error bars represent standard errors. Asterisks between bars indicate significant differences ($P < 0.05$) between location for a given species. Insets show statistical results (χ^2 values of likelihood ratio tests) for the effects of location, species and their interaction; ns refers to non-significant effect and ** and *** indicate a significant effect $P < 0.01$ and $P < 0.001$, respectively.

Given that trees of the two locations (mesic and dry) belong to the same provenance, i.e., they have a common genetic origin, the increase in NSC concentration observed in response to drought is clearly a phenotypic response (i.e., environmentally mediated). Our results also show that disparate growth parameters, like the BAI and the needle length, were significantly reduced in the dry location independently of species. Noteworthy, a consistent root C accumulation was observed in the dry location for both species. Roots constitute a typical storage tissue in many tree species (Fajardo et al. 2011, Martínez-Vilalta et al. 2016). Different C allocation patterns in species or populations subjected to contrasting water regimes have been widely demonstrated in seedlings

under controlled watering conditions. For mature trees in natural conditions, evidence is scarcer and often local adaptation cannot be distinguished from short-term responses to drought (e.g., Sala et al. 2012). Particularly for *P. ponderosa* and *P. contorta*, our studied species, genetic differences in growth responses to precipitation have been reported among populations from different climates (Zhang et al. 1997, Rehfeldt et al. 1999). By contrast, our results of growth reductions and C accumulation under drier conditions were not influenced by local adaptation to a specific precipitation regime. By working with same-provenance afforestations, we can confidently assert that drought exerts direct effects (i.e., environmentally driven phenotypes) on the C balance and growth dynamics of mature trees, as has been previously hypothesized (McDowell 2011, Muller et al. 2011, Hoch 2015). Therefore, even when drought-adapted populations of a given species could show higher C allocation to storage regarding drought-sensitive populations (Sala et al. 2012, Wiley and Helliker 2012, Dietze et al. 2014), our study indicates the existence of a direct environmental effect. Precipitation is the most conspicuous environmental difference between the two studied locations, and hence the most plausible environmental factor explaining our results.

The environmental effect that we detected on growth and carbohydrate concentrations is consistent with the hypothesis that growth is more sensitive to water deficit than photosynthesis, and that growth reduction provokes a C reserve accumulation (Boyer 1970, Muller et al. 2011, Hoch 2015). Although some growth could have occurred during late-summer, spring is the period of maximal growth in Patagonia (Rusch 1993, Veblen et al. 1996). Thus, our results suggest that carbohydrate accumulation and reduced growth simultaneously co-occurred in the dry location. On the other hand, our results could also be explained by a preferential carbohydrate accumulation occurring in 'competition' with growth, either by a direct up-regulation of carbohydrate storage (Sala et al. 2012, Wiley and Helliker 2012), or indirectly, by down-regulation of growth (Dietze et al. 2014). Such 'preferential' carbohydrate accumulation has been hypothesized to occur to prevent C starvation in the long term or to warrant the osmotic function (Sala et al. 2012, Wiley and Helliker 2012, Dietze et al. 2014). The single time point for the carbohydrate reserve assessment in the current study is probably insufficient to be conclusive in this regard (Hoch 2015). Non-structural carbohydrate concentrations are highly dynamic in time (Rosas et al. 2013, Martínez-Vilalta et al. 2016), and thus our results should be interpreted with caution in this respect. It remains also possible that drought effects on growth-carbohydrate relations vary seasonally and annually. Nonetheless, the contrasting NSC composition that we found between locations may be informative on the drivers behind C allocation. Starch, an osmotically inert compound with no other known function than storage (Chapin et al. 1990, Hoch et al. 2003), can be particularly expected to accumulate when growth is impeded (i.e., insufficient cell turgor

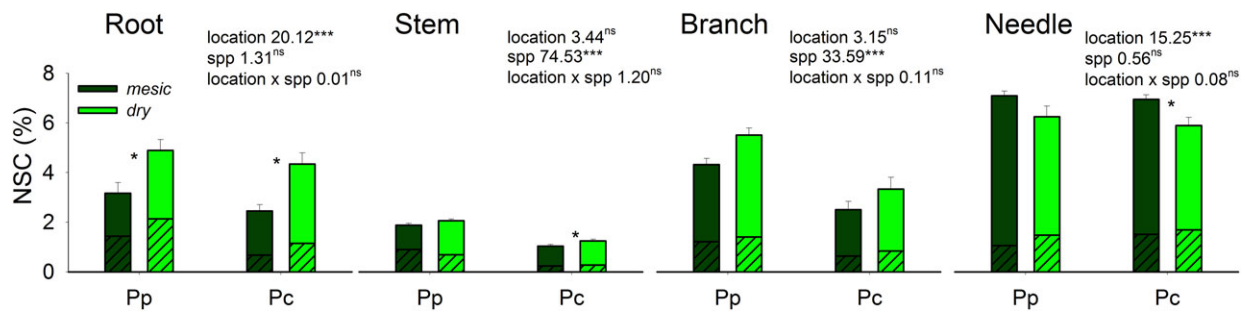


Figure 5. Non-structural carbohydrate concentrations in different organs of *Pinus ponderosa* (Pp) and *Pinus contorta* (Pc) trees from single seed source afforestations in a mesic and a dry location in the Aysén Region, Patagonia, Chile. Asterisks between bars indicate significant differences between species in a given location. Insets show statistical results (χ^2 values of likelihood ratio tests) for the effects of location, species and their interaction; ns refers to non-significant effect and *** indicates a significant effect at $P < 0.001$. Hatched and non-hatched areas of the bars represent starch and low-molecular weight sugars, respectively.

Table 2. Effects of location, species and the interaction of both (χ^2 (P -value)), and mean \pm SE values of sugar and starch concentrations (as % of d.wt.) in different organs of *Pinus ponderosa* (Pp) and *Pinus contorta* (Pc) trees from single seed source afforestations in a mesic and a dry location in the Aysén Region, Patagonia, Chile. Different letters stand for significantly different ($P < 0.05$) mean values among treatments.

	Branches	Roots	Stems	Needles
Sugars (%)				
Location	6.02 (0.014)	28.94 (<0.001)	21.81 (<0.001)	23.22 (<0.001)
Species	32.50 (<0.001)	0.10 (0.75)	20.14 (<0.001)	5.74 (0.016)
Spp \times location	0.05 (0.81)	0.50 (0.48)	2.13 (0.14)	0.65 (0.42)
Pp mesic	3.10 \pm 0.13a	1.73 \pm 0.24a	0.98 \pm 0.05a	6.04 \pm 0.14a
Pc mesic	1.96 \pm 0.30b	1.47 \pm 0.10a	0.80 \pm 0.04b	5.44 \pm 0.14ab
Pp dry	4.10 \pm 0.15a	2.75 \pm 0.27b	1.37 \pm 0.04c	4.77 \pm 0.12bc
Pc dry	2.50 \pm 0.32b	3.20 \pm 0.33b	0.97 \pm 0.03a	4.21 \pm 0.34c
Starch (%)				
Location	0.43 (0.51)	6.39 (0.011)	0.04 (0.83)	0.20 (0.65)
Species	14.25 (<0.001)	7.35 (0.007)	36.54 (<0.001)	0.71 (0.40)
Spp \times location	1.02 (0.31)	0.94 (0.33)	1.31 (0.25)	0.70 (0.40)
Pp mesic	1.22 \pm 0.14a	1.44 \pm 0.25a	0.90 \pm 0.05a	1.05 \pm 0.15a
Pc mesic	0.69 \pm 0.12b	0.78 \pm 0.13a	0.24 \pm 0.06b	1.51 \pm 0.14a
Pp dry	1.41 \pm 0.20a	2.14 \pm 0.21b	0.69 \pm 0.06a	1.48 \pm 0.36a
Pc dry	0.86 \pm 0.20b	1.15 \pm 0.19a	0.29 \pm 0.05b	1.74 \pm 0.27a

for expansion and differentiation). Likewise, starch can be expected to be the main storage compound if carbohydrate accumulation is driven by a precautionary strategy that involves changing allocation from growth to storage under stressful conditions to prevent chronic C starvation in the long term (Wiley and Helliker 2012, Dietze et al. 2014). In these cases, carbohydrate accumulation should involve main storage tissues, which in conifers include needles (Chapin et al. 1990, Hoch et al. 2003, Martínez-Vilalta et al. 2016). Contrary to these expectations, we found that, except for the roots of *P. ponderosa*, starch concentrations were never significantly higher in the dry than in the mesic location. It is also remarkable that needles were the only tissue where NSC concentrations were lowest in the dry site. These results, along with the overall minor starch proportion as a component of NSC, appear to argue against the explanation of a purely 'passive' carbohydrate reserve accumulation due to drought-limited C sink activity (Muller et al. 2011, Dietze et al. 2014), but

also against the explanation of 'purely active' carbohydrate reserve formation as a precautionary strategy (Wiley and Helliker 2012, 'quasi-active' sensu Dietze et al. 2014).

It has been proposed that storage can be up-regulated under drought to warrant the osmotic function, and hence to maintain turgor and long-distance vascular integrity in xylem and phloem (Sala et al. 2012, Dietze et al. 2014). This hypothesis implies a 'competition' for carbohydrates between storage and growth and therefore C limitation compatible with carbohydrate accumulation. In line with this hypothesis, we found that low-molecular weight sugars were significantly accumulated in the dry location in both species and all woody tissues (Table 2), while leaf sugar concentrations were lower in the dry than in the mesic location. Probably needles continued exporting photosynthates that eventually could not be invested in new growth. Ultimately, the osmotic necessities seemed to be more important in heterotrophic tissues (e.g., wood), consistent with the importance of

the maintenance of the vascular integrity in dry climates (Sala et al. 2012, Brodersen and McElrone 2013). Nevertheless, it is important to note that we did not find a significant decrease of starch between the mesic and the dry locations, as can be expected from an imperative osmotic demand that comes at the cost of reduced growth. In fact, in most woody tissues, starch concentrations tended to be higher at the dry than at the mesic site (Table 2), with a single significant difference in roots of *P. ponderosa*. As a result, the overall net C balance was more positive at the dry compared with the mesic location, consistent with a stronger drought-induced growth reduction than the reduction of C uptake on the dry site. We therefore hypothesize that carbohydrate accumulation and osmotic adjustment co-occurred in our study, in line with the idea that 'passive' and 'active' C reserve pool changes can be present simultaneously (Dietze et al. 2014).

Conclusions

The effects of drought on C allocation in plants have been documented by many studies thus far, mostly in experimental conditions in early stages of tree development. Under natural conditions, however, it remained uncertain whether local adaptation could influence growth and C balance patterns when populations or species from different climates were compared. By examining mature trees with a common provenance that were planted in locations of contrasting climates, we found growth reductions and carbohydrate accumulation under dry conditions. Given the common origin of these trees, it is highly unlikely that the trends we found are explained exclusively by genetic variation (i.e., local adaptation). Rather, our results appear attributable to a physiological response of trees to lower precipitation. In the same line, Fajardo et al. (2012) found that low temperature had similar effects on the C balance of trees.

Prevailing hypotheses about why plants reduce growth and increase C accumulation in response to drought consider that storage is the main destination of C accumulation (Wiley and Helliker 2012, Palacio et al. 2014). These views conceive that C accumulation will have an important use in the future under climate change scenarios. An alternative hypothesis is that carbohydrates are required 'as such' in higher concentrations under drought or low temperature in osmoregulation, mainly as low-molecular weight sugars (Sala et al. 2012, Brodersen and McElrone 2013). In this case, C accumulation is having an immediate important function. In strong support of this hypothesis, a recent large-scale comparison showed that plants worldwide maintain their levels of soluble sugars at high minimum values during the growing season, while starch can be almost completely depleted (Martínez-Vilalta et al. 2016). Our study shows that at the peak of growth demand, drier conditions led to sugar accumulation in trees. Thus, we add a new piece of evidence for the primordial role of sugars in the tree C balance

under drought conditions. However, since sugar accumulation and growth reduction at the growing season peak did not occur along with starch reductions, our study does not support the hypothesis that osmoregulation requirements may cause limiting C supply for growth (Sala et al. 2012, Wiley and Helliker 2012).

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Conflict of interest

None declared.

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