Title: Insight into Canary Island pine physiology provided by stable isotope patterns of water and plant tissues along an altitudinal gradient

José Carlos Miranda^{1,2}, Marco M. Lehmann¹, Matthias Saurer¹, Jan Altman^{1,3}, Kerstin Treydte¹.

¹Swiss Federal Institute for Forest, Snow and Landscape Research WSL, 8903 Birmensdorf, Switzerland.

²Universidad Politécnica de Madrid, 28034 Madrid, Spain.

³Institute of Botany of the Czech Academy of Science, 25243 Průhonice, Czech Republic

Author of correspondence:

Jose Carlos Miranda

Tel: +34 687770851

Email: jc.miranda@upm.es

Abstract

The Canary Islands, an archipelago east of Morocco's Atlantic coast, present steep altitudinal gradients covering various climatic zones from hot deserts to subalpine Mediterranean, passing through fog-influenced cloud forests. Unlike the majority of the Canarian flora, *Pinus canariensis* grow along most of these gradients, allowing the study of plant functioning in contrasting ecosystems. Here we assess the water sources (precipitation, fog) of *P. canariensis* and its physiological behavior in its different natural environments. We analyzed carbon and oxygen isotope ratios of water and organics from atmosphere, soil and different plant organs and tissues (including 10-year annual time series of tree-ring cellulose) of six sites from 480 to 1990 m asl on the Canary island La Palma. We found a decreasing $\delta^{18}\text{O}$ trend in source water that was overridden by an increasing $\delta^{18}\text{O}$ trend in needle water, leaf assimilates and tree-ring cellulose with increasing altitude, suggesting site-specific tree physiological responses to relative humidity. Fog-influenced and fog-free sites showed similar $\delta^{13}\text{C}$ values, suggesting photosynthetic activity to be limited by stomatal closure and irradiance at certain periods. Besides, we observed an ^{18}O -depletion (fog-free and timberline sites) and ^{13}C -depletion (fog-influenced and fog-free sites) in latewood compared to earlywood caused by seasonal differences in: (i) water uptake (i.e. deeper ground water during summer drought, fog water frequency and interception) and (ii) meteorological conditions (stem radial growth and latewood $\delta^{18}\text{O}$ correlated with winter precipitation). In addition, we found evidence for foliar water uptake and strong isotopic gradients along the pine needle axis in water and assimilates. These gradients are likely the reason for an unexpected underestimation of pine needle water $\delta^{18}\text{O}$ when applying standard leaf water $\delta^{18}\text{O}$ models. Our results indicate that soil water availability and air humidity

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conditions are the main drivers of the physiological behavior of pine along the Canary
Island's altitudinal gradients.

Keywords

Canary Islands, cloud forest, drought, *Pinus canariensis*, relative humidity,
stable isotopes, tree rings, vapor pressure deficit.

For Peer Review

51 Introduction

52 The Canary Archipelago is a natural laboratory for the study of plant physiology
53 and plant responses to climate, due to its strong climate variability (dry, temperate,
54 continental and polar climates; AEMET-IM 2012) across relatively small space
55 (averaging 1000 km² per island). This is caused by the mixed effect of the wide
56 altitudinal range (0 to 3718 m asl) and the influence of the humid trade winds, and
57 triggers an altitudinal-belt structured flora (Aguilar et al. 2010). Northeastern trade
58 winds influence the windward slopes by increasing air humidity, causing orographic
59 precipitation and leading to fog formation (more frequent in summer) at mid altitudes
60 (~1250 m asl), while leeward slopes face drier conditions (Máyer and Marzol 2013).
61 Fog increases vegetation water availability by means of fog interception that in optimal
62 conditions can reach three times the amount of precipitation water (Aboal et al. 2013;
63 Marzol and Máyer 2012). Interestingly, the main landscape-forming tree species Canary
64 Island pine (*Pinus canariensis* C. Sm. ex DC. in Buch) with an European
65 paleodistribution (Miranda 2017), spreads over several altitudinal climate-driven flora
66 belts, growing from 200 to 2200 m asl on both windward and leeward slopes, with
67 precipitation ranging from 300 to 3000 mm/year and annual average temperatures from
68 10 to 20 °C (Climent et al. 1996; Pérez-de-Paz et al. 1994). Thus, Canary Island pine is
69 an ideal candidate to study tree physiological responses to climate variability and
70 species-specific strategies to survive under a wide climatic range. Previous studies on
71 this species revealed species-specific strategies to cope with drought (López et al.
72 2013), frost (Luis et al. 2007), volcanic eruptions (López de Heredia et al. 2014;
73 Miranda et al. 2020; Rodríguez Martín et al. 2013) and fires (Rozas et al. 2013),
74 demonstrating its plasticity. Those studies were, however, restricted to short time
75 periods or single locations, and therefore have not assessed at once the full ecological

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3 76 spectrum of the species along altitudinal gradients so far, leaving open how the
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5 77 physiology of the species adapts to such a wide range of climatic conditions.
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8 78 Carbon ($\delta^{13}\text{C}$) and oxygen ($\delta^{18}\text{O}$) stable isotope ratios in plant tissue, like leaves
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10 79 and tree rings, can be used as a proxy to assess both plant physiology and plant water
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12 80 relations (Farquhar et al. 2007; Gessler et al. 2014; McCarroll and Loader 2004). $\delta^{18}\text{O}$
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14 81 of plant tissue depends on meteorological, physical, physiological and morphological
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16 82 processes and variables at leaf level (Brinkmann et al. 2018; Cernusak et al. 2016;
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18 83 Farquhar et al. 2007; Tang and Feng 2001). In leaves, water inputs from diverse
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20 84 sources, which can have different isotope composition, occur under dew, fog and
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22 85 rainfall conditions (Burgess and Dawson 2004; Kim and Lee 2011). The isotopic
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24 86 signature of the leaf water is transferred on leaf assimilates (Lehmann et al. 2017). In
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26 87 the phloem, newly produced sugars mix with older carbohydrate reserves dampening
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28 88 the isotopic signature of the sugar pool (McCarroll and Loader 2004; Sternberg and
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30 89 Deniro 1983). Finally, during tree-ring cellulose biosynthesis oxygen atom exchange
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32 90 occurs between phloem sugars and ^{18}O -depleted xylem water (Sternberg et al. 2006).
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35 91 Conversely, $\delta^{13}\text{C}$ in tree samples is determined by the intracellular CO_2
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37 92 concentration during leaf carbohydrate synthesis, which is under strong control of
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39 93 stomatal conductance and photosynthetic rate and therefore reflecting intrinsic water use
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41 94 efficiency (Gessler et al. 2014; McCarroll and Loader 2004), with both processes being
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43 95 regulated by environmental and species-specific conditions. After photosynthesis,
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45 96 further isotope fractionations can occur at the leaf and stem levels together with mixing
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47 97 of sugar pools with different assimilation times or storage (Gessler and Treydte 2016),
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49 98 causing generally a basipetal ^{13}C enrichment in phloem sugars (Boegelein et al. 2019;
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51 99 Brueggemann et al. 2011; Offermann et al. 2011). Although the mentioned isotope
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53 100 fractionations and mixing processes occur during the vertical transport of sugars, tree-
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ring cellulose $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ values can be strongly correlated with the isotopic signature of leaf sugars and reflect physiological processes at the leaf level, specifically in evergreen species (Gessler et al. 2014).

Thus, $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ values of plant compounds can be a useful tool to assess plant responses to altitude-driven environmental variability. The carbon isotopic composition in plants is known to become generally ^{13}C -enriched with increasing altitude due to decreasing temperatures, and CO_2 and O_2 atmospheric partial pressures (Körner et al. 1991), but this could be modified by concurrent changes in humidity. Variations of $\delta^{18}\text{O}$ values of tree tissues in relation to altitude are not so well studied. Plant compounds are thought to be ^{18}O -depleted with increasing altitude following the isotopic patterns of meteoric water (Barbour 2007; Burk and Stuiver 1981; Naoe et al. 2016; Terwilliger et al. 2002). However, under strong influence on leaf water ^{18}O enrichment, opposite trends have also been reported (Kahmen et al. 2011). Both tree-ring $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ also respond to seasonal environmental or source water variations (Gessler et al. 2014; Treydte et al. 2014) and hence, to seasonal physiological variations.

Besides, stands influenced by recurrent seasonal fog events can present inter and intra-annual variation in $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ due to isotopically differentiated source water inputs (Prada et al. 2015, Johnstone et al. 2013) and due to changes in temperature, relative humidity and irradiation affecting isotope fractionation processes (Ritter et al. 2019). Trees can intercept fog water, this is, small fog droplets impact on their leaves, branches and stems, and merge into bigger drops (Ritter et al. 2008). Needle-like leaves are better fog droplet interceptors than other leaf morphologies or bigger vegetative surfaces (Shuttleworth 1977), and the capacity of Canary Island pine to intercept large amounts of fog water (2 times the precipitation water input) has been already assessed

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126 (Aboal et al. 2000; Aboal et al. 2013). The intercepted fog drops increase tree water
127 availability by either flowing down and dripping to the ground where roots can take it
128 up, or by directly transferring into the leaves as observed for several cloud forest species
129 (Goldsmith et al. 2013).

130 Here we aim at understanding Canary Island pine physiology and its relation to
131 hydroclimatic conditions as a function of altitude. For this goal, we investigate the
132 influence of altitude-driven climate and its seasonal variations on $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ of
133 twigs, needles, and early- and latewood cellulose of pine trees and on the isotopic
134 signature of the source water on the island of La Palma. Sampling was performed at five
135 sites along an altitudinal gradient on the windward slope (fog influence at mid-altitudes)
136 and at one site on the leeward slope (fog-free). We hypothesize (i) a site-specific
137 strategy to avoid water losses (higher water use efficiency at drier sites triggered by
138 lower transpiration rates), allowing the species to grow in a wide range of relative
139 humidity conditions. This would imply higher $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ values in plant tissues with
140 increasing altitude according to Scheidegger's et al (2000) conceptual model. We also
141 hypothesize (ii) seasonal differences in average stomatal conductance at locations where
142 summer drought is not alleviated by fog influence; and in photosynthetic rates at
143 locations where frequent fog events occur seasonally. This would mean differences in
144 both $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ in earlywood (formed in spring and early summer) compared to
145 latewood (formed in summer and autumn). Finally, we hypothesize (iii) an influence of
146 fog intercepted water/foliar water uptake on tree rings from the mid-altitude site and,
147 thus, the capability of this species' needles to take up water. This would mean an ^{18}O
148 enrichment, as fog water was reported to be enriched compared to precipitation water
149 (Prada et al. 2015).

Materials and Methods

Site description

The study was performed on La Palma (Canary Islands) on Canary Island pine (*Pinus canariensis* C. Sm. ex DC. in Buch) along an altitudinal gradient ranging from 480 to 1990 m asl. Climate of the Canary Islands is strongly modulated by altitude and exposure (windward and leeward slopes). Under stable atmospheric conditions, northeastern humid trade winds affect mostly mid-altitude locations typically between 1200-1300 m asl, exceptionally extending to 800-1800 m asl (Dorta 1985; Marzol 2008; Torres et al. 2001; Valladares 1995). Above the trade wind influence, a subsidence temperature inversion avoids water vapor to rise up, causing its condensation and resulting in fog conditions - enabling fog water interception by vegetation and even foliar water uptake - and orographic precipitation at mid altitudes (Herrera et al. 2001). Thus, precipitation (mainly occurring during the wet season, from October to March) increases from coastal to mid altitudes and decreases above the trade wind zone (Fig. 1), where it is completely absent from June to August (Máyer and Marzol 2013). Temperatures usually decrease with increasing elevation (Fig. 1), except during subsidence temperature inversion periods (Bechtel 2016).

Five sites were selected along an altitudinal transect on an NNE slope at the following altitudes (Fig. 1): 480 m (28° 49' 25.16" N, 17° 50' 37.12" W), 970 m (28° 48' 31.16" N, 17° 50' 13.86" W), 1250 m (28° 47' 50.80" N, 17° 50' 47.42" W), 1800 m (28° 46' 48.71" N, 17° 50' 48.98" W) and 1990 m (28° 46' 19.39" N, 17° 51' 18.07" W). Trade wind and hence, fog influence, is strongest at the mid-altitude site (fog influenced, 1250 m), rare at the 1800 and 970 m sites, and absent at the timberline (1990 m) and the lowest, 480 m site. A sixth site was assessed on the WNW slope (leeward) at 1550 m (28° 45' 21.94" N, 17° 55' 57.26" W) that is not influenced by the

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175 trade winds, holding as a fog-free control in the altitudinal range where fog commonly
176 occurs on the windward slope.

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178 *Sampling of plant tissues, soil and water*

179 Samples from different pine organs and tissues, as well as soil and atmospheric
180 water vapor samples, were taken to perform $\delta^{18}\text{O}$, $\delta^{13}\text{C}$ and dendrochronological
181 analyses at two sampling days between 10 am and 3 pm (Eidg. Forschungsanstalt WSL
182 2018). During the first sampling day on the 7th of March 2018, soil, stem, twig and
183 needle material were sampled from five trees at each of the five NNE slope sites. Soil
184 samples (S) were collected at 10 cm depth. In each tree, a stem xylem piece (SX) of 5-8
185 cm length was collected with a 0.5 cm increment borer (Haglof, Sweden). A 1×8 cm
186 piece of stem phloem (SP) was cut using a chisel. Two twigs of five to eight cm length
187 from the lower part of the canopy were collected with a pruner, and their bark (TB) and
188 xylem (TX) were separated and collected together with previous growing season
189 needles from 2017 (Ne). Samples were placed in gas-tight 12 ml glass vials
190 ("Exetainer", Labco, Lampeter, UK), stored in cooling bags with ice packs and then
191 frozen after the field work.

192 During the second sampling day on the 9th of March 2018, additional needle
193 samples from previous growing season (2017) were taken at the 1250 and 1990 m sites,
194 divided in three equal parts from the brachyblast to the tip (i.e. "bottom", "mid", and
195 "tip") and collected separately in Exetainers (full length of the needles varied from 15 to
196 30 cm). During the second sampling day, also water vapor (WV) was sampled in
197 cryogenic traps at the 480 m, 1250 m and 1990 m sites at two consecutive times (10:30
198 am -12:30 pm and 12:30-2:30 pm). U-glass traps were connected to a 3 mm tube on one
199 side (inlet) and a small air pump (1 L/min) on the other side (outlet) and submerged into

a dry ice/ethanol slurry in dewar flasks in order to condensate all water vapor flowing through the glass trap for 2 h.

Later during the fieldtrip (second week of March 2018), four cores per tree were sampled from 30 trees at the fog-influenced 1250 m and timberline 1990 m sites (NNE slope) and at the fog-free 1550 m site (WNW slope) with 0.5 cm diameter increment borers for tree-ring analyses (tree-ring width, earlywood and latewood $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$).

Sample processing and stable isotope analyses

Water from soil, stem xylem and phloem, twig xylem and bark, and needles was cryogenically extracted according to West et al. (2006): Sample tubes were placed into a 80°C water bath, connected to a vacuum system including water traps cooled with liquid nitrogen. Captured water was then pipetted to 2 ml vials. $\delta^{18}\text{O}$ values of all these samples and water vapor samples were determined with a high-temperature conversion/elemental analyzer coupled via a ConFlo III referencing interface to a Delta^{Plus}XP isotope ratio mass spectrometer (TC/EA-IRMS, all supplied by Finnigan MAT, Bremen, Germany), using a glassy carbon reduction method as previously described (Gehre et al. 2004). Oxygen isotope ratios were measured on the resulting gas CO and referenced to VSMOW (Vienna Standard Mean Ocean Water, IAEA, Vienna, Austria). Measurement precision of quality control standards were <0.2 ‰ (standard deviation).

All dry needle material from the sites at 1250 and 1990 m was ground to a fine powder using a steel ball-mill. Aliquots (1 mg) of needle organic matter (Ne-OM) were weighted in silver capsules. Needle water-soluble compounds (Ne-WSC), which mostly but not exclusively reflect the photosynthetic assimilates, were extracted from 100 mg needle organic matter in a 2 ml vial in a water bath at 85 °C for 30 min using 1.5 ml

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3 225 deionized water (Lehmann et al. 2015). Subsequently, samples were centrifuged (2 min,
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5 226 10,000 g) and the supernatant contacting the Ne-WSC was transferred to a new vial.
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7 227 Aliquots (c. 1 mg) were then pipetted to silver capsules, frozen at -20°C, and freeze-
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9 228 dried. Both $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ of Ne-OM and Ne-WSC were measured by TC/EA-IRMS
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11 229 (vario PYRO cube, Elementar, Hanau, Germany; coupled to the same ConFlo and IRMS
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13 230 as for water analysis) and referenced to VPDB and VSMOW, respectively. Laboratory
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15 231 control standard was typically measured with a precision (standard deviation) of <0.3 ‰
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17 232 for $\delta^{13}\text{C}$ and 0.2 ‰ for $\delta^{18}\text{O}$.

21 233 For dendrochronological analyses, the surface of all sampled cores was cut with
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23 234 a microtome (Gartner and Nievergelt 2010), and polished afterwards with a 600 grained
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25 235 sandpaper to maximize visibility of the tree rings. Tree-ring widths were measured with
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27 236 0.01 mm resolution with a LINTAB measuring device (Rinntech, Heidelberg, Germany)
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29 237 and cross-dated (Stokes and Smiley 1996) visually and statistically with the software
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31 238 COFECHA (Holmes 1983). Standard dendrochronological procedures were followed to
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33 239 accurately date the tree rings. In order to analyze intra-seasonal tree-ring $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$
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35 240 variations, three different trees per site and one core per tree were selected (fog-
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37 241 influenced at 1250 m, fog-free at 1550 m and timberline at 1990 m). We avoided cores
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39 242 with distinct compression wood, though it has been reported recently for other conifer
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41 243 species that compression wood only has a minor if any effect on the stable isotope
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43 244 signature of tree ring cellulose (Janecka et al. 2020). Earlywood (EW) and latewood
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45 245 (LW) of each ring for the period 2008-2017 was split off with a scalpel under a
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47 246 stereomicroscope. EW and LW samples were transferred to Teflon filter bags (Ankom
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49 247 Technology, Macedon, NY, USA) and holocellulose was extracted using a two-step
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51 248 digestion method (NaOH and NaClO₂) following Boettger et al. (2007). Extracted
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cellulose was homogenized following Laumer et al. (2009) and weighted (1 mg) into silver capsules for $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ analyses as described above.

Stomatal conductance measurements

During the first sampling day (7th of March 2018), stomatal conductance (g_s), and leaf temperature measurements were performed 4 times in 2017 needles on each of the studied trees at the 1250 m, 1800 m, and 1990 m sites on the same twigs collected for isotope analyses using an SC-1 leaf porometer (Decagon Devices, Washington, USA).

Foliar water uptake (FWU)

In order to test the foliar water uptake capacity of Canary Island pine, 0.8-1 m long branches were taken in the field on the 7th of March 2018 at 1250 m and 1990 m asl from the same five study trees used for the previously described tree tissues' sampling. Branches were placed in black plastic bags to avoid transpiration and water loss. After the fieldwork, branches were re-cut underwater to 0.4 m length, kept overnight in a bucket and again placed in black plastic bags. On the following day, leaf water potential was measured on two needle bundles of every branch with a Scholander pressure chamber (PMS Instrument Company, Albany, OR, USA) to ensure that branches and needles were fully rehydrated (i.e. water potential was 0 MPa). Subsequently, ten needle bundles from 2017 (3 needles per bundle) per tree were sampled all over the branch plus another ten of the needle bundles from 2016 of the trees at the 1250 m site (needles of 2016 were not always present on the trees at the 1990 m site). We applied the gravimetric method described in Limm et al. (2009) using a scale (Mettler Toledo Switzerland accuracy 0.01g), submerging the needles in

labeled water mixed with tap water ($\delta^{18}\text{O} = 55.2 \text{ ‰}$). The bottom of the needle bundle was enclosed by parafilm to avoid water loss and kept over the water surface to avoid water entering the needle through the bundle. Uptake and increase of leaf water content were calculated and corrected for leaf surface water capacity after Limm et al. (2009). Finally, needles were transferred to Exetainers for water extraction.

Mechanistic modeling

Modeling of evaporative needle water ^{18}O enrichment above source water (twig xylem water) was performed using the Craig Gordon (1965) model as modified for application to leaves and given by Farquhar et al. (2007) :

$$\Delta^{18}\text{O}_{\text{Ne-Wat}} = (1 + \varepsilon_{eq}) \left[(1 + \varepsilon_k) \left(1 - \frac{w_a}{w_i} \right) + \frac{w_a}{w_i} (1 + \Delta^{18}\text{O}_{\text{WV}}) \right] - 1 \quad \text{Eq. 1}$$

with ε_{eq} and ε_k being equilibrium and kinetic fractionation respectively, w_a/w_i the ratio of water vapor partial pressures outside and inside the leaf, and $\Delta^{18}\text{O}_{\text{WV}}$ the ^{18}O enrichment of water vapor ($\delta^{18}\text{O}_{\text{WV}}$) above twig xylem representing the source water ($\delta^{18}\text{O}_{\text{TX}}$) (Cernusak et al. 2016):

$$\Delta^{18}\text{O}_{\text{WV}} = \frac{\delta^{18}\text{O}_{\text{WV}} - \delta^{18}\text{O}_{\text{TX}}}{1 + \delta^{18}\text{O}_{\text{TX}}} \quad \text{Eq. 2}$$

Equilibrium and kinetic fractionation factors as well as water vapor partial pressures were calculated after Cernusak et al. (2016). Air temperature, relative humidity, leaf temperature and g_s were measured in situ at 1250, 1800 and 1990 m sites. At 480 and 970 m sites, atmospheric variables were obtained from a station as described in the following section, leaf temperature was approximated to air temperature and g_s was approximated to the average g_s at the 1250 m site (Table S1). Modeled $\delta^{18}\text{O}_{\text{Ne-Wat}}$ values were derived using Eq 2 from $\Delta^{18}\text{O}_{\text{Ne-Wat}}$ and $\delta^{18}\text{O}_{\text{TX}}$.

To assess the robustness of modeled values, different scenarios were simulated, i.e. varying leaf temperature (measured leaf temperature in each individual ± 0.5 , ± 1 and ± 2 standard deviations of the values measured at each site) and relative humidity (average site value ± 2.5 , ± 5 and ± 10 %).

In addition, we calculated the apparent biosynthetic fractionation factor (ϵ_{bioapp}) as the observed $\delta^{18}\text{O}$ differences between the water-soluble compounds and needle water:

$$\epsilon_{\text{bioapp}} = \delta^{18}\text{O}_{\text{Ne-WSC}} - \delta^{18}\text{O}_{\text{Ne-Wat}} \quad \text{Eq. 3}$$

Meteorological data

To study the influence of climatic variables on tree radial growth and $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ of tree-ring cellulose, we used temperature and precipitation data from stations at locations with climate conditions comparable to our study sites: One station at 1380 m on a NE slope on La Palma (4.3 km away from our 1250 m site), two stations at 2137 and 2340 on a NE slope on Tenerife (140 km away from 1990 m site), and one station at 1032 m on a WSW slope on Tenerife (130 km away from 1550 m site) (Table 1); the first two stations belong to the Spanish Meteorological Agency (AEMET), and the third one to the local Tenerife Island Government (Cabildo de Tenerife). Relative air humidity at the same stations and further calculation of vapor pressure deficit (VPD) as saturated minus actual vapor pressure, were used to characterize wet and dry seasons at the fog-influenced, fog-free and timberline sites (Table1). Monthly sea-level pressure (SLP) data were taken from the website of the Royal Netherlands Meteorological Institute (Harris et al. 2014).

Additionally, sampling day temperatures and relative humidity were measured/estimated. In situ measurements were conducted during the two sampling

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3 322 days at the 1250, 1800 and 1990 m sites with a SC-1 leaf porometer. Both were
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5 323 calculated as the mean of measurements taken immediately before and after the
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7 324 sampling at each site (and after the initial calibration at each site). Meteorological
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10 325 values for the 480 m site at sampling days were gathered from a meteorological station
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12 326 at La Palma (528 m - NE slope, 0.5 km away; AEMET). Values for 970 m site were
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14 327 interpolated from the 480 and the 1250 m sites. (Table 2).
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17 328 18 19 329 *Statistical analyses*

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21 330 Linear regression models were applied to test for $\delta^{18}\text{O}$ differences among plant
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23 331 tissues, soil and water samples at each site, and among altitudes for each sample type; as
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25 332 well as along needle gradient (bottom, mid and tip parts). The effect of relative humidity
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27 333 at sampling time on needle water $\delta^{18}\text{O}$ was also tested by linear models, as well as the
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29 334 effects of altitude and year on the increase of leaf water content and $\delta^{18}\text{O}$ after
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31 335 submerging the needles. A linear mixed model accounting for tree identity as a random
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33 336 factor was performed to check differences in g_s among altitudes; and accounting for tree
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35 337 identity and year of ring formation as random factors to test for $\delta^{18}\text{O}$ differences in EW
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37 338 or LW, sites, and their interactions. In the case of tree-ring cellulose $\delta^{13}\text{C}$ values,
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39 339 differences between seasonal wood formation were tested independently at each site
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41 340 with a linear mixed model accounting for tree identity and year of ring formation as
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43 341 random factors. Finally, linear models testing the effects of mean annual relative
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45 342 humidity and VPD on $\delta^{18}\text{O}$ of tree-ring cellulose were performed. The non-parametric
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47 343 Kruskal-Wallis test was applied when data did not meet assumptions of normality and
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49 344 homoscedasticity of residuals. This was the case for $\delta^{18}\text{O}$ of SX and SP among altitudes,
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51 345 ring widths among sites and tree-ring $\delta^{13}\text{C}$ among sites for each season of wood
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53 346 formation. To test for differences between groups, Tukey and Dunn post-hoc tests were
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performed in the case of parametric (linear regression and linear mixed models) and non-parametric (Kruskal-Wallis) analyses, respectively. To determine whether the increase in leaf water content was significantly above 0 after submerging the needles, a one-sample t-test was applied. The same was also applied to test for significant differences between observed and modeled/simulated $\delta^{18}\text{O}$ values of needle water.

Pearson's correlation coefficients were calculated between climate data (temperature, precipitation, sea-level pressure and North Atlantic Oscillation) and tree-ring width and EW and LW isotope data, respectively. Correlations were performed with monthly mean climatic values, and with trimester means coinciding with the 3-month drought period for the previous and current growing seasons (September₋₁-October₋₁-November₋₁ - SON₋₁, December₋₁-January-February - DJF₋₁, March-April-May - MAM, June-July-August - JJA, September-October-November - SON).

Statistical analyses were carried out in R version 3.6.0 (R Core Team 2019) with the package nlme (Pinheiro et al. 2020) to perform linear mixed models. Figures were produced using the package ggplot2 (Wickham 2009).

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Results

$\delta^{18}\text{O}$ of soil, water and plant tissues along the altitudinal gradient

$\delta^{18}\text{O}$ values in water decreased significantly (P-value < 0.001) and linearly with increasing altitude for soil (-1.7 ‰ km⁻¹), twig xylem (-1.6 ‰ km⁻¹) and twig bark (-1.4 ‰ km⁻¹) and not in a linear way for stem xylem (from -3.1 to -5.2 ‰) and stem phloem (from -3.0 to -5.5 ‰), with no statistically significant differences within each site (Fig. 2, Table 3). In contrast, $\delta^{18}\text{O}$ values in needle water increased with altitude from 5.4 to 21.4 ‰ (P-value < 0.001), in an exponential way, with relatively little change at the lower sites. $\delta^{18}\text{O}$ values in needle organic matter and water-soluble compounds increased with increasing altitude and were higher compared to needle water: 17.0 and 20.2 ‰ higher at the 1250 site and 12.6 ‰ and 14.5 ‰ higher at the 1990 m site, respectively. Besides, Ne-WSC were ¹⁸O-enriched compared to the 10-year average of tree-ring cellulose, by 1.9 ‰ at the 1250 site and 1.7 ‰ at the 1990 site. $\delta^{18}\text{O}$ values of water vapor averaged -16.8 ‰, but did not change significantly along altitude (P-value > 0.1). Relative humidity at sampling time significantly influenced $\delta^{18}\text{O}$ values in needle water (decreasing $\delta^{18}\text{O}$ with increasing humidity) explaining 87.3 % of its variability (P-value < 0.001).

Stomatal conductance (g_s) significantly decreased along the altitudinal gradient (P-value < 0.001) averaging 405.9, 322.7 and 210.0 mmol·m⁻²·s⁻¹ at 1250 m, 1800 m and 1990 m sites respectively (Table 2). Meteorological conditions at the first sampling day represented a typical wet season day, preceded by heavy rains during the previous weeks. On the second sampling day, however, atmospheric instability caused an inversion in humidity (Table 1 and 2).

386 *Needle water $\delta^{18}\text{O}$: Gradients, mechanistic modeling and FWU*

387 Needle water and Ne-WSC showed a significant (P-value < 0.001 for both
388 compounds) ^{18}O enrichment along the needle length (~15 to 30 cm) from the bottom to
389 the tip at both study sites. $\delta^{18}\text{O}$ values of needle water were 17.0 ‰ higher at the tip
390 than at the bottom of needles at 1250 m, but only 5.3 ‰ higher at 1990 m. In contrast,
391 the $\delta^{18}\text{O}$ range of Ne-WSC along the needles was lower (7.2 ‰) at 1250 m than at 1990
392 m (11.5 ‰; see differences between Ne-WSC and needle water in Fig. 3). Accordingly,
393 the apparent biosynthetic fractionation factor (ebio_{app}) showed opposed trends at the two
394 altitudes. Values of ebio_{app} decreased from the bottom to the tip (25.2 to 15.4 ‰) at
395 1250 m, but increased from 30.0 to 36.1 ‰ at 1990 m.

396 Modeled $\delta^{18}\text{O}$ values of needle water at the study sites based on Eq. 1 fitted with
397 the observed values, except for those at 1800 m (Fig. 4). Besides, there was a higher
398 variability in observed values compared to modeled ones (average standard deviation of
399 2.19 in observed values vs. 1.26 in modeled ones). Nevertheless, modeled and observed
400 values were significantly correlated ($r = 0.88$, P-value < 0.01). The simulations varying
401 leaf temperature input, showed higher dispersions at 480, 970 and 1250 m sites
402 compared to 1800 and 1990 m sites (Fig. S1). Compared to these simulations,
403 dispersions due to relative humidity sensitivity analysis were similar or lower. At 1800
404 m site, observed values were significantly lower than simulated values (P-value <
405 0.05), except for the higher leaf temperature scenario (+ 2 standard deviations) and the -
406 5 and -10% relative humidity scenarios.

407 The capacity of Canary Island pine needles for foliar water uptake (FWU) was
408 demonstrated with needles from two years (2016 and 2017) and two sites (1250 and
409 1990 m), as the increase in leaf water content (LWC) was significantly above 0 after
410 performing the gravimetric method (P-value < 0.05, Table 4). An increase in LWC > 3

411 % was observed for the 2017 needles at both sites. 2016 needles (only present at 1990
412 m) tended to have a 6.4% LWC increase, which was, however, not significant (P-value
413 > 0.05). In addition, $\delta^{18}\text{O}$ values of needle water indicated ^{18}O -label uptake after FWU,
414 but showed no significant difference between needle years and altitude (P-value > 0.05).

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416 *Tree-ring cellulose $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ records*

417 Mean $\delta^{18}\text{O}$ values of tree-ring cellulose followed the $\delta^{18}\text{O}$ trend in needle water
418 and assimilates, showing a significant increase along the altitudinal gradient for both
419 EW and LW (Fig. 5a, d). EW increased from 30.3 to 35 ‰ and LW from 29.9 to 33.3
420 ‰ from the 1250 to the 1990 site. In addition, $\delta^{18}\text{O}$ differences between EW and LW
421 indicated seasonal variations. The $\delta^{18}\text{O}$ values of EW cellulose were significantly higher
422 than LW at the fog-free 1550 m site (0.85 ‰ P-value < 0.001) and timberline 1990 m
423 (1.6 ‰, P-value < 0.001; Fig. 5a, d), while no significant differences were observed at
424 the fog-influenced 1250 m site. Altitudinal-driven environmental changes and seasonal
425 variations in EW vs. LW together explained 82 % of the variability of tree-ring $\delta^{18}\text{O}$
426 (marginal $R^2 = 0.82$).

427 $\delta^{13}\text{C}$ values of tree-ring cellulose also increased with altitude by 0.81 ‰ for EW
428 and 0.99 ‰ for LW, with higher $\delta^{13}\text{C}$ values at 1990 m compared to both 1250 and
429 1550 m sites (P-value < 0.001 for both EW and LW) (Fig. 5b, d). In contrast to $\delta^{18}\text{O}$,
430 $\delta^{13}\text{C}$ values of EW were consistently higher compared to LW at 1250 m and 1550 m (P-
431 value < 0.05), but not at 1990 m.

432 No altitudinal gradient in growth was detected, as tree-ring widths of the 10
433 years used for isotope analysis were not significantly different among sites. However,
434 tree-ring cellulose $\delta^{18}\text{O}$ increased significantly (P-value < 0.001) with decreasing mean
435 annual relative humidity and with increasing mean annual VPD (Fig. 6). VPD explained

68.5 and 66.4 % of $\delta^{18}\text{O}$ variability in EW and LW cellulose respectively, whereas relative humidity explained 82.2 and 78.5 %.

We further investigated the climate sensitivity of the tree-ring parameters over the 10-year study period (Fig. 7, S2). Tree-ring width at all sites was positively correlated to previous winter precipitation (DJF₋₁, r between 0.41 and 0.60, P -value < 0.05 for this and following correlations). At the 1550 m fog-free site it was also correlated to spring precipitation (MAM, $r = 0.58$), and at the 1250 m fog site negatively correlated to autumn precipitation (SON, $r = -0.50$). LW $\delta^{18}\text{O}$ at 1250 and 1990 m were negatively correlated to previous winter precipitation (DJF₋₁, $r = -0.48$ and -0.71 , respectively), and also LW $\delta^{13}\text{C}$ at the 1990 m timberline site was negatively correlated to previous winter precipitation (DJF₋₁, $r = -0.38$). Regarding sea-level pressure (SLP) negative correlations were found for tree-ring width and previous winter SLP at all sites (DJF₋₁, r between -0.45 and -0.55) and at the 1250 m site also to spring SLP (MAM, $r = -0.53$). LW $\delta^{18}\text{O}$ was positively correlated to winter SLP at 1250 and 1990 m ($r = 0.50$ and 0.66 respectively) and at 1250 m also to spring (MAM, $r = 0.50$) and summer SLP (JJA, $r = 0.44$). Positive correlation was also found between summer SLP and LW $\delta^{13}\text{C}$ at the fog-free site at 1550 m.

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453 **Discussion**

454 *$\delta^{18}\text{O}$ variation in soil and plant tissues along the altitudinal gradient during the wet*
455 *season*

456 Soil water $\delta^{18}\text{O}$ values decreased with increasing altitude (Fig. 2) as expected
457 due to Rayleigh distillation known to occur for precipitation (Poage and Chamberlain
458 2001; Rozanski et al. 1993; Siegenthaler and Oeschger 1980). As no isotope
459 fractionation is assumed to occur during root water uptake and transport in the xylem,
460 $\delta^{18}\text{O}$ values in stem and twig tissues followed this decrease. Stem and twig water in
461 both xylem and phloem/bark tissues had similar isotope values, indicating water
462 exchange between both tissues. The slight ^{18}O enrichment in water from twig tissues at
463 the highest site (1990 m) might be a consequence of mixing with ^{18}O -enriched needle
464 water and potentially enhanced twig evaporation due to lower relative humidity and/or
465 higher VPD (Treydte et al. 2014). In contrast to soil and xylem water, $\delta^{18}\text{O}$ values in
466 needle water strongly increased along the altitudinal gradient. This is likely caused by
467 the meteorological conditions at sampling time (a typical winter day with trade wind
468 influence; Table 1,2), especially decreasing relative humidity and air temperature with
469 increasing altitude. This was also predicted by the Craig-Gordon model for leaf isotope
470 enrichment, where relative humidity and air temperature are important driving variables
471 (Eq. 1). It further leads to the observed reduction of g_s and transpiration rates, even if
472 soil water availability was probably high due to recent precipitation events (Table 2).
473 Canopy conductance in this species declines with increasing evaporative demand,
474 especially in absence of soil drought conditions (Brito et al. 2014; Luis et al. 2005).
475 Moreover, the increasing ^{18}O enrichment in needle water along the altitudinal gradient
476 was most probably imprinted on leaf assimilates (Lehmann et al. 2017). Our
477 observation for the leaf level is supported by the altitudinal increase in $\delta^{18}\text{O}$ values of

tree-ring cellulose (Fig. 2 and Fig. 5). Finally, no differences were observed in $\delta^{18}\text{O}$ values of water vapor along the altitudinal gradient during the fog-free sampling day. During foggy days, however, more variability may be found due to the temperature inversion towards higher altitudes preventing water vapor from rising up. This would in consequence also affect needle water $\delta^{18}\text{O}$ values. In our case, we expect $\delta^{18}\text{O}$ variations of tree-ring cellulose to be mainly modulated by the strong effect of evaporative enrichment, and further oxygen atom exchange with xylem water rather than by $\delta^{18}\text{O}$ variation in water vapor. However, more detailed measurements of $\delta^{18}\text{O}$ values of atmospheric water vapor during different meteorological conditions at different altitudes should be performed in future studies.

Besides the influence of environmental conditions, the observed variability of all studied tissues along the altitudinal gradient could also partly be caused by genetic variability (Prunier et al. 2016). Other studies on *P. canariensis*, however, emphasize that its genetic diversity and adaptations are mostly related to volcanism or extreme arid conditions at low south exposed slopes rather than on altitudinal gradients (López de Heredia et al. 2014; López et al. 2016; Navascués et al. 2006).

Mechanistic modeling of needle water $\delta^{18}\text{O}$ and the effect of needle anatomy

The general trend of increasing needle water ^{18}O enrichment with decreasing humidity expected from the Craig-Gordon model was well reflected in our empiric data (despite the assumptions made for the 480 and 970 sites). However, modeled $\delta^{18}\text{O}$ values of needle water at the 1800 m site were significantly lower than the observed $\delta^{18}\text{O}$ values (P-value <0.05; Fig. 4). This result was consistent with the simulated leaf and air humidity simulations, and it could only be explained in the case of increased leaf temperatures in 2 standard deviations or with relative humidity values of -5 % or less

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3 503 below the measured conditions (Fig. S1). Even in those scenarios, values fell into the
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5 504 1:1 line, that was also the case of the observed values elsewhere, meaning that the
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8 505 average oxygen composition of the whole needle water was similar to that at the
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10 506 evaporative site. This is untypical for conifer species as a thick-walled endodermis
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12 507 (Casparian strip) in needles is thought to isolate the source water of vascular bundle and
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14 508 transfusion tissue from evaporative enriched water (Roden et al. 2015; Timofeeva et al.
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17 509 2020; Zwieniecki et al. 2007). Observed values might then be related to the progressive
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19 510 ^{18}O enrichment (Fig. 3) that the exceptional long Canary Island pine needles (up to 30
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21 511 cm length) showed, which is potentially driven by the diffusion of the heavy
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23 512 isotopologue from evaporative sites back to xylem water (through aquaporins), further
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25 513 enriching the longitudinal flow towards the tip (Cernusak et al. 2016; Helliker and
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27 514 Ehleringer 2002; Kannenberg et al. 2021). Differentiated transpiration rates along the
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29 515 needle surface could also trigger the longitudinal enrichment (Shu et al. 2008). Specific
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31 516 climatic or physiologic conditions (Table S1) might therefore play a role on discussed
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33 517 processes leading to a higher mixture of needle water and, thus, making isotope
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35 518 modeling particularly challenging in the extraordinary long Canary island pine needles.
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38 519 The ^{18}O enrichment along needle gradients was higher at 1250 m than at 1990 m
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40 520 (Fig. 3) and is likely explained by lower relative humidity at the mid-altitude site at the
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42 521 given sampling day (9th of March), in contrast to inverse conditions of the previous day
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44 522 (7th of March) due to changes in weather conditions (Table 2). The changes in relative
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46 523 humidity likely affected the needle water $\delta^{18}\text{O}$ values more rapidly than the $\delta^{18}\text{O}$ values
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48 524 of assimilates, so that the latter rather might reflect needle water $\delta^{18}\text{O}$ values from
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50 525 previous days, which were clearly more ^{18}O -enriched at the highest altitude (Fig. 2).
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52 526 This may also explain the unexpected opposite bottom-to-tip trends in the apparent
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54 527 biosynthetic oxygen isotope fractionation ($\epsilon_{\text{bio_app}}$) between needle water and Ne-WSC
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at both altitudes (Fig. 3). Nevertheless, the isotope trends in organic matter and tree-ring cellulose were similar (Fig. 2), which is important to know for the interpretation of the tree-ring isotope signals. Future studies on progressive ^{18}O enrichment in needles in controlled conditions could decipher whether climate conditions (relative humidity), plant physiology (stomatal conductance), anatomy (Casparian strip and aquaporins) or a mix of these factors trigger the observed gradients.

Foliar water uptake and impact of fog on tree-ring isotope patterns

On the Canaries, a distinct layer of fog typically develops during atmospheric stability and high atmospheric pressure in summer at mid altitude. We assessed for the first time the foliar water uptake capacity of the species (Table 4), implying an alternative way of water uptake that may relief water stress (e.g. summer drought) in its crown as already reported for other species (Burns Limm et al. 2009; Eller et al. 2013). However, further studies should be conducted to understand the mechanism that allows this process to occur.

Although fog-influenced and fog-free sites were not at the same altitude, we expected main differences to be caused by the contrast of fog presence/absence due to the slope orientation rather than other factors related to altitudinal difference (300 m). Higher tree-ring $\delta^{18}\text{O}$ values of Canary Island pine at the fog-free (1550 m, WNW) than at the fog-influenced site (1250 m, NNE) are likely caused by lower stomatal conductance under lower relative humidity in fog-free conditions (Fig. 5, Table 1). Interestingly, fog water (with a closer geographic origin) was found to be ^{18}O -enriched compared to advective precipitation during the wet season (with a further geographic origin) (Prada et al. 2015; Scholl et al. 2011). However, foggy environmental conditions (lower temperatures and irradiance, higher relative humidity and thus lower VPD)

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3 553 seemed to have a stronger effect on $\delta^{18}\text{O}$ variations in needle water at mid-term than the
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5 554 ^{18}O -enriched fog water input, leading to the impossibility to trace the $\delta^{18}\text{O}$ of fog water
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8 555 in wood cellulose. The expected lower stomatal conductance at the fog-free site did not
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10 556 result in lower ^{13}C -discrimination as tree-ring $\delta^{13}\text{C}$ values were similar to the fog-
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12 557 influenced site. Likely, the fog-free site experienced also lower photosynthetic rates due
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14 558 to stomatal closure as previously observed in this species (Brito et al. 2014; Luis et al.
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16 559 2005; Peters et al. 2003), causing similar intercellular CO_2 concentrations and thus ^{13}C
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18 560 discrimination at both sites. This would agree with Scheidegger's et al (2000) model:
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20 561 Similar $\delta^{13}\text{C}$ and higher $\delta^{18}\text{O}$ values at the fog-free site would theoretically mean a
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22 562 reduction in both average maximum net photosynthesis and stomatal conductance
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24 563 (Table S2).

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28 564 Tree rings at the timberline site (1990 m, NNE) were clearly enriched in both
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30 565 ^{18}O and ^{13}C compared to the fog-influenced and fog-free site (Fig. 5, Table 1). This site
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32 566 faces lower temperatures and drier air (i.e. lower relative humidity than the fog-free site,
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34 567 Table 1) and relatively low fog frequency and precipitation (similar to fog-free site). We
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36 568 therefore assume that low relative humidity caused low stomatal conductance (Table
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38 569 S2) and that this response was not compensated by the reduced photosynthetic rates,
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40 570 resulting in the highest $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ values of all sites in agreement with Scheidegger
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42 571 et al (2000).

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49 573 *Seasonal isotopic variations and climate sensitivity of tree-ring parameters*

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51 574 The seasonal variation of $\delta^{18}\text{O}$ at fog-free and timberline sites, with lower LW
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53 575 than EW $\delta^{18}\text{O}$ values might be a result of changes in source water uptake from ^{18}O -
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55 576 enriched (shallow) to ^{18}O -depleted (deep) soil water, with the latter potentially
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57 577 originating from ^{18}O -depleted winter precipitation (Allen et al. 2019; Allison et al.

1983; Goldsmith et al. 2012; Sarris et al. 2013; Treydte et al. 2014). This is also in agreement with the correlation of tree-ring width and LW $\delta^{18}\text{O}$ with precipitation and SLP (Fig. 7). The role of winter precipitation on latewood formation is also supported by its negative correlation with LW $\delta^{13}\text{C}$, as higher soil water reserves might trigger a lower intrinsic water use efficiency in the pine needles and thus, a decrease in LW $\delta^{13}\text{C}$.

Seasonal $\delta^{13}\text{C}$ differences were observed for tree rings at both the fog-influenced and fog-free sites (Fig. 5 b, d), with lower LW $\delta^{13}\text{C}$ values compared to EW. At the fog-influenced site this could be caused by higher CO_2 stomatal conductance and lower photosynthetic rates during the frequent foggy conditions in summer, thus more humid and with a lower irradiance (Marzol 2008; Ritter et al. 2019). Leaf gas exchange measurements during foggy conditions would need, however, to be performed to confirm this directly. At the fog-free site lower LW $\delta^{13}\text{C}$ values compared to EW may be caused by the strong influence of stomatal closure on photosynthetic rates as discussed in the previous section and reported for this species (Brito et al. 2014; Luis et al. 2005; Peters et al. 2003; Scheidegger et al. 2000) (Table S2), with higher photosynthetic rates occurring during the winter days (Peters et al. 2008). Finally, lower temperatures, shorter growing seasons and generally lower photosynthetic activity at the timberline (Weigel et al. 2018) in addition to VPD conditions during latewood formation that reduced stomatal conductance and photosynthetic rate (Peters et al. 2003) (Table S2) may cause reduced intra-annual differences between mean EW and LW $\delta^{13}\text{C}$ values. Thus, our results may primarily show that carbon and oxygen isotope patterns in tree rings of Canary Island pine reflect a strong interplay of plant physiology (stomatal regulation, water use efficiency) with environmental conditions (altitude-driven climate and seasonal variations).

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EW and LW $\delta^{18}\text{O}$ values converged at/in the more humid site/years whereas they diverged at/in drier sites/years. This is probably caused by lower humidity during LW formation. In correspondence to Kahmen et al. (2011) who explained 91 % of the variability of tree-ring cellulose $\delta^{18}\text{O}$ with mean annual VPD, also a significant portion of the variability in EW and LW $\delta^{18}\text{O}$ of our samples (68,5 % and 66.5 % respectively) was related to mean annual VPD. We, however, found mean annual relative humidity to have an even stronger effect on EW and LW $\delta^{18}\text{O}$ (82.2 % and 78.5 % respectively) (Fig.6). This might be related to the different environmental conditions in both study regions with generally lower water availability and lower temperatures on the Canary Islands compared to Hawaii (Kahmen et al. 2011).

613 Conclusions

614 Assessment of the carbon and oxygen isotope composition of various Canary
615 Island pine tissues along an altitudinal gradient on La Palma showed the plasticity of
616 physiological responses in this species to the highly variable environmental conditions
617 along its broad climatic range of distribution. Our study demonstrates that changes in
618 relative humidity along the altitudinal gradient exert the strongest influence on oxygen
619 stable isotopes both at short (needle water) and midterm (tree-ring cellulose of 10
620 years). This, together with *in situ* measurements showed that the capacity of this species
621 to adapt to the different studied environments is mainly triggered by the regulation of
622 the water losses according to the local humidity conditions. The strong isotopic
623 heterogeneity within the needle triggered by the relatively long needle morphology of
624 the species, makes leaf water isotope modeling challenging. The similar $\delta^{13}\text{C}$ values and
625 thus water use efficiency during the wet and dry season at fog-free and fog-influenced
626 sites show as well the adaptation of this pine to different environments. In addition,
627 seasonal $\delta^{18}\text{O}$ variations showed the ability of *P. canariensis* to uptake water from
628 different soil water pools if needed. Moreover, we assessed for the first time the
629 capability of this species to benefit from fog water by means of foliar uptake. However,
630 fractionations derived from foggy environmental conditions left a greater imprint on
631 cellulose $\delta^{18}\text{O}$ than fog ^{18}O -enriched water itself.

632 Plasticity of water loss regulation and water use efficiency of *P. canariensis* can
633 partly explain its wide distribution across an archipelago with limited water input except
634 at foggy sites. This broad distribution can also be a consequence of the little competition
635 with other species on the Island and by the *P. canariensis* physiology, which is well-
636 adapted to severe perturbances allowing it to prosper on volcanically active islands
637 (López de Heredia et al. 2014; Miranda et al. 2020). The extreme physiological

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638 plasticity of *P. canariensis* could also allow the species to well-adapt to climatic
639 changes in the near future.

For Peer Review

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646 **Conflict of interest**

647 None declared.

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Figure legends

Figure 1: Canary Islands and study sites on La Palma. a) Location of the Canary Islands, b) La Palma island, contour lines (500 m) and selected study sites (green dots), c) schematic diagram of site locations along the altitudinal gradient, aspect, fog influence (blue color) and annual average temperatures and precipitation at low, mid and high altitudes.

Figure 2: Oxygen isotope composition ($\delta^{18}\text{O}$) of atmospheric water and extracted water of soil and Canary Island pine samples along an altitudinal gradient on La Palma. Abbreviation: water vapor (WV), soil water (S), stem xylem water (SX), stem phloem water (SP), twig xylem water (TX), twig bark water (TB), needle water (Ne-Wat), needle organic matter (Ne-OM), needle water-soluble compounds (Ne-WSC), and 10-year average of tree-ring cellulose (TR). Dotted lines show linear model calculated for $\delta^{18}\text{O}$ values (left) of soil water and $\log(\delta^{18}\text{O})$ values of Ne-Wat (right). Letters indicate statistically significant differences between tissues at each altitude (Tukey test $P < 0.05$). Mean values $\pm$ standard error (WV $n = 2$; TR $n = 3$, other compounds $n = 5$).

Figure 3: Oxygen isotope composition ($\delta^{18}\text{O}$) of Canary Island pine needle water and assimilates from the two sites at 1250 and 1990 m asl. $\delta^{18}\text{O}$ values of needle water (Ne-Wat), needle water-soluble compounds (Ne-WSC), and the biosynthetic fractionation factor ($\epsilon_{\text{bio_app}}$) of three needle segments (bottom, mid, tip) are given. Needles ranged from 15 to 30 cm in length. Mean values $\pm$ standard error ($n = 5$).

Figure 4: Observed vs. modeled $\delta^{18}\text{O}$ values in needle water of Canary Island pine along an altitudinal gradient on La Palma. Black dotted line represents the 1:1 line.

Figure 5: Carbon and oxygen isotope variations of tree-ring cellulose of the Canary Island pines studied on La Palma. Mean a) $\delta^{18}\text{O}$ and b) $\delta^{13}\text{C}$ values of tree-ring cellulose for early (EW) and latewood (LW) of 10-year chronologies (2008-2017) from three sites (fog-influenced at 1250 m, fog-free at 1550 m and timberline at 1990 m). c) Mean ring-width series per year at each site. d) $\delta^{18}\text{O}$ - $\delta^{13}\text{C}$ representation of early- (EW) and latewood (LW) at each site. Greek letters indicate significant differences in $\delta^{18}\text{O}$ values between sites and seasons. Latin letters indicate significant differences in $\delta^{13}\text{C}$ values between seasons at each site (lower case), and between sites for each season (upper case). Both ribbons and error bars show $\pm$ standard errors ($n = 3$).

Figure 6: Linear regressions between oxygen isotope values ($\delta^{18}\text{O}$) of Canary Island pine tree-ring cellulose from earlywood (EW) and latewood (LW) and annual mean relative humidity (left) and vapor pressure deficit (VPD; right), and in comparison to values from Kahmen et al (2011) (right). Points represent mean annual $\delta^{18}\text{O}$ per season ($n=3$).

Figure 7: Statistical relationships between tree-ring variables and climate of the Canary Island pines studied on La Palma. Pearson's correlation coefficients of ring widths and latewood oxygen and carbon isotope composition (LW $\delta^{18}\text{O}$, LW $\delta^{13}\text{C}$) of tree-ring cellulose of the studied period (2008-2017) from three sites (fog-influenced at 1250 m, fog-free at 1550 m and timberline at 1990 m) with three-month averages of precipitation and sea-level pressure (SLP): previous growing season September,

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987 October, November (SON₋₁); December, January, February (DJF₋₁); current growing
988 season March, April, May (MAM); June, July, August (JJA); September, October,
989 November (SON). Filled bars represent significant correlations (P-value<0.05).
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For Peer Review

Table 1: Meteorological data of dry and wet seasons for the fog-influenced, fog-free and timberline sites. Mean temperature (Temp), precipitation (Prec), relative humidity (rH) and vapor pressure deficit (VPD) at the tree-ring sites during the dry (April-September) and wet (October-March) seasons over the study period (2008-2017) are given.

Site	Temp (°C)		Prec (mm)		rH (%)		VPD (kPa)	
	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet
1990-timberline	14.3	6.9	36.6	361.3	30.6	47.4	1.17	0.52
1550 - fog-free	17.8	12.2	41.9	291.6	61.6	67	0.85	0.46
1250- fog-influenced	17.32	13.78	188.1*	1091.2*	75.53	81.5	0.47	0.24

* precipitation data does not include water inputs by means of fog water interception that could double or triple annual precipitation

Table 2: Meteorological data of the sampling dates for the study sites.

Temperature (Temp), relative humidity (rH), vapor pressure deficit (VPD) and stomatal conductance on 2017 needles (g_s) of the two days at the time of water and tissue sampling (water vapor, soil, stem xylem and phloem, twig xylem and bark, and needles) are given, as well as the previous month precipitation decile of the last 30-year February precipitation ($Prec_{Feb}$).

Date	Sampled tissue	Site	Temp (°C)	rH (%)	VPD (kPa)	g_s (mmol·m ⁻² ·s ⁻¹)	$Prec_{Feb}$ (decile)
07.03.2018	Water and tissue samples	1990 m	11.2 ¹	41 ¹	0.78	210.0 ⁺	7
		1800 m	16.4 ¹	56 ¹	0.82	322.7 ⁺	-
		1250 m	17.4 ¹	66 ¹	0.68	405.9 ⁺	9
		970 m	18.2 ²	69 ²	0.65	-	-
		480 m	19.5 ³	74 ³	0.59	-	8
09.03.2018	Needles and water vapor	1990 m	9.8	74	0.31	-	7
		1250 m	18.3	48	1.09	-	9
	Water vapor	480 m	24.5	52	1.47	-	8

¹ Values measured *in situ* with SC-1 leaf porometer

² Values interpolated from upper and lower sites.

³ Values gathered from a meteorological station 500 m away

Other meteorological values collected from stations or calculated as indicated in Materials and methods.

⁺ g_s differences in altitude were significant according to the fitted linear mixed model accounting for tree identity as a random factor (P - value < 0.001).

Table 3: Statistics for the analysis of altitudinal effects on the oxygen isotopic

composition of water and assimilates of the different tree tissues and organs. Slope and intercept of the applied linear model are given. LM: linear model, K-W: Kruskal-Wallis. Compounds: soil water (S), stem xylem water (SX), stem phloem water (SP), twig xylem water (TX), twig bark water (TB), needle water (Ne-Wat), needle organic matter (Ne-OM) and water-soluble compounds from needles (Ne-WSC).

Response variable	Compound	no. Sites	Analysis	Slope	Intercept	Adjusted R ²	P-value
$\delta^{18}\text{O}$ (‰)	S	5	LM	$-1.67 \cdot 10^{-3}$	-2.017	0.64	<0.001
	SX	5	K-W	-	-	-	<0.001
	SP	5	K-W	-	-	-	<0.01
	TX	5	LM	$-1.56 \cdot 10^{-3}$	-1.842	0.65	<0.001
	TB	5	LM	$-1.37 \cdot 10^{-3}$	-1.831	0.66	<0.001
	Ne-Wat	5	LM *	$9.72 \cdot 10^{-4*}$	1.015*	0.75	<0.001
	Ne-OM	2	LM	$7.10 \cdot 10^{-3}$	19.906	0.99	<0.001
	Ne-WSC	2	LM	$5.25 \cdot 10^{-3}$	25.490	0.88	<0.001

* Logarithmic transformation was applied to $\delta^{18}\text{O}$ Ne-Wat to adjust the linear model

Table 4: Foliar water uptake (FWU) of the studied Canary Island pine needles.

Needles from two sites and of two years have been used. ΔLWC : increase in needle water content, $\delta^{18}\text{O}_{\text{FWU}}$: $\delta^{18}\text{O}$ values of needle water after foliar water uptake. Mean values $\pm$ standard error ($n = 5$).

Site	Needle cohort	FWU (gram)	ΔLWC (%)	$\delta^{18}\text{O}_{\text{FWU}}$ (‰)
1990 m	2017	0.071 ± 0.014	3.8 ± 1.9	35.0 ± 4.5
1250 m	2017	0.081 ± 0.018	3.5 ± 1.7	33.3 ± 3.5
1250 m	2016	0.124 ± 0.028	6.4 ± 1.4	36.3 ± 5.0

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Figure 1: Canary Islands and study sites on La Palma. a) Location of the Canary Islands, b) La Palma island, contour lines (500 m) and selected study sites (green dots), c) schematic diagram of site locations along the altitudinal gradient, aspect, fog influence (blue color) and annual average temperatures and precipitation at low, mid and high altitudes.

82x62mm (300 x 300 DPI)

Figure 2: Oxygen isotope composition ($\delta^{18}\text{O}$) of atmospheric water and extracted water of soil and Canary Island pine samples along an altitudinal gradient on La Palma. Abbreviation: water vapor (WV), soil water (S), stem xylem water (SX), stem phloem water (SP), twig xylem water (TX), twig bark water (TB), needle water (Ne-Wat), needle organic matter (Ne-OM), needle water-soluble compounds (Ne-WSC), and 10-year average of tree-ring cellulose (TR). Dotted lines show linear model calculated for $\delta^{18}\text{O}$ values (left) of soil water and log($\delta^{18}\text{O}$) values of Ne-Wat (right). Letters indicate statistically significant differences between tissues at each altitude (Tukey test $P < 0.05$). Mean values $\pm$ standard error (WV $n = 2$; TR $n = 3$, other compounds $n = 5$).

Figure 3: Oxygen isotope composition ($\delta^{18}\text{O}$) of Canary Island pine needle water and assimilates from the two sites at 1250 and 1990 m asl. $\delta^{18}\text{O}$ values of needle water (Ne-Wat), needle water-soluble compounds (Ne-WSC), and the biosynthetic fractionation factor (ϵ_{bioapp}) of three needle segments (bottom, mid, tip) are given. Needles ranged from 15 to 30 cm in length. Mean values $\pm$ standard error ($n = 5$).

Figure 4: Observed vs. modeled $\delta^{18}\text{O}$ values in needle water of Canary Island pine along an altitudinal gradient on La Palma. Black dotted line represents the 1:1 line.

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Figure 5: Carbon and oxygen isotope variations of tree-ring cellulose of the Canary Island pines studied on La Palma. Mean a) $\delta^{18}\text{O}$ and b) $\delta^{13}\text{C}$ values of tree-ring cellulose for early (EW) and latewood (LW) of 10-year chronologies (2008-2017) from three sites (fog-influenced at 1250 m, fog-free at 1550 m and timberline at 1990 m). c) Mean ring-width series per year at each site. d) $\delta^{18}\text{O}$ - $\delta^{13}\text{C}$ representation of early- (EW) and latewood (LW) at each site. Greek letters indicate significant differences in $\delta^{18}\text{O}$ values between sites and seasons. Latin letters indicate significant differences in $\delta^{13}\text{C}$ values between seasons at each site (lower case), and between sites for each season (upper case). Both ribbons and error bars show $\pm$ standard errors ($n = 3$).

Figure 6: Linear regressions between oxygen isotope values ($\delta^{18}\text{O}$) of Canary Island pine tree-ring cellulose from earlywood (EW) and latewood (LW) and annual mean relative humidity (left) and vapor pressure deficit (VPD; right), and in comparison to values from Kahmen et al (2011) (right). Points represent mean annual $\delta^{18}\text{O}$ per season ($n=3$).

Figure 7: Statistical relationships between tree-ring variables and climate of the Canary Island pines studied on La Palma. Pearson's correlation coefficients of ring widths and latewood oxygen and carbon isotope composition (LW $\delta^{18}\text{O}$, LW $\delta^{13}\text{C}$) of tree-ring cellulose of the studied period (2008-2017) from three sites (fog-influenced at 1250 m, fog-free at 1550 m and timberline at 1990 m) with three-month averages of precipitation and sea-level pressure (SLP): previous growing season September, October, November (SON-1); December, January, February (DJF-1); current growing season March, April, May (MAM); June, July, August (JJA); September, October, November (SON). Filled bars represent significant correlations (P-value < 0.05).

Supplementary data

Figure S1: Averaged observed vs. modeled $\delta^{18}\text{O}$ values in needle water of Canary Island pine along an altitudinal gradient on La Palma when a) simulating a range of leaf temperatures above and below 2 standard deviations and b) simulating a range of relative humidity of -10 to +10 % changes. Black dotted line represents the 1:1 line. Empty circles represent significant differences (P-value <0.05) between simulated and observed values. Filled circles represent similar simulated and observed values.

Figure S2: Climate sensitivity of Canary Island pine tree rings on La Palma. Pearson's correlation coefficients of ring widths, early and latewood oxygen and carbon isotope composition (EW $\delta^{18}\text{O}$, LW $\delta^{18}\text{O}$, EW $\delta^{13}\text{C}$, LW $\delta^{13}\text{C}$, respectively) of tree-ring cellulose of the studied period (2008-2017) from three sites (fog-influenced at 1250 m, fog-free at 1550 m and timberline at 1990 m) with three-month average of temperature, precipitation, sea-level pressure (SLP) and North Atlantic Oscillation (NAO): previous growing season September, October, November (SON₋₁); December, January, February (DJF₋₁); current growing season March, April, May (MAM); June, July, August (JJA); September, October, November (SON). Filled bars represent significant correlations (P<0.05).

Table S1: Mean input and output values of CG-model per site. T_{air} : Air temperature, T_{leaf} : leaf temperature, rH: relative humidity, g_s : stomatal conductance, $\delta^{18}\text{O}_{\text{WV}}$: water vapour $\delta^{18}\text{O}$, $\delta^{18}\text{O}_{\text{TX}}$: twig xylem water $\delta^{18}\text{O}$, ϵ_{eq} : equilibrium fractionation factor, ϵ_k : kinetic fractionation factor, w_a/w_i : ratio of water vapor partial pressures outside and inside the leaf, $\delta^{18}\text{O}_{\text{Ne-Wat}}$: needle water $\delta^{18}\text{O}$.

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1015 **Table S2:** Average number of days per month in which a vapor pressure deficit > 2.5
1016 kPa is reached. According to Peters Morales and Jiménez (2003), stomatal conductance
1017 decreases dramatically above the 2.5 kPa threshold, reaching zero conductance at a
1018 VPD of 4.5 kPa.
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