



Stable Water Use Efficiency under Climate Change of Three Sympatric Conifer Species at the Alpine Treeline

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The ability of treeline associated conifers in the Central Alps to cope with recent climate warming and increasing CO₂ concentration is still poorly understood. We determined tree ring stable carbon and oxygen isotope ratios of *Pinus cembra*, *Picea abies*, and *Larix decidua* trees from 1975 to 2010. Stable isotope ratios were compared with leaf level gas exchange measurements carried out *in situ* between 1979 and 2007. Results indicate that tree ring derived intrinsic water-use efficiency (iWUE) of *P. cembra*, *P. abies* and *L. decidua* remained constant during the last 36 years despite climate warming and rising atmospheric CO₂. Temporal patterns in $\Delta^{13}\text{C}$ and $\Delta^{18}\text{O}$ mirrored leaf level gas exchange assessments, suggesting parallel increases of CO₂-fixation and stomatal conductance of treeline conifer species. As at the study site soil water availability was not a limiting factor iWUE remained largely stable throughout the study period. The stability in iWUE was accompanied by an increase in basal area increment (BAI) suggesting that treeline trees benefit from both recent climate warming and CO₂ fertilization. Finally, our results suggest that iWUE may not change species composition at treeline in the Austrian Alps due to similar ecophysiological responses to climatic changes of the three sympatric study species.

Keywords: stable isotopes, intrinsic water use efficiency, tree growth, climate change, treeline, Central Alps

INTRODUCTION

High-altitude forest ecosystems at the timberline-treeline transition have raised concern as they may undergo significant alterations due to climate warming and changes in ground-level air chemistry (Holtmeier and Broll, 2007; Wieser et al., 2009). Dendroclimatological studies conducted within the treeline ecotone of the Central European Alps have shown radial stem growth to be limited by low summer temperature (Carrer and Urbinati, 2004; Oberhuber, 2004; Büntgen et al., 2005; Frank and Esper, 2005; Oberhuber et al., 2008). During recent decades, several authors report treeline-associated conifers to reflect increased radial growth, putatively related to climate warming (Graumlich et al., 1989; Peterson et al., 1990; Jacoby and D'Arrigo, 1997; Rolland and Florence-Schueller, 1998; Bunn et al., 2005). Moreover, increasing atmospheric CO₂ concentration may act in concert with climate warming to increase carbon accumulation within the treeline ecotone (cf. Graumlich, 1991; Saurer et al., 1997; Duquesnay et al., 1998; Sidorova et al., 2009).

Notwithstanding, reduced radial growth has been attributed to late-summer drought under increasing treeline temperature in the European Alps (Büntgen et al., 2006; Carrer and Urbinati, 2006; Oberhuber et al., 2008; Wieser et al., 2009).

Stable isotope ratios of carbon and oxygen, i.e., $^{13}\text{C}/^{12}\text{C}$ and $^{18}\text{O}/^{16}\text{O}$, respectively, may serve as dendrochronological proxies that facilitate mechanistic understanding of climate-related influences on physiological processes such as leaf gas exchange and stem wood formation (Loader et al., 2007; Weigt et al., 2015; and further references therein). In plant organic matter, $\delta^{13}\text{C}$ expressing the $^{13}\text{C}/^{12}\text{C}$ ratio in relation to an international standard (Pee Dee Belemnite) depends on variables such as leaf conductance for water vapor (g_w) that modify the net CO_2 uptake rate (A ; Farquhar et al., 1989). In addition, $\delta^{13}\text{C}$ of plant organic matter ($\delta^{13}\text{C}_p$) is a function of atmospheric $\delta^{13}\text{C}$, which is accounted for by calculating discrimination of photosynthesis against ^{13}C ($\Delta^{13}\text{C}$; Farquhar and Richards, 1984) in relation to intrinsic water-use efficiency (iWUE), i.e., the ratio of A (rate of net CO_2 fixation) versus g_w (leaf conductance for water vapor). For a review see Brugnoli and Farquhar (2000).

When analyzing $\delta^{13}\text{C}_p$ alone, the impacts of A (demand of CO_2) and g_w (supply of CO_2) on iWUE are difficult to separate (Saurer et al., 2008). The oxygen isotope ratio ($\delta^{18}\text{O}$), however, may allow a distinction between biochemical and stomatal limitations of photosynthesis as it is not affected by the photosynthetic CO_2 carboxylation but linked to g_w (Barbour et al., 2000; Grams et al., 2007). It is, therefore, an ideal covariable to estimate to what degree photosynthesis and stomatal conductance modify $\delta^{13}\text{C}_p$ (Scheidegger et al., 2000; Werner et al., 2012). Originally, this dual isotope approach was introduced for photosynthetic tissue and only recently tested conceptually for the interpretation of tree-ring data (Roden and Farquhar, 2012), although several critical points should be taken into account during interpretation. Among the most important issues that need to be considered are the facts that the $\delta^{18}\text{O}$ of source and atmospheric water can vary spatially and temporarily and that post-photosynthetic and post-evaporative oxygen atom exchange processes could affect the initial leaf-level isotope signal (see below).

At the leaf level, $\delta^{18}\text{O}$ of photoassimilates derive primarily from leaf water, typically being enriched in ^{18}O compared to the source water (i.e., xylem water) through evaporative enrichment at the site of transpiration. This enrichment is counteracted by the so-called Péclet effect and transpiratory leaf cooling (for a review see Barbour, 2007), which may result in the above mentioned negative correlation between $\delta^{18}\text{O}_p$ and g_w (e.g., Barbour et al., 2000; Grams et al., 2007). However, to an extent that may depend on species and site conditions, the signal is dampened by oxygen exchange with source water during biomass formation at the stem level (Gessler et al., 2013). This causes, at least partially, a decoupling between oxygen isotopic signatures of photoassimilates and the tree ring organic matter. However, in a recent report Weigt et al. (2015) confirmed that information may be exploited to relate ecophysiological responses of trees to environmental changes for both total sapwood organic matter and extracted cellulose. In any case, it appears advisable to

confirm interpretation from the dual isotope approach by gas exchange assessments whenever possible.

Previous tree-ring carbon isotope studies carried out in tropical, arid, Mediterranean, temperate and boreal forest ecosystems have identified an increase in iWUE over the past 40 years in response to increasing atmospheric CO_2 concentration (Penuelas et al., 2011; Saurer et al., 2014) and just recently supported by tree-ring $\delta^{13}\text{C}$ dynamic global vegetation models comparisons (DGVMs; Frank et al., 2015). Tree growth on the contrary, remained stable or even declined, suggesting local site conditions to override a potential CO_2 -induced increase in growth (Penuelas et al., 2011; Silva and Anand, 2013; Levesque et al., 2014). Increasing iWUE accompanied by a reduced productivity has been attributed to the combined effect of elevated CO_2 and climate change-induced soil drying (Penuelas et al., 2011; Saurer et al., 2014).

The impact of the steadily increasing CO_2 level and concurrent climate change, however, still awaits clarification for treeline-associated conifers in the Central Austrian Alps where low temperatures limit tree growth (Oberhuber, 2007; Wieser et al., 2009). At treeline in the Central Austrian Alps ample precipitation during the growing season prevails every third to fourth day on average (Wieser, 2012), so that soil water limitation stays absent, allowing trees to meet their water demand (Tranquillini, 1979; Mayr, 2007; Matyssek et al., 2009). Hence, whole-tree conductance stays high and mainly depends on the evaporative demand in terms of irradiance and vapor pressure deficit (Wieser, 2012). Therefore, we hypothesize that treeline trees passively respond to the increasing atmospheric CO_2 level (C_a), so that their leaf-intercellular CO_2 concentration (C_i) rises in parallel, while iWUE remains unchanged. The hypothesis was evaluated by stable carbon and oxygen isotope sampling and radial growth analysis over the past 36 years (1975–2010) in stems of mature *Pinus cembra*, *Picea abies* and *Larix decidua* trees growing at the treeline of Mt. Patscherkofel in the Central Tyrolean Alps. Observed long-term trends in $\delta^{13}\text{C}_p$, $\delta^{18}\text{O}_p$ in tree rings and iWUE were at least for some years compared with *in situ* leaf-level gas exchange data, assessed at the same study site between 1979 and 2007 in adult *P. cembra* and *L. decidua* trees. Results are discussed in view of tree response to climate warming at the treeline ecotone.

MATERIALS AND METHODS

Study Site, Climatic Data, and Tree Species

The study was conducted in a scattered stand at the lower edge of the treeline ecotone at 1950 m a.s.l. on Mt. Patscherkofel (47°12'37'' N, 11°27'07'' E), south of Innsbruck, Austria. The site is characterized by a cool subalpine climate, the possibility of frost during the entire year and a continuous snow cover from October through April. We used monthly mean temperatures and monthly total precipitation from 1975 to 2010 from a weather station nearby (Klimahaus Research Station and Alpengarten; 1950 m a.s.l.) for our analysis. Mean annual precipitation averaged 878 mm, with 58% falling during

the growing season (May through September). Mean annual air temperature averaged 2.4°C, with summer maxima of up to 27°C and winter minima of −28°C.

The geology of Mt. Patscherkofel is dominated by gneisses and schist. The soil at the study site is a haplic podzol, being a typical soil type of the treeline ecotone in the Central Tyrolean Alps (Neuwinger, 1972). The water holding capacity of the soil (at 5–65 cm depth) at saturation (−0.001 MPa) averages 0.60 m³ m^{−3}. Due to frequent precipitation during the growing season soil water potential rarely drops below −0.01 MPa, approximating soil water contents above 0.35 m³ m^{−3} (Wieser, 2012; including the dry summer of 2003, unpublished data).

The stand is composed of the dominant tree species *Pinus cembra*, accounting for 84% of the tree population, and accompanied by *Larix decidua* (9%) and *Picea abies* L. Karst (7%) at some locations. Trees grew either as isolated trees or in groups of four to five. The distance between single trees or tree groups was 20–30 m. From 20 *P. cembra* trees cored at the lower edge of the treeline ecotone Oberhuber et al. (2008) derived an expressed signal population (EPS) value of 0.94, reflecting a strong climate signal in the site chronology. From these trees we selected five trees which had the strongest correlation to the site specific mean tree-ring chronology, no missing rings, and regular ring boundaries. In addition we cored five dominant *P. abies* and *L. decidua* trees each to account for potential inter-specific differences of the three associated treeline species. In 2010 the trees were 69 ± 9 years old, with stem heights averaging 12 ± 1.3 m. The stem diameter at breast height (DBH) averaged 22 ± 3.2 cm.

Tree Ring and Basal Stem Area Increment

In fall 2010 we obtained two increment cores per trees at DBH using a 5-mm-diameter increment bore. For contrast enhancement of tree ring boundaries the cores were dried in the laboratory, non-permanently mounted on a holder, and the surface was prepared with a razor blade (Pilcher, 1990). Ring widths were measured to the nearest 1 μm using a reflecting microscope (Olympus SZ61) and the software package TSAP WIN Scientific. Ring widths of both cores from each sample tree were averaged and individual tree ring chronologies were then checked for dating accuracy using the COFECHA software (Holmes, 1994; Grissino-Mayer, 2001). As ring width may be biased by a negative correlation with the time course during tree maturation, ring width was converted to basal stem area increment (BAI) according to:

$$\text{BAI} = 3.14 (R_n^2 - R_{n-1}^2) \quad (1)$$

where R is stem radius inside tree bark and n is the year of tree ring formation (Fritts, 1976). Bark thickness was subtracted from stem radius. Finally BAI of each year were averaged over the five sample trees of each species.

Stable Isotope Analysis

$\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ analyses for the years 1975–2010 were performed on the same cores as used for BAI assessment. Annual rings

(early wood plus late wood) were cut exactly at ring boundaries by use of a scalpel and a reflecting microscope (Wild 308700). For each of the five study trees per species the two samples per tree ring were pooled and homogenized with a swing mill (Retsch MM301, Retsch Haan, Germany). In a subsample, we compared isotope signatures in bulk wood with those in cellulose for determining the necessity of cellulose extraction in our study trees. Cellulose extraction was performed using a modified version of the method of Brendel et al. (2000). The methodological comparison corroborated significant correlations in the cases of $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ (Figure 1) as reported earlier from coniferous and other tree species (Jaggi et al., 2002; Sohn et al., 2013) and is in accordance with a recent report (Weigt et al., 2015). On average, $\delta^{13}\text{C}$ in cellulose was 1.0–1.1‰ higher than in bulk wood (Table 1), being smaller than 1.3–1.4‰ found in *Picea abies* by Borella et al. (1998) and Sohn et al. (2013). Mean $\delta^{18}\text{O}$ in cellulose was 4.3–4.9‰ higher than in bulk wood (Table 1) being somewhat lower than 5.9‰ in bulk wood of *Picea abies* (Sohn et al., 2013). Based on these findings and in accordance with a recent methodological study (Weigt et al., 2015), we used bulk wood samples rather than extracted cellulose for isotope analysis.

Regarding $\delta^{13}\text{C}$, 2.0 ± 0.02 mg of homogenized samples were weighed into tin capsules each (3.5 × 5 mm, IVA Analysentechnik e.K., Meerbusch, Germany) and combusted to CO₂ in an elemental analyzer (Eurovector EA3000) connected to an isotope ratio mass spectrometer (Isoprime, Elementar, Hanau, Germany). For $\delta^{18}\text{O}$ analysis 0.7 ± 0.05 mg were weighed into silver capsules each (3.5 × 5 mm, IVA Analysentechnik e.K., Meerbusch, Germany) to obtain CO at 1,430°C in a high-temperature pyrolysis system (HTO, HekaTech, Wegberg, Germany) which was connected via an open-split interface (Conflo III; Finnigan MAT, Bremen, Germany) to an isotope ratio mass spectrometer (Delta Plus; Finnigan MAT, Bremen, Germany). Isotope abundances were expressed using the δ -notation in ‰ relative to the international standards:

$$\delta_{\text{sample}} = (R_{\text{sample}}/R_{\text{standard}} - 1) * 1000 \quad (2)$$

where R_{sample} is the molar fraction of the $^{13}\text{C}/^{12}\text{C}$ or $^{18}\text{O}/^{16}\text{O}$ ratio of the sample, and R_{standard} that of the international IAEA standards V-PDB for carbon and V-SMOW for O. The analytical precision was <0.12‰ and <0.28‰ regarding $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$, respectively (expressed as standard deviation of the internal laboratory standard at the same sample mass).

Isotope Discrimination and iWUE

Tree ring specific $\delta^{13}\text{C}_{\text{trng}}$ were corrected for the progressive decline in atmospheric $\delta^{13}\text{C}_{\text{atm}}$ through calculating ^{13}C discrimination ($\Delta^{13}\text{C}$):

$$\Delta^{13}\text{C}(\text{‰}) = (\delta^{13}\text{C}_{\text{atm}} - \delta^{13}\text{C}_{\text{trng}})/(1 + \delta^{13}\text{C}_{\text{trng}}/1000) \quad (3)$$

To this end, $\delta^{13}\text{C}_{\text{atm}}$ with its nearly linear time course during 1980 through 2010¹ was extrapolated for the years 1975 through 1979. In a simplified model, Farquhar et al. (1982) related $\Delta^{13}\text{C}$

¹www.scrippsco2.ucsd.edu/home

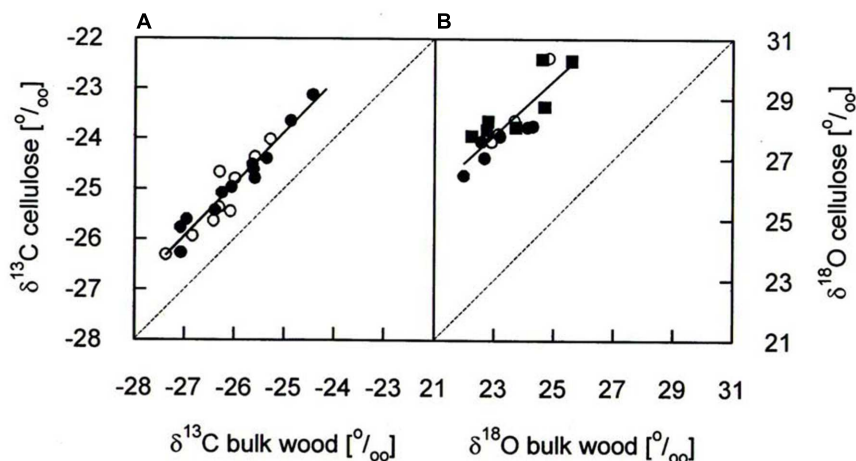


FIGURE 1 | Comparison of (A) $\delta^{13}\text{C}$ and (B) $\delta^{18}\text{O}$ in bulk wood and cellulose of annual growth rings of *P. cembra* (solid circle), *P. abies* (solid square) and *L. decidua* (open circle). Dashed lines reflect the one-to-one lines for comparison and the solid lines correspond to the linear regression analyses of the data points: $\delta^{13}\text{C}$: $y = 1.03x + 1.86$, $r^2 = 0.92$, $P < 0.001$, $n = 36$; $\delta^{18}\text{O}$: $y = 0.92x + 6.65$, $r^2 = 0.73$, $P < 0.001$, $n = 18$.

through plant physiological processes during CO_2 fixation in C3 plants with the ratio of intercellular to ambient CO_2 partial pressure (C_i/C_a):

$$\Delta^{13}\text{C} = a + (b - a) * C_i/C_a \quad (4)$$

where a ($=4.4\text{‰}$) refers to the slower diffusivity of $^{13}\text{CO}_2$ relative to $^{12}\text{CO}_2$ in air and b ($=27\text{‰}$) is the isotopic fractionation caused by enzymatic C fixation. C_a was obtained from published data². It should be noted that $\Delta^{13}\text{C}$ is determined by the ratio of chloroplast to the ambient CO_2 mole fraction (C_c/C_a) rather than C_i/C_a , as used in equation 3, making the here calculated value sensitive to mesophyll conductance (g_m ; Seibt et al., 2008). The latter varies in accordance to changes in environmental conditions such as temperature, irradiance, water and CO_2 availability (Flexas et al., 2008). Consequently using C_a may be problematic if g_m to CO_2 is not constant (Seibt et al., 2008). However, as information on mesophyll conductance of the three conifers under study is not available and published means of g_m would not improve results (Cernusak et al., 2013), we chose using the simplified linear model of Farquhar et al. (1982). Hence, iWUE, i.e., the ratio of the net carbon gain (A) versus leaf conductance for water vapor (g_w), was calculated as follows:

$$\text{iWUE} = A/g_w = C_a(b - \Delta^{13}\text{C}/C_a)/1.6 * (b - a) \quad (5)$$

where 1.6 is the ratio between the diffusivities of water vapor and CO_2 in air.

Enrichment in ^{18}O in tree rings over source water ($\Delta^{18}\text{O}$), resulting from incorporation of ^{18}O -enriched photoassimilates into stem biomass, was calculated from $\delta^{18}\text{O}$ of tree ring organic matter ($\delta^{18}\text{O}_{\text{trng}}$) and precipitation ($\delta^{18}\text{O}_{\text{prep}}$) according to:

$$\Delta^{18}\text{O}(\text{‰}) = (\delta^{18}\text{O}_{\text{trng}} - \delta^{18}\text{O}_{\text{prep}})/(1 + \delta^{18}\text{O}_{\text{prep}}/1000) \quad (6)$$

²<http://cdiac.ornl.gov/trends/co2/sio-mlo.html>

TABLE 1 | $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ difference between cellulose and bulk wood in annual growth rings of *Pinus cembra*, *Picea abies* and *Larix decidua*.

Species	$\delta^{13}\text{C}$ [‰]	$\delta^{18}\text{O}$ [‰]
<i>P. cembra</i>	1.1 ± 0.0	4.4 ± 0.4
<i>P. abies</i>	1.0 ± 0.1	5.0 ± 0.6
<i>L. decidua</i>	1.1 ± 0.1	4.9 ± 0.4

Precipitation is the only source of water on Mt. Patscherkofel and thus was assumed to reflect source water of trees. Furthermore, as there is evidence from a treeline in the Central Swiss Alps that mean values of $\delta^{18}\text{O}_{\text{prep}}$ and $\delta^{18}\text{O}$ of soil water do not differ significantly, although soil water $\delta^{18}\text{O}$ carries a distinct signal from snow melt water far into the growing season (Treydte et al., 2014), we used annual means of $\delta^{18}\text{O}_{\text{prep}}$ sampled on top of Mt. Patscherkofel (2246 m a.s.l.) (Umweltbundesamt Austria; personal communication) approximately 300 m south of the selected study trees for our $\Delta^{18}\text{O}$ calculation. We elevationally corrected $\delta^{18}\text{O}_{\text{prep}}$ by a factor of 0.17‰ per 100 m of elevation (Umweltbundesamt Austria; personal communication) which is considerably lower than the global mean of 0.28‰ per 100 m of elevation (Poage and Chamberlain, 2001).

Leaf Level Gas Exchange Data

To illustrate long term trends in foliar CO_2 and H_2O gas exchange we compiled published gas exchange data of mature, field grown *P. cembra* and *L. decidua* trees carried out at our study site between 1979 and 2007 (Table 2). Maximum net photosynthetic capacity at ambient CO_2 (A_{max} ; sensu Larcher, 2001) was assessed of sun exposed twigs from the upper canopy. Employed *in situ* were thermoelectrically climate-controlled cuvettes (Walz, Effeltrich, Germany) or a portable exchange system (CIRAS 1, PP Systems, Hitchin, Hertfordshire, UK). For methodological details see publications given in Table 2.

TABLE 2 | A comparison of published maximum net photosynthetic capacity ($A_{\max}$) and leaf conductance for water vapor of sun exposed shoots of mature *Pinus cembra* and *Larix decidua* trees at the lower end of the treeline ecotone on Mt. Patscherkofel.

Species	Year	Measured trees	$A_{\max}$ [$\mu\text{mol m}^{-2} \text{s}^{-1}$]	g_w [$\text{mmol m}^{-2} \text{s}^{-1}$]	Reference
<i>P. cembra</i>	1979	1	3.4	n d	Havranek, 1981
<i>P. cembra</i>	2002	2	4.6 ± 0.2	n d	Wieser et al., 2005
<i>P. cembra</i>	2007	3	5.2 ± 0.7	n d	Wieser et al., 2010
<i>L. decidua</i>	1980	1	3.3	48	Benecke et al., 1981
<i>L. decidua</i>	1993	4	5.6 ± 0.9	85 ± 14	Volgger, 1995

n d, not determined.

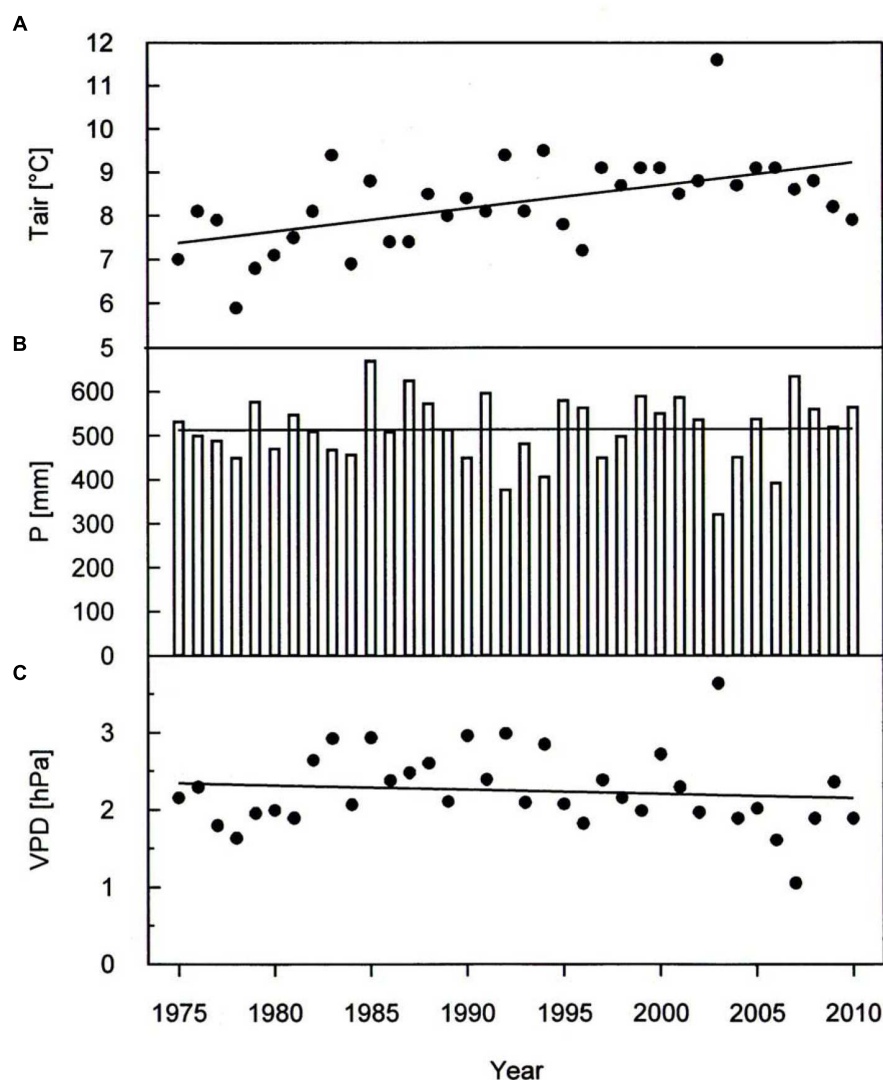


FIGURE 2 | Temporal variation in (A) growing season mean air temperature (T_{air}), (B) total growing season precipitation (P) and (C) growing season mean vapor pressure deficit (VPD) during the period 1975 throughout 2010. Data were fit by linear regression analysis: $T_{\text{air}}: y = 0.053x - 97.0$, $r^2 = 0.30$, $P < 0.001$; $P: y = 0.075x - 365.3$, $r^2 = 0.00$, $P = 0.95$; $\text{VPD}: y = -0.055x + 13.3$, $r^2 = 0.01$, $P = 0.49$.

Statistical Analysis

Temperature, precipitation, vapor pressure deficit, $\Delta^{13}\text{C}$, $\Delta^{18}\text{O}$, BAI, C_i , C_i/C_a and iWUE trends were calculated for the time period 1975–2010 by least-squares linear regression analysis.

For a given variable, differences among trends (slopes) between *P. cembra*, *P. abies* and *L. decidua* were assessed by the two-slope comparison test (Zar, 1999). We used repeated measures ANOVA to detect significant differences in the mean values

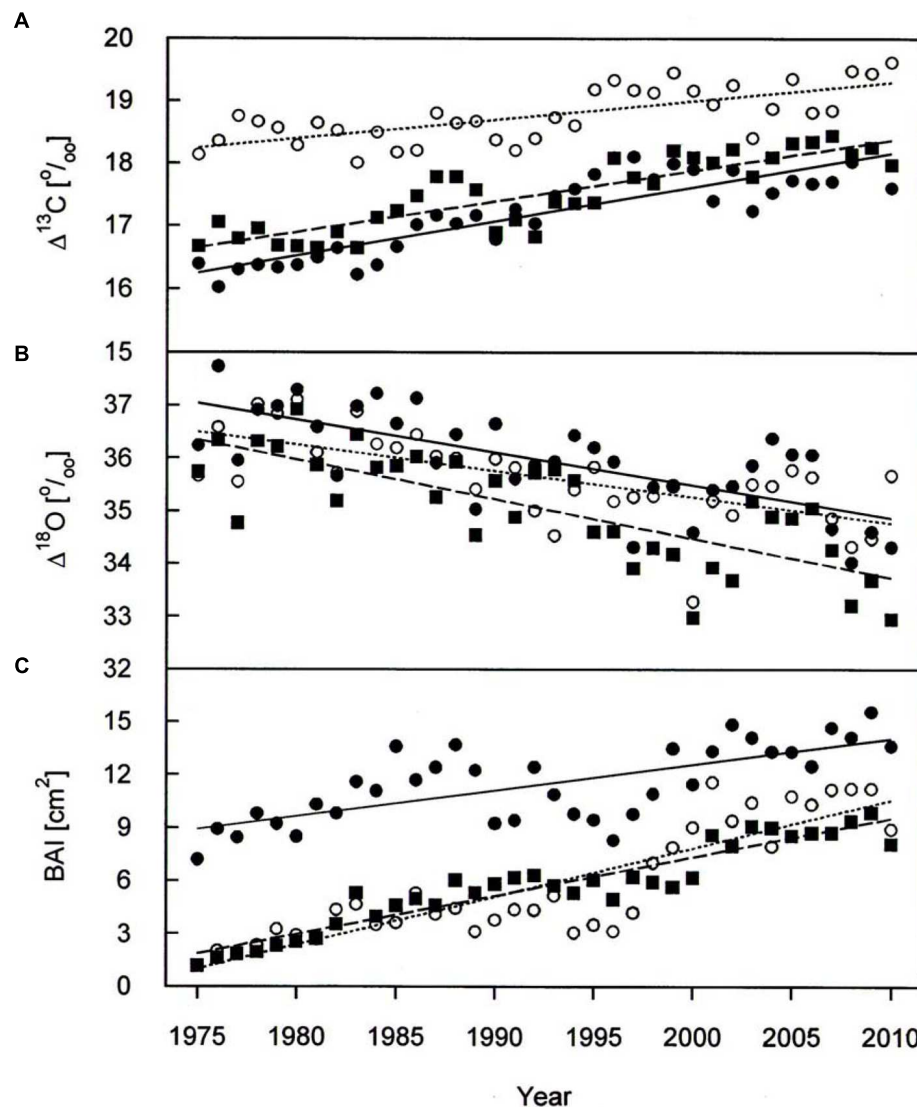


FIGURE 3 | $\Delta^{13}\text{C}$ (A), $\Delta^{18}\text{O}$ (B), and BAI (C) chronologies of *P. cembra* (solid circle, solid line), *P. abies* (solid square, dashed line) and *L. decidua* (open circle, dotted line) between 1975 and 2010. See Table 2 for regression information.

(1975–2010) of $\Delta^{13}\text{C}$, $\Delta^{18}\text{O}$, BAI, C_i , C_i/C_a and iWUE of *P. cembra*, *P. abies* and *L. decidua*. Following Kunter et al. (2004) we used multiple least-squares linear regression models to assess the influence of atmospheric CO_2 concentration (C_a) and mean growing season (May–Sep) air temperature (T_{veg}) and their interactions (explanatory variables) on tree-ring variables. For assessing the climatic impact on tree ring variables (BAI, $\Delta^{13}\text{C}$, and $\Delta^{18}\text{O}$) statistical analyses were based on mean monthly air temperature (°C) and total monthly precipitation (mm) throughout the study period (1975–2010). For each species Pearson's correlation coefficients between BAI, isotope chronologies and both climate variables were calculated from August of the year prior to growth to September of the growth year. All the statistical analysis were conducted by use of the SPSS 16 software package (SPSS, Inc. Chicago, IL, USA), and

a probability level of $P < 0.05$ was considered as statistically significant. As suggested by Sarris et al. (2013) we did not remove any age related trend from our tree-ring chronologies by conventional detrending procedures, thus avoiding the risk of removing any environmental signal or trend captured by our tree-ring series.

RESULTS

Inter-annual Trends in Climate and Tree-Ring Indices

A warming trend is reflected at our treeline site during the growing seasons (0.50°C per decade $P < 0.001$) of 1975–2010, without concurrent trends in precipitation and vapor pressure

deficit (Figure 2). During the whole study period $\Delta^{13}\text{C}$ and $\Delta^{18}\text{O}$ chronologies were synchronized between the three studied species. In each species $\Delta^{13}\text{C}$ increased over time (Figure 3A; Table 3) whereas $\Delta^{18}\text{O}$ decline (Figure 3B; Table 3). The increase in $\Delta^{13}\text{C}$ was accompanied by rising of A_{max} for both *P. cembra* (1979–2007) and *L. decidua* (1980–1993) by about 50%. Likewise, g_w increase by about 75% in *L. decidua* (Table 3).

The mean tree-ring $\Delta^{13}\text{C}$ was highest in *L. decidua*, although the increase was significantly higher in *P. cembra* and *P. abies* (Table 4). Temporal changes in $\Delta^{18}\text{O}$ by contrast, did not differ significantly between the tree species (Table 4). *P. cembra* showed the highest $\Delta^{18}\text{O}$ while *P. abies* presented the lowest $\Delta^{18}\text{O}$ and *L. decidua* displayed an intermediate mean (Table 4). On average, growth of *P. cembra* was significantly higher than growth of *P. abies* and *L. decidua* (Figure 3C; Table 4). During the whole study period all three species showed an increase in growth expressed as BAI, being significantly lower in *P. cembra* than *P. abies*, and *L. decidua* (Figure 3C; Table 4).

Paralleling atmospheric CO_2 enhancement (Figure 4A), tree ring derived C_i increased from 1975 through 2010 from 180 to 234 $\mu\text{mol mol}^{-1}$ in *P. cembra*, from 185 to 227 $\mu\text{mol mol}^{-1}$ in *P. abies* and from 213 to 256 $\mu\text{mol mol}^{-1}$ in *L. decidua* (Figure 4A; Table 2). Although species specific differences in the temporal change of C_i were not statistically significant different from each other, mean C_i was significantly lower in *P. cembra* and *P. abies* as compared to *L. decidua* (Table 4). Averaged over the study period *P. cembra* showed the lowest and *L. decidua*, the highest C_i/C_a , while the C_i/C_a of *P. abies* was intermediate (Table 4). The increase in C_i/C_a over time (Figure 4B; Table 3) was significantly higher in *P. cembra* and *P. abies* than in *L. decidua* (Table 4). In all the three species iWUE had remained stable during the study period (Figure 4C; Table 3). However, we observed statistically significant between species, with *P. cembra* showing the highest and *Larix decidua* showing the lowest iWUE averaged over the study period (Table 4).

TABLE 3 | Regression information for Figures 3 and 4.

Variable	Species	Equation	r^2	P-value
$\Delta^{13}\text{C}$	<i>P. cembra</i>	$y = 0.054x - 91.3$	0.77	<0.001
	<i>P. abies</i>	$y = 0.049x - 79.2$	0.76	<0.001
	<i>L. decidua</i>	$y = 0.030x - 40.1$	0.49	<0.001
$\Delta^{18}\text{O}$	<i>P. cembra</i>	$y = -0.062x + 159.9$	0.50	<0.001
	<i>P. abies</i>	$y = -0.075x + 184.7$	0.60	<0.001
	<i>L. decidua</i>	$y = -0.056x + 148.0$	0.51	<0.001
BAI	<i>P. cembra</i>	$y = 0.147x - 281.9$	0.50	<0.001
	<i>P. abies</i>	$y = 0.219x - 430.8$	0.90	<0.001
	<i>L. decidua</i>	$y = 0.273x - 538.4$	0.77	<0.001
C_i	<i>P. cembra</i>	$y = 1.81x - 3392.5$	0.94	<0.001
	<i>P. abies</i>	$y = 1.75x - 3296.0$	0.94	<0.001
	<i>L. decidua</i>	$y = 1.56x - 2873.3$	0.94	<0.001
C_i/C_a	<i>P. cembra</i>	$y = 0.002x - 4.33$	0.77	<0.001
	<i>P. abies</i>	$y = 0.002x - 3.88$	0.76	<0.001
	<i>L. decidua</i>	$y = 0.001x - 2.18$	0.62	<0.001
iWUE	<i>P. cembra</i>	$y = -0.085x + 249.4$	0.09	0.067
	<i>P. abies</i>	$y = -0.061x + 199.5$	0.06	0.148
	<i>L. decidua</i>	$y = 0.045x - 21.9$	0.05	0.215

TABLE 4 | Tree-ring carbon isotope characteristics ($\Delta^{13}\text{C}$, $\Delta^{18}\text{O}$, BAI, C_i , C_i/C_a , and iWUE) in *P. cembra*, *P. abies*, and *L. decidua* during the period 1975–2010.

	<i>P. cembra</i>		<i>P. abies</i>		<i>L. decidua</i>	
	Change	Average ($\pm\text{SE}$)	Change	Average ($\pm\text{SE}$)	Change	Average ($\pm\text{SE}$)
$\Delta^{13}\text{C}$ [‰]	1.9^a	17.2 $\pm$ 0.7 ^a	1.8^a	17.3 $\pm$ 0.6 ^a	1.1^b	18.8 $\pm$ 0.484 ^b
$\Delta^{18}\text{O}$ [‰]	-2.2^a	35.9 $\pm$ 0.9 ^a	-2.7^a	35.0 $\pm$ 1.052 ^b	-2.0^a	35.6 $\pm$ 0.883 ^c
BAI [cm ²]	5.3^a	7.6 $\pm$ 3.8 ^a	7.9 ^b	5.7 $\pm$ 2.473 ^b	9.8^b	5.8 $\pm$ 3.3 ^b
C_i [$\mu\text{mol mol}^{-1}$]	65.2^a	203.5 $\pm$ 19.6 ^a	63.06^a	208.5 $\pm$ 19 ^a	56.2^a	228.8 $\pm$ 16.9 ^b
C_i/C_a	0.1^a	0.6 $\pm$ 0.03 ^a	0.1^a	0.6 $\pm$ 0.03 ^b	0.00^b	0.6 $\pm$ 0.03 ^c
iWUE [$\mu\text{mol mol}^{-1}$]	-3.1 ^a	81.0 $\pm$ 2.9 ^a	-2.2 ^a	78.4 $\pm$ 2.6 ^b	-1.6 ^a	67.8 $\pm$ 2.2 ^c

Significant changes from 1975–2010 at $P < 0.05$ are in bold and italics. Between species differences in change and average ($\pm\text{SE}$) are marked with different letters. Change values were calculated as the slope of the corresponding least-squares linear regressions (Table 2) multiplied by the number of years of the study period 1975–2010 (=36).

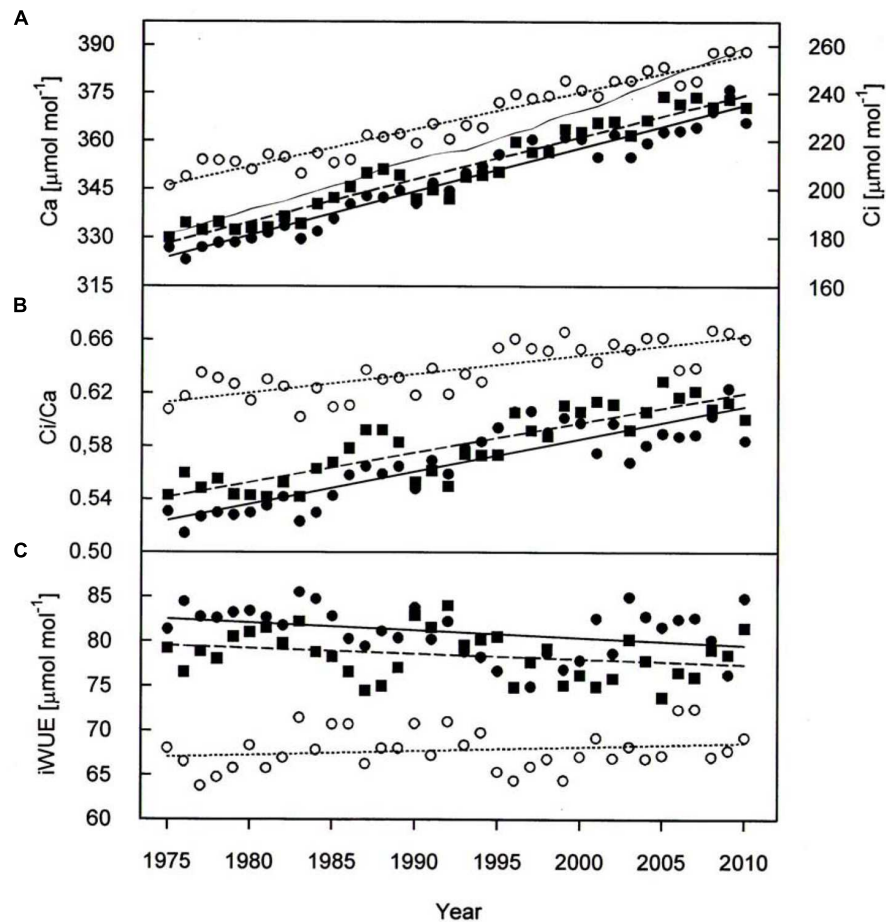


FIGURE 4 | (A) Ambient atmospheric CO₂ concentration (C_a, thin solid line) C_i, **(B)** C_i/C_a, and **(C)** iWUE chronologies of *P. cembra* (solid circle, solid line), *P. abies* (solid square, dashed line) and *L. decidua* (open circle, dotted line) between 1975 and 2010. See **Table 2** for regression information.

Effects C_a and T_{veg} on Δ¹³C, Δ¹⁸O, BAI, C_i, C_i/C_a and iWUE

Multiple linear regression analysis show that Δ¹³C and Δ¹⁸O of all species significantly increased with increasing ambient CO₂ concentration (C_a) while growing season mean air temperature (T_{veg}) had no effect on Δ¹³C and on Δ¹⁸O (**Table 5**). Growth (BAI) of *P. cembra*, and *P. abies* significantly increased with increasing C_a and T_{veg}. *L. decidua* presented a significant increase in BAI at increasing C_a without any response to T_{veg} (**Table 5**). For all species we found a significant increase in C_i and C_i/C_a at higher C_a but not at higher T_{veg} (**Table 5**). iWUE of *P. cembra*, *P. abies*, and *L. decidua*, however, did not significantly respond to increasing C_a and T_{veg} (**Table 5**).

Δ¹³C, Δ¹⁸O, and BAI Response to Climate (Climate-Growth Relationships)

Climate-response relationships of Δ¹³C, Δ¹⁸O, and BAI differed both in time and in signal strength (**Figures 5 and 6**). In all three species Δ¹³C was significantly positive correlated with April

throughout June temperatures (**Figure 5A**) and significantly negative correlated with January precipitation (**Figure 6A**). Previous-year August and October temperature also favored Δ¹³C in *P. cembra* and in *P. abies*, respectively (**Figure 5A**), whereas previous- and current-year August precipitation did so in *P. abies* (**Figure 6A**).

The effects of temperature and precipitation on Δ¹⁸O were clearly in opposite directions (**Figures 5B and 6B**). In all the three species tree-ring Δ¹⁸O was negative correlated to air temperature from April to June of the current year and significantly positive correlated to previous-year December temperature (**Figure 5B**) as well as to January precipitation (**Figure 6B**). From **Figure 5B**, previous- year August temperature had a negative correlation with *L. decidua* and current-year January temperature showed a negative correlation with *L. decidua*. Previous-year November and current-year March precipitation showed a negative correlation with Δ¹⁸O in *L. decidua*, as did June precipitation in *P. cembra* (**Figure 6B**).

We also found significant positive correlations between BAI and temperature during April, May, and June in

TABLE 5 | Summary of multiple linear regression models fitted to explain inter-annual changes (1975–2010) in $\Delta^{13}\text{C}$, $\Delta^{18}\text{O}$, BAI, C_i , C_i/C_a , and iWUE of *P. cembra*, *P. abies*, and *L. decidua* in response to atmospheric CO_2 concentration (C_a) and mean growing season (May–Sep) air temperature (T_{veg}).

Species	Variable	coefficient	SE	β	t-value	r-value partial	P-value
$\Delta^{13}\text{C}$							
<i>P. cembra</i>	Intercept	5.483	1.225		4.477		<0.001
	C_a	0.034	0.004	0.903	8.584	0.831	<0.001
	T_{veg}	−0.058	0.067	−0.091	−0.865	0.149	0.393
<i>P. abies</i>	Intercept	6.797	1.054		6.446		<0.001
	C_a	0.031	0.003	0.925	9.187	0.848	<0.001
	T_{veg}	−0.064	0.058	−0.111	−1.106	−0.189	0.277
<i>L. decidua</i>	Intercept	11.883	1.027		11.572		<0.001
	C_a	0.023	0.003	0.901	6.917	0.769	<0.001
	T_{veg}	−0.164	0.056	−0.378	−2.900	−0.451	0.007
$\Delta^{18}\text{O}$							
<i>P. cembra</i>	Intercept	49.624	2.360		21.030		<0.001
	C_a	−0.039	0.008	−0.742	−5.158	−0.668	<0.001
	T_{veg}	0.054	0.130	0.060	0.419	0.073	0.678
<i>P. abies</i>	Intercept	51.708	2.363		21.886		<0.001
	C_a	−0.050	0.008	−0.842	−6.513	−0.750	<0.001
	T_{veg}	0.143	0.130	0.143	1.0103	0.189	0.278
<i>L. decidua</i>	Intercept	45.863	2.219		20.671		<0.001
	C_a	−0.026	0.007	−0.574	−3.671	−0.538	0.001
	T_{veg}	−0.094	0.122	−0.120	−0.768	−0.0132	0.448
BAI							
<i>P. cembra</i>	Intercept	−19.964	5.482		−3.642		0.001
	C_a	0.079	0.018	0.627	4.472	0.614	<0.001
	T_{veg}	0.357	0.301	0.166	1.184	0.202	<0.001
<i>P. abies</i>	Intercept	−40.583	2.643		−15.354		<0.001
	C_a	0.120	0.009	0.855	13.990	0.925	<0.001
	T_{veg}	0.401	0.145	0.169	2.761	0.433	0.009
<i>L. decidua</i>	Intercept	−53.532	5.382		−9.947		<0.001
	C_a	0.159	0.017	0.844	9.128	0.846	<0.001
	T_{veg}	0.272	0.296	0.085	0.919	0.158	0.365
C_i							
<i>P. cembra</i>	Intercept	−188.830	19.045		−9.915		<0.001
	C_a	1.119	0.062	0.991	18.130	0.953	<0.001
	T_{veg}	−1.033	1.047	−0.054	−0.987	−0.169	0.331
<i>P. abies</i>	Intercept	−177.500	17.308		−10.255		<0.001
	C_a	1.100	0.056	0.996	19.623	0.960	<0.001
	T_{veg}	−1.014	0.952	−0.054	−1.065	−0.182	0.294
<i>L. decidua</i>	Intercept	−113.938	14.692		−7.755		<0.001
	C_a	0.987	0.048	1.011	20.726	0.964	<0.001
	T_{veg}	−1.313	0.808	−0.079	−1.625	−0.272	0.114
C_i/C_a							
<i>P. cembra</i>	Intercept	0.037	0.054		0.680		0.501
	C_a	0.002	0.000	0.906	8.752	0.836	<0.001
	T_{veg}	−0.003	0.003	−0.087	−0.843	−0.145	0.405
<i>P. abies</i>	Intercept	0.087	0.049		1.795		0.082
	C_a	0.001	0.000	0.918	9.118	0.864	<0.001
	T_{veg}	−0.003	0.003	−0.096	−0.995	−0.164	0.347
<i>L. decidua</i>	Intercept	0.321	0.041		7.817		<0.001
	C_a	0.001	0.000	0.887	7.240	0.783	<0.001
	T_{veg}	−0.004	0.002	−0.0193	−1.574	−0.264	0.125
iWUE							
<i>P. cembra</i>	Intercept	98.731	9.964		9.909		<0.001
	C_a	−0.062	0.032	−0.372	−1.915	−0.316	0.064
	T_{veg}	0.534	0.548	0.189	0.974	0.161	0.337
<i>P. abies</i>	Intercept	92.903	9.052		10.263		<0.001
	C_a	−0.053	0.029	−0.351	−1.798	−0.299	0.081
	T_{veg}	0.534	0.534	0.210	1.072	0.184	0.291

(Continued)

TABLE 5 | Continued

Species	Variable	coefficient	SE	β	t-value	r-value partial	P-value
<i>L. decidua</i>	Intercept	59.644	7.661		7.785		<0.001
	C_a	0.007	0.025	0.052	0.270	0.047	0.789
	T_{veg}	0.695	0.421	0.317	1.650	0.276	0.108

Explanatory variables significantly influencing $\Delta^{13}C$, $\Delta^{18}O$, BAI, C_i , C_i/C_a , and iWUE at $P < 0.05$ are in bold and italics. Multiple linear regressions to obtain a relationships between (dependent) tree-ring variables and the explanatory variables C_a and T_{veg} were calculated according to: Tree-ring variable = $a + (b \cdot C_a) + (c \cdot T_{veg})$, where a , b , and c are fitting coefficients. Note that the β -coefficient expresses the relative importance of each explanatory variable in standardized terms. The direction of the relationships between variables (plus or minus) of the β -coefficients and the Pearson's correlation coefficient indicates whether the relationship between the explanatory variable and the dependent variable is positive or negative.

P. cembra, *P. abies*, and *L. decidua* (Figure 5C). Current-year August temperature also favored BAI in *L. decidua* and *P. abies*, and also previous-year October temperature in *P. abies* (Figure 5C). The correlations between BAI and precipitation were weak, except for significant positive correlations in February and current-year August in *P. cembra* and a significant negative correlation in January in *P. abies* (Figure 6C).

DISCUSSION

Similar growth and $\Delta^{13}C$, and $\Delta\delta^{18}O$ responses were found over time in *P. cembra*, *P. abies* and *L. decidua* at the lower edge of the treeline ecotone in the central Austrian Alps. From 1975 throughout 2010 the three species increased $\Delta^{13}C$ and BAI, while $\Delta^{18}O$ showed a declining trend. Apparently, underlying response mechanisms were similar across the three studied species.

Our observed correlations for temperature and precipitation for $\Delta^{13}C$ and $\Delta^{18}O$ (Figures 5 and 6) are consistent with results reported for oak and pine trees at temperate sites in Switzerland (Saurer et al., 2008). Weather conditions prevailing during April through June predominantly were responsible for variations in tree-ring $\Delta^{13}C$, $\Delta^{18}O$, and BAI of *P. cembra*, *P. abies*, and *L. decidua*. We found positive correlations between April to June temperatures and $\Delta^{13}C$. $\Delta^{13}C$ is strongly affected by net CO_2 uptake rates, which at treeline are governed by both photon flux density and temperature (Treydte et al., 2001; McCarroll and Pawellek, 2004; Kress et al., 2011) as well as enhanced plant transpiration (Liu et al., 2015). Moreover, at our study site *in situ* net photosynthetic capacity of sun exposed twigs from the upper canopy of mature *P. cembra* and *L. decidua* trees measured under clear summer days also tended to increase between 1979 and 2007 (Table 2), which might be attributed to both the observed increase in atmospheric C_a and T_{air} . Additionally, the temperature optimum of A_{max} for *P. cembra* increased from 12.5°C in 1956 (Pisek et al., 1969, 1973) to 15.0°C in 2002 (Wieser, 2004) and to 17.1°C in 2007 (Wieser et al., 2010), matching the observed increase in mean growing season air temperature of 0.9°C per decade (Figure 2). An increase in net photosynthetic rates under elevated CO_2 was also observed in *P. mugo* and *L. decidua* after nine years of free-air CO_2 enrichment at the Swiss treeline (Dawes et al., 2013; Streit et al., 2014). Three years of ecosystem warming also increased carbon

uptake of *Pinus cembra* at treeline in the Austrian Alps (Wieser et al., 2015).

Our precipitation signals suggest that trees do not suffer from moisture stress. Indeed the observed declining trend $\Delta^{18}O$ and the strong negative correlations between $\Delta^{18}O$ and growing season precipitation is consistent with the physiological isotopic responses (Barbour and Farquhar, 2000), suggesting that stomatal conductance is increased during the study period. Although a leaf physiological signal in $\delta^{18}O$ will be dampened at the level of tree rings due to oxygen exchange with source water during cellulose biosynthesis, impact of g_w on ^{18}O in tree rings may be still detectable, even in whole wood analyses (Weigt et al., 2015). Ecosystem warming accompanied by unchanged VPD also increased g_w and hence also transpiration in boreal *Picea abies* (Bergh and Linder, 1999), *Pinus sylvestris* (Kellomäki and Wang, 1998), *Picea mariana* (Van Herk et al., 2011), *Pinus cembra* at treeline (Wieser et al., 2015), and *Populus deltoides* (Barron-Gafford et al., 2007). Thus, it seems that in cold environments under non-limiting water availability increasing temperatures counteract the diminishing effect of rising CO_2 on leaf conductance (Saurer et al., 2014).

The observed positive correlations between BAI and April–June temperatures are also reflected in wood formation. At the study site wood formation of larch, spruce and pine generally starts in May, reaches its maximum in June, and terminates in August (Havranek, 1981; Loris, 1981; Gruber et al., 2009). Beside summer temperatures (Figure 5C) other climatic variable like winter and August precipitation (Figure 6C) are also known to influence radial growth at treeline as shown for *P. cembra* by Oberhuber (2004), reflecting minor soil water effects on tree growth at treeline (Tranquillini, 1979; Wieser, 2004). Although treeline trees are saturated with carbohydrates (Gruber et al., 2011), growth of trees at treeline is primarily affected by temperature dependent carbon sink activity during tissue formation (Hoch and Körner, 2003, 2012). The observed increase in BAI (Figure 3C) suggests that our treeline trees benefit from climate warming, although effects of CO_2 fertilization on growth may not completely ruled out (Table 5). Four years of experimental air warming with open-top-chambers also stimulated radial growth of *Picea glauca* seedlings at the subarctic treeline in southwest Yukon, Canada (Danby and Hik, 2007). Thus, when growth is stimulated and there is plenty of water g_w can increase as indicated by a decline in $\Delta^{18}O$ (Figure 3B) along with increasing A , resulting in a constant iWUE (Figure 4C).

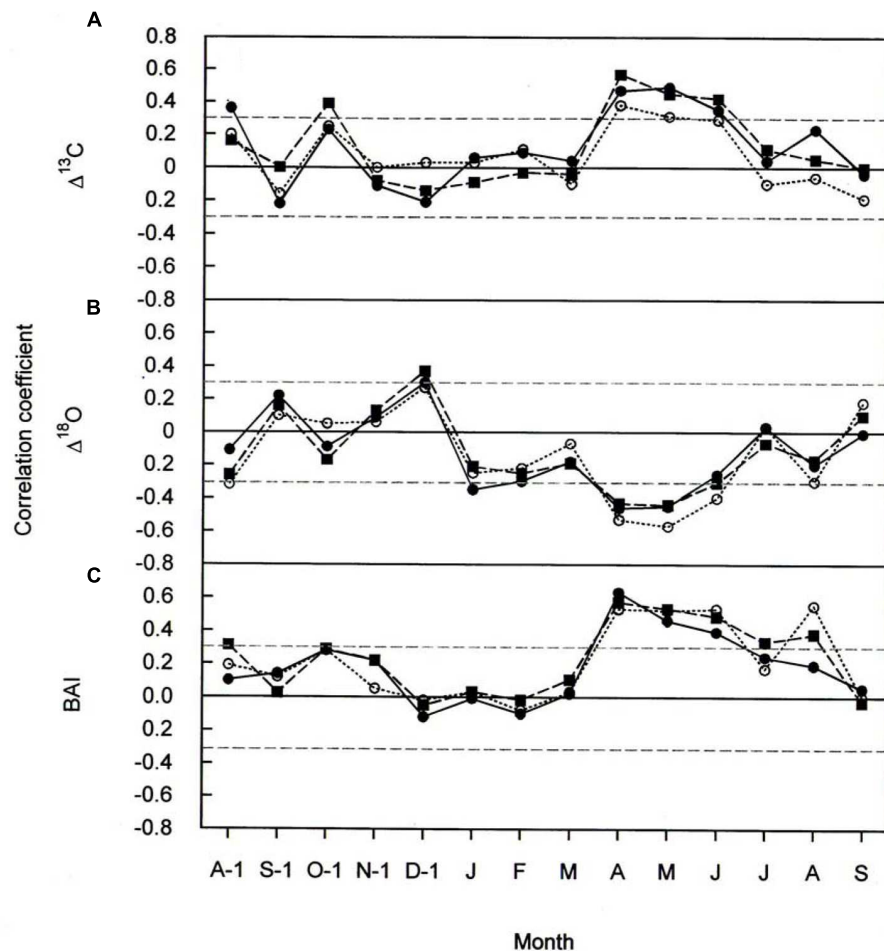


FIGURE 5 | Pearson correlation coefficients between (A) $\Delta^{13}\text{C}$ (B) $\Delta^{18}\text{O}$, and (C) BAI, and monthly mean temperature for *P. cembra* (solid circle, solid line), *P. abies* (solid square, dashed line) and *L. decidua* (open circle, dotted line) between 1975 and 2010. Horizontal gray lines indicate $P < 0.05$.

Elevated atmospheric CO_2 is expected to affect plant carbon-water relationships, as a decline in stomatal conductance is often observed when plants are exposed to elevated CO_2 (Battipaglia et al., 2013). If stomatal conductance declines under increasing CO_2 in combination with an increased or unchanged carbon assimilation, this will decrease the C_i to C_a ratio and thus decrease $\Delta^{13}\text{C}$. Conversely, in all three study species $\Delta^{13}\text{C}$ increased from 1975 throughout 2010, while tree-ring derived iWUE remained stable (Figure 4C) although ambient CO_2 concentration increased by $60 \mu\text{mol mol}^{-1}$ (Figure 2B). The stability of iWUE resulted as C_i drifted upward paralleling the rise in C_a (Figure 2B). Likewise, in *Picea schrenkiana* at treeline in the western Tianshan Mountains in China iWUE remained also unchanged from 1985 to 2010 (Wu et al., 2015). No change in iWUE (i.e., homeostasis) over the last 100 years was also reported for three conifer species in the Selkirk Range (Rocky Mountains, Idaho, ID, USA) by Marshall and Monserud (1996). Other studies by contrast observed a 20% increase in iWUE from the 1960 throughout 2000 in mature trees in tropical, arid, Mediterranean, wet temperate and boreal forests distributed

through Europe, Asia, Africa, America, and Oceania (Penuelas et al., 2011; Saurer et al., 2014; Frank et al., 2015). In these latter studies, increasing iWUE was attributed to the combined effect of increasing CO_2 and climate change-induced soil drying that reduced stomatal aperture. Soil drought can be ruled out along the treeline ecotone of the Central Alps (Mayr, 2007; Wieser, 2012). Occurrence of soil drought strongly depends on site conditions such as precipitation patterns, water holding capacity of the soil, and evaporative demand. Ample precipitation and moderate evaporative demand in general cause soil water availability to be sufficiently high to meet the trees' water demand at treeline in the Central Tyrolean Alps (Mayr, 2007; Wieser et al., 2009). As a consequence, treeline trees are rarely forced to restrict transpiration (Tranquillini, 1979; Benecke et al., 1981; Matyssek et al., 2009; Wieser and Leo, 2012; Wieser et al., 2014, 2015). Given the ample soil water availability whole-tree conductance of *P. cembra*, *P. abies*, and *L. decidua* remains high for CO_2 uptake because leaf conductance for water vapor depends only on the evaporative demand driven by irradiance and vapor pressure deficit (Wieser, 2012).

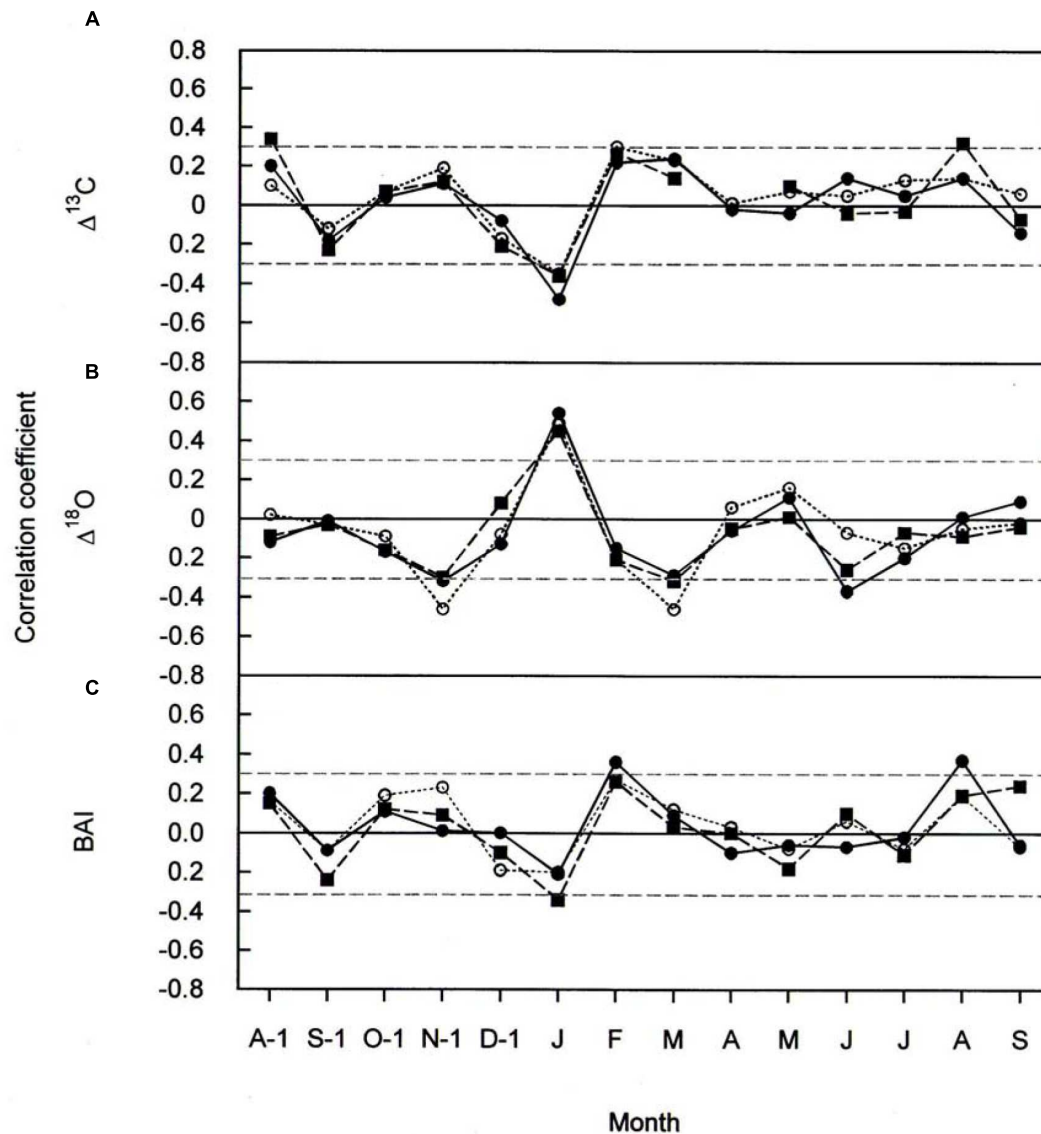


FIGURE 6 | Pearson correlation coefficients between (A) $\Delta^{13}\text{C}$ (B) $\Delta^{18}\text{O}$, and (C) BAI, and monthly total precipitation for *P. cembra* (solid circle, solid line), *P. abies* (solid square, dashed line) and *L. decidua* (open circle, dotted line) between 1975 and 2010. Horizontal gray lines indicates $P < 0.05$.

Beside climate warming and increasing C_a , nitrogen deposition could also be important for explaining the observed increase in tree growth as increasing nitrogen deposition during the 1980ties (Smidt and Mutsch, 1993) has been suggested as a possible growth stimulator. However, there is evidence that nitrogen contents per needle dry mass are higher in trees at treeline as compared to trees growing at lower elevation sites (Körner, 1989; Birmann and Körner, 2009). Furthermore, since 1988, nitrogen deposition at treeline in the Tyrolean Alps is steadily declining (Amt der Tiroler Landesregierung, 2015), and a nitrogen fertilizer experiment at the alpine treeline in the Swiss Alps showed little or no growth stimulation (Keller, 1970). Thus, it seems that presently nitrogen deposition is insufficient to explain observed growth trends at treeline as

reported previously by Tranquillini (1979) and Nicolussi et al. (1995).

CONCLUSION

Treeline trees respond to the increasing atmospheric CO_2 level (C_a) in a way that their leaf-intercellular CO_2 concentration (C_i) drifted upward paralleling the rise in atmospheric CO_2 while iWUE remained stable over the last 36 years. The stability in iWUE was accompanied by an increase in BAI suggesting that treeline trees benefit from both recent climate warming and CO_2 fertilization. In addition, treeline trees are rarely forced to restrict

transpiration due to ample soil water availability (Tranquillini, 1979; Matyssek et al., 2009; Wieser et al., 2015). A stable iWUE suggests an increase of both carbon gain and leaf conductance for water vapor as also indicated by stable C and O isotope analysis and direct gas exchange assessments. Furthermore, iWUE may not change species composition at treeline in the Austrian Alps due to similar ecophysiological responses to climatic changes of the three sympatric study species. Our finding that growth of treeline associated conifers increases with slowly rising ambient CO₂ concentration and warming may be relevant for assessing complex growth models with empirical data, finally leading to model improvements and better estimations of forest-climate feedbacks.

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AUTHOR CONTRIBUTIONS

GW, RM, and TG conceived and designed the experiment. WO, AG, and ML performed the experiment. GW, WO, AG, and ML analyzed the data. GW, RM, and TG wrote the manuscript and WO and AG provided editorial advice.

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