

Effects of climate change on treeline trees in Sagarmatha (Mt. Everest, Central Himalaya)

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Abstract

Question: Tree growth at high altitude in the Himalayan region is limited by cold temperatures and also strongly influenced by the seasonality of the Asian monsoon. Understanding whether the ongoing increase in temperatures and changes in precipitation regimes in the Himalayan region can stimulate or limit tree growth is of particular importance to predict the local treeline dynamics.

Location: Altitudinal treeline (~4000 m a.s.l.) in the Sagarmatha (Mt. Everest) National Park (Central Himalaya, Eastern Nepal).

Methods: We assessed the relationships between ring widths and monthly precipitations and mean temperatures, and analysed cellulose stable isotopes ($\delta^{13}\text{C}$ and $\delta^{18}\text{O}$) and their derived C discrimination ($\Delta^{13}\text{C}$), and intrinsic water use efficiency (iWUE) in *Abies spectabilis* and *Betula utilis* at the Himalayan treeline.

Results: Growth of *A. spectabilis* strongly depended on summer temperatures, whereas that of *B. utilis* on spring precipitation. $\delta^{13}\text{C}$ and iWUE increased with time in both species, especially in *A. spectabilis*. The long-term decrease in $\Delta^{13}\text{C}$ was accompanied by an increase in $\delta^{18}\text{O}$ in both species, thus suggesting an increase in photosynthetic efficiency rather than a stronger stomatal control of transpiration.

Conclusions: Climate change is progressively reducing the physiological limitations due to low temperatures and low spring precipitations at the Central Himalayan tree-line, thus potentially facilitating a further altitudinal forest advance.

KEYWORDS

Abies spectabilis, *Betula utilis*, climate change, stable isotopes, tree growth, treeline

1 | INTRODUCTION

The limit to the tree growth form towards the forest limit at high altitude and latitude coincides approximately to the average temperature of $\sim 6^\circ\text{C}$ during the growing season (Körner and Paulsen, 2004). In such environments, increasing air temperature can have an important influence on plant metabolism, biomass production and natural forest dynamics. Because of climate warming, the length of

winter dormancy is commonly becoming shorter and the vegetative period longer (Chmielewski and Rötzer, 2001), determining a global trend of current treeline shifts towards higher latitudes and altitudes (Kullman, 1998; Körner and Paulsen, 2004; Harsch *et al.*, 2009). However, increasing air temperatures may also become limiting for plant survival and recruitment in cold environments with reduced water availability, as increasing air dryness determines higher evaporation rates from soil and leaf evapotranspiration, as recently observed in the southern Tibetan Plateau (Lyu *et al.*, 2019).

Himalayan regions are one of the most critical hotspots of climate change, with annual temperatures increasing more than three times the average rate of warming at a global scale, i.e. $+0.4^{\circ}\text{C}$ per decade (Xu *et al.*, 2009). As a consequence, ice caps and mountain glaciers are receding (Xu *et al.*, 2009), and the growing season is becoming longer (Shrestha and Gautam, 2012). However, tree growth at the Himalayan treeline is not only limited by cold air temperature, but also strongly influenced by the East Asian and Southwest Monsoons (Liang *et al.*, 2014), which determine arid conditions during the cold winter and abundant precipitation in summer (Qi *et al.*, 2013). Consequently, the treeline shifts towards higher elevations can be limited by precipitation during the beginning of the vegetative season (Sigdel *et al.*, 2018). Recent dendrochronological analyses on dominant tree species at the Central Himalayan treeline reported a positive effect of summer temperatures on tree growth, especially in *Abies spectabilis* (D. Don) Mirb., but also a certain degree of growth sensitivity to the amount of precipitation falling at the beginning of the growing season, especially in *Betula utilis* D. Don (Gaire *et al.*, 2014; Pandey *et al.*, 2018). Consequently, these studies raised the question whether conditions of pronounced air and soil dryness due to the low precipitation amounts during these periods of the year can potentially expose plants to drought stress, with negative effects on growth and seedlings establishment (Liang *et al.*, 2014; Pandey *et al.*, 2018). On the contrary, it could also be questioned whether warmer oceans due to climate change anticipate the monsoon season (Loo and Billa, 2015), thus determining conditions of overall better water availability at the beginning of the growing season, with a positive stimulus for tree physiology and growth.

Analysis of stable C and O isotopes in tree rings is a consolidated method to retrospectively investigate the tree physiological responses to climate variability (McCarroll and Loader, 2004; Barbour and Song, 2014; Gessler *et al.*, 2014). The ratio of heavier to lighter C stable isotopes ($\delta^{13}\text{C} = {}^{13}\text{C}/{}^{12}\text{C}$) in the plant biomass is lower compared to that in the atmosphere due to isotope fractionation by air diffusion through the stomata (i.e. CO_2 molecules containing ${}^{12}\text{C}$ diffuse more easily than CO_2 with ${}^{13}\text{C}$) and by the enzymatic discrimination of Rubisco against ${}^{13}\text{C}$ during CO_2 fixation (Farquhar *et al.*, 1989a). Dry environmental conditions can induce a reduction in stomatal conductance (g_s) to prevent excessive water losses with transpiration and/or the development of excessive xylem tension potentially causing the deterioration of the whole-tree hydraulic system (Liu *et al.*, 2015; Wolf and Anderegg, 2016). Consequently, CO_2 diffusion is limited, $\delta^{13}\text{C}$ of the air contained in the leaves increases progressively due to CO_2 fixation, but Rubisco's discrimination against ${}^{13}\text{C}$ becomes less effective, thus maximising the fixation of C relatively enriched in ${}^{13}\text{C}$ in the plant biomass. Alternatively, $\delta^{13}\text{C}$ of the plant biomass can increase under conditions favouring the enzymatic activity of Rubisco and a relative increase in the rate of CO_2 fixation, like nitrogen fertilization (Makino, 2003; Balster and Marshall, 2009), or an increase in temperature not associated with drought (Gessler *et al.*, 2014; Altieri *et al.*, 2015). Due to the impact of physiological

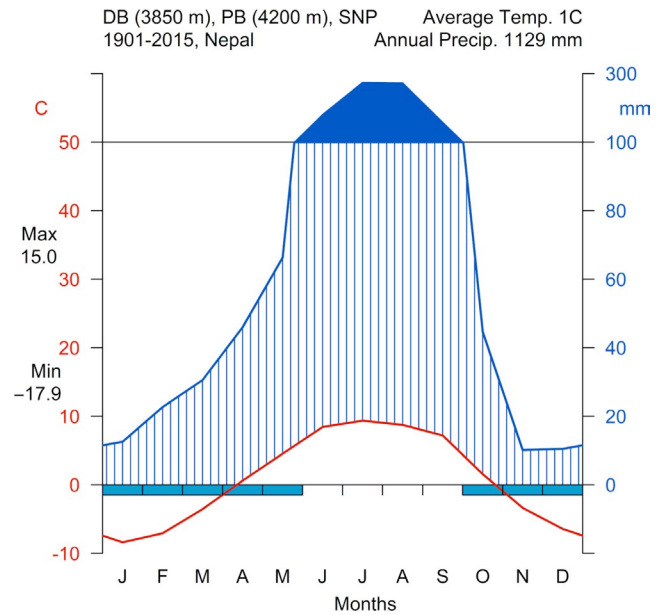


FIGURE 1 Climate diagram for the study area of Deboche (DB) and Pangboche (PB) based on the 1901–2015 weather records downloaded from the CRU dataset [Colour figure can be viewed at wileyonlinelibrary.com]

processes on carbon isotope fractionation, there is also a clear link between $\delta^{13}\text{C}$ and the water-use efficiency, i.e., the ratio between loss of water per carbon gained by the plant (Farquhar *et al.*, 1989b).

Analysis of the ratio of stable O isotopes ($\delta^{18}\text{O} = {}^{18}\text{O}/{}^{16}\text{O}$) can be a useful analytical tool to determine whether a variation in $\delta^{13}\text{C}$ in the biomass produced by a plant is due either to hydraulic limitations imposing an overall reduction in g_s or to an increase in C assimilation efficiency with photosynthesis (Saurer *et al.*, 2002; Battipaglia *et al.*, 2008; Leonelli *et al.*, 2017). Fractionation of the isotopes in water molecules occurs primarily during changes of its physical state, with molecules enriched in lighter isotopes evaporating relatively more easily, and those enriched in heavier isotopes condensating comparatively more easily. Therefore, assuming no changes in the isotopic composition of the source water, conditions favouring high water losses with transpiration rates (particularly dry air) will leave the produced plant biomass depleted in ${}^{16}\text{O}$ (i.e., $\delta^{18}\text{O}$ will become relatively higher). On the contrary, $\delta^{18}\text{O}$ will become relatively lower in humid conditions, which are usually accompanied by reduced transpiration.

In this study, we investigated tree physiological responses to the ongoing climate change at one of the world's highest treelines and climate change hotspots, the Khumbu Valley (Central Himalaya, Nepal). We retrospectively analysed tree-ring width, $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ time series to better understand whether tree physiology and growth can be further stimulated by an increase in precipitation at the beginning of the growing season or whether a warming-induced anticipation of spring phenology towards the dry winter could expose trees to higher risks of drought stress with negative effects on growth.

2 | METHODS

Our study area is located within the Sagarmatha (Mt. Everest) National Park (Central Himalaya, Eastern Nepal), where two permanent plots of 1 ha (100 m × 100 m) were established close to the altitudinal treeline at Deboche (DB, 27.83° N, 86.77° E, 3,850 m a.s.l.) and Pangboche (PB, 27.85° N, 86.79° E, 4,200 m a.s.l.). The climate of the region is strongly influenced by the subtropical Asian monsoon, with over 80% of the total annual precipitation falling in summer between June and September (Figure 1). Forest stands at both sites are dominated by two species (the target of this study), *Betula utilis* D.Don and *Abies spectabilis* (D.Don) Mirb., with a minor occurrence of *Juniperus recurva* Buch-Ham. ex D.Don, *Sorbus microphylla* (Wall. ex Hook.f.) Wenz., *Acer campbellii* Hook.f. & Thomson ex Hiern and an increasing presence of dwarf *Rhododendron campanulatum* D.Don towards the border with the alpine meadows (Singh and Singh, 1987). *Betula utilis* has a high freezing tolerance (Zobel and Singh, 1997), and in the Central Himalaya is mainly distributed on the moist areas of north-facing slopes from approximately 2,700 m a. s. l. up to the treeline (approximately 4,300 m a.s.l.). Instead, *Abies spectabilis* develops on mesic north-facing slopes between 2,800 up to more than 4,000 m a.s.l. (Shrestha and Chhetri, 2017), and is susceptible to frost damage (Tiwari *et al.*, 2017).

Sampling was performed in May 2016. For each species in both plots (DB and PB), we selected all trees with stem diameter >15 cm (145 *Abies spectabilis* and 113 *Betula utilis*) and extracted one core (0.5 cm in diameter) at breast height (1.3 m height) perpendicular to the direction of maximum slope steepness to avoid the presence of reaction wood.

2.1 | Ring-width measurements

On each tree core, ring widths (RW) were measured to the nearest 0.01 mm using a LINTAB table fitted with a stereoscope and equipped with TsapWin software (Rinntech, Heidelberg, Germany). To ensure correspondence between each annual ring and the calendar year of formation, the ring-width series were first visually and then statistically cross-dated using COFECHA software (Stokes and Smiley, 1968; Holmes, 1983). To assess inter-annual variability of RW we: (i) removed size/age trends commonly observed in RW (Carrer *et al.*, 2015) by fitting with a cubic smoothing spline with 50% frequency cut-off equal to 67% of the series length; (ii) computed the ratio between observed and fitted values to obtain detrended series (Cook and Kalriukstis, 1990); and (iii) averaged the detrended series from the trees by bi-weight robust mean (Cook, 1985) to obtain species-specific indexed chronologies of RW using the R-package dplR (Bunn, 2008). Pearson's correlation was then assessed for the relationships of the bi-weight robust mean of ring width (iRW) with meteorological data, i.e. total monthly precipitation and average monthly temperatures.

2.2 | Analyses of carbon and oxygen stable isotopes

Analyses of stable isotopes were conducted for a subset of cores (five per species per site) having the highest correlation between their RW chronologies. Cores were split into segments of 10-year xylem (i.e., produced in 10 years, accounting for missing rings potentially present) moving from bark to pith. These segments were then ground using a centrifugal mill (Mixer Mills, MM 200, Retsch, Haan, Germany) to a powder with a grain size of <0.05 mm diameter. Ground samples (approximately 10 mg) were packed in porous fibre filter bags (F57, 25-micron porosity, ANKOM Technology) and processed for cellulose extraction following established procedures. For each sample, we performed two washes with 5% sodium hydroxide (NaOH) solution for 2 hr at 60°C followed by a wash with 7% sodium chloride (NaClO₂) for 30 hr at 60°C in a Memmert water bath for better homogenization of cellulose. For each sample, approximately 1 mg of cellulose was weighed with a MX5 microbalance (Mettler Toledo) and then enclosed in a sliver capsule (3.3 × 5 mm). The abundance of stable isotopes of carbon ($\delta^{13}\text{C}$, i.e. the relative deviation of $^{13}\text{C}/^{12}\text{C}$ of the sample from the international standard Vienna Pee Dee Belemnite scale) and oxygen ($\delta^{18}\text{O}$, i.e. the relative deviation of $^{18}\text{O}/^{16}\text{O}$ of the sample from the international standard Vienna Standard Mean Ocean Water scale) was measured using a Continuous Flow Isotopic Ratio Mass Spectrometer (CF-IRMS) based on pyrolysis at 1,450°C (PYRO-cube, Elementar), coupled with a mass spectrometer (IRMS Delta V Advantage, Thermo Fisher Scientific) via a variable open split interface (Conflo III) (Woodley *et al.*, 2012). The analytical errors (standard deviations) of the isotope measurements were less than 0.2‰ and 0.3‰ for $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$, respectively.

$\delta^{13}\text{C}$ data were then detrended for the declining pattern of $\delta^{13}\text{C}$ in atmospheric CO₂ due to the increased fossil fuel emissions since the beginning of the industrial revolution (Keeling and Mook, 1979; Francey *et al.*, 1999), and the corrected $\delta^{13}\text{C}$ ($\delta^{13}\text{C}_{\text{corr}}$) was calculated as (McCarroll and Loader, 2004):

$$\delta^{13}\text{C}_{\text{corr}} = \delta^{13}\text{C} - (\delta^{13}\text{C}_{\text{air}} + 6.4) \quad (1)$$

where $\delta^{13}\text{C}$ and $\delta^{13}\text{C}_{\text{air}}$ are the ratio of carbon stable isotopes ($^{13}\text{C}/^{12}\text{C}$) of the tree ring sample and air, respectively, in a given year. A baseline of $\delta^{13}\text{C}_{\text{air}} = -6.4\text{‰}$ is assumed as the pre-industrial baseline value (Saurer and Aellen, 1997; McCarroll and Loader, 2004).

2.3 | Estimate of carbon discrimination ($\Delta^{13}\text{C}$) and intrinsic water-use efficiency (iWUE)

The total discrimination against ^{13}C relative to a given tree ring ($\Delta^{13}\text{C}$) was calculated as:

$$\Delta^{13}\text{C} = \frac{\delta^{13}\text{C}_{\text{air}} - \delta^{13}\text{C}}{1 + \frac{\delta^{13}\text{C}}{1,000}} \quad (2)$$

Since $\Delta^{13}\text{C}$ depends on the isotopic fractionation due to CO_2 diffusion from the atmosphere to the substomatal cavities of the mesophyll through stomata (a) and due to enzymatic fractionation by Rubisco (b), it can be calculated according to Farquhar and O'Leary (1982) as:

$$\Delta^{13}\text{C} (\%) = a + (b - a) \times \frac{C_i}{C_a} \quad (3)$$

where C_i and C_a are the CO_2 concentration in the leaf mesophyll and free atmosphere, respectively.

Combining Equations 2, and 3, and assuming a fixed C isotopic fractionation by air diffusion and by Rubisco's carboxylation of $a = 4.4\text{‰}$ and $b = 27\text{‰}$ (Farquhar *et al.*, 1982; Farquhar *et al.*, 1989b), respectively, it follows that C_i can be calculated as:

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

Since the stomatal conductance to water vapour (g_w) is 1.6 times higher than that of CO_2 (g_c), the intrinsic water-use efficiency (iWUE) can be calculated as:

$$\text{iWUE} = \frac{A}{g_w} = \frac{A}{1.6g_c} = \frac{g_c (C_a - C_i)}{1.6g_c} = \frac{(C_a - C_i)}{1.6} \quad (5)$$

where A is the C assimilation with photosynthesis.

The time series of $\delta^{13}\text{C}_{\text{corr}}$, $\Delta^{13}\text{C}$, iWUE and $\delta^{18}\text{O}$ were expressed based on anomaly data calculated at an individual level: for each tree, anomalies were calculated by subtracting the average of the reference period (1956–2015) from the actual data. Anomalies were then averaged for species and site and ultimately added to the average value calculated for each species and site over the same reference period.

2.4 | Climatic data

Data of mean monthly temperatures and total monthly precipitation were obtained from the Climatic Research Unit (CRU) TS 4.01 data set with a grid with a spatial resolution of $0.5^\circ \times 0.5^\circ$ (Harris *et al.*, 2014). These CRU meteorological data have been reported to correlate well with data of mean monthly temperatures and total monthly precipitation recorded at the Pyramid Research Station (at 5,050 m a. s. l. in the Khumbu Valley, Sagarmatha National Park, Nepal) of the Ev-K2 (Centro Nazionale Ricerche – CNR, Italy) and NAST (Nepal Academy of Science and Technology, Nepal) institutes (Pandey *et al.*, 2018).

2.5 | Statistical analysis

Quality of the iRW chronologies was statistically checked. Mean sensitivity (MS), expressed population signal (EPS), first-order

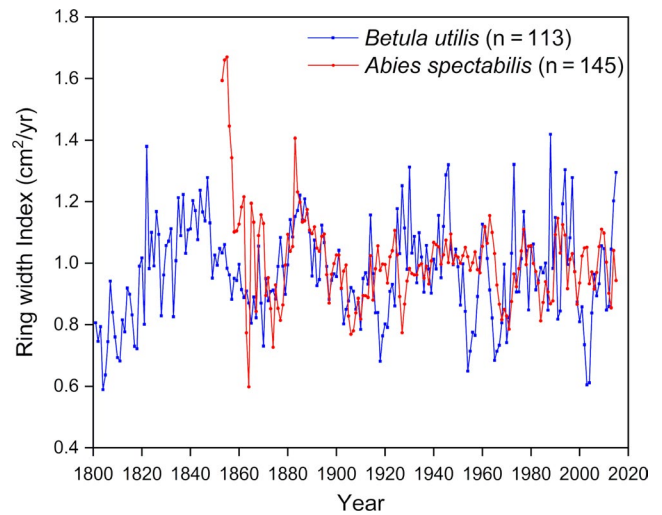


FIGURE 2 Tree ring width chronologies of *Abies spectabilis* and *Betula utilis* from the study area [Colour figure can be viewed at wileyonlinelibrary.com]

TABLE 1 Chronology statistics of ring-width chronologies for *Abies spectabilis* and *Betula utilis*, Nepal Himalaya from 1901 to 2015

Statistics	<i>Abies spectabilis</i>	<i>Betula utilis</i>
Number of trees (cores)	145	113
Standard deviation (SD)	0.08	0.16
Mean sensitivity (MS)	0.15	0.12
Expressed population signals (EPS)	0.94	0.94
First-order correlation (AC-1)	0.59	0.46
Series inter-correlation (Rbar)	0.18	0.17

autocorrelation (AC-1) and mean inter-series correlation (R_{bar}) were calculated for each species (Fritts, 1976; Holmes, 1983; Wigley and Briffa, 1984). Pearson's correlation coefficient was assessed between the iRW series and monthly data of average temperature and total precipitation from July of the previous year to September of the current year, and for the seasonal temperature and total precipitation calculated for the winter (DJF), pre-monsoon (MAM) and monsoon (JJAS) periods. Pearson's correlation coefficient was also computed between the anomaly data ($\delta^{13}\text{C}$, $\Delta^{13}\text{C}$, iWUE and $\delta^{18}\text{O}$) and monthly and seasonal data of mean monthly temperature and total monthly precipitation averaged for the same time interval (i.e., 10 years).

3 | RESULTS

3.1 | Association of iRW chronologies with climate

The detrended ring width (iRW) chronologies extended from 1858 to 2015 for *Abies spectabilis* and from 1805 to 2015 for *Betula utilis* (Figure 2). Statistical parameters were calculated for the maximum

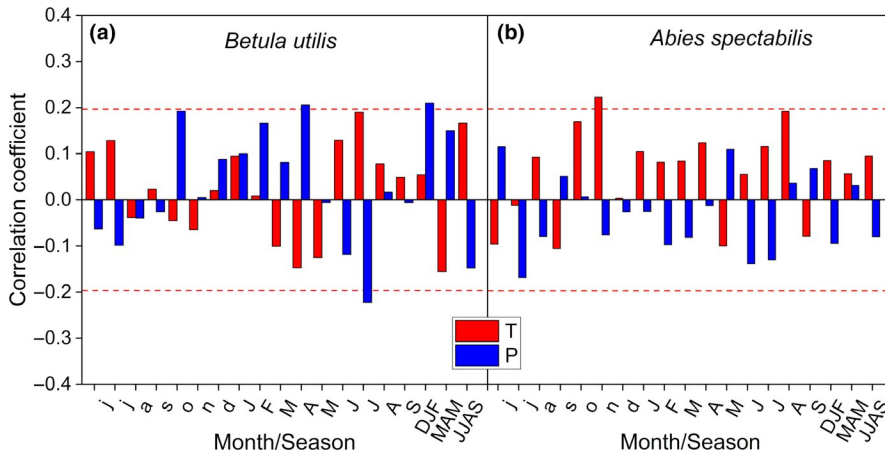


FIGURE 3 Pearson's correlation between mean ring width indices of *Abies spectabilis* and *Betula utilis* and monthly climate data. The horizontal dashed line indicates the 95% significance threshold. DJF, MAM, and JJAS represent the seasonal average climate of winter (December to February), spring (March to May) and summer (June to September), respectively [Colour figure can be viewed at wileyonlinelibrary.com]

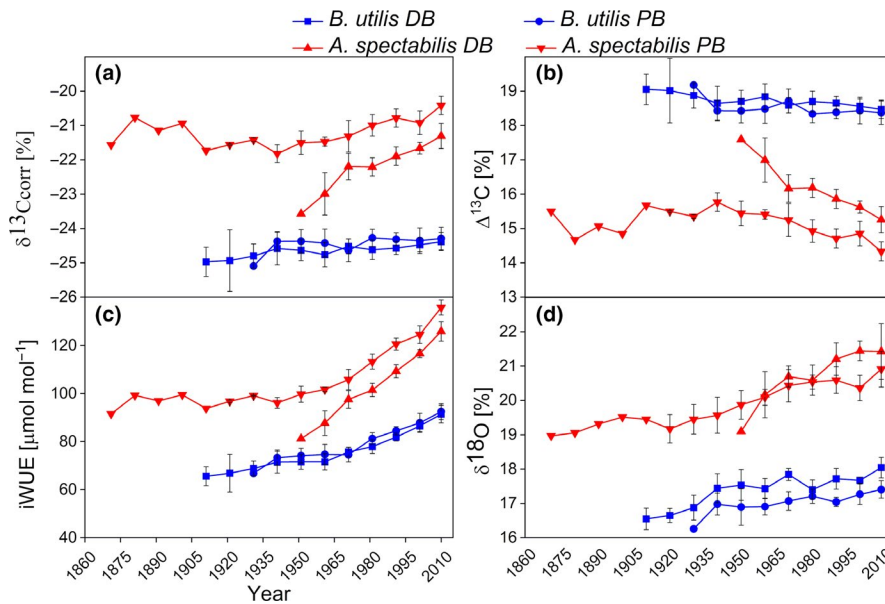


FIGURE 4 Long-term trend of $\delta^{13}\text{C}$ (isotopic fractionation of ^{13}C), $\Delta^{13}\text{C}$ (discrimination against ^{13}C), iWUE (intrinsic water use efficiency) and $\delta^{18}\text{O}$ (isotopic fractionation of ^{18}O) for *Abies spectabilis* and *Betula utilis* tree ring cellulose from 1866 to 2015 from Deboche and Pangboche. Error bars represent standard errors [Colour figure can be viewed at wileyonlinelibrary.com]

period common to all cores (1901–2015, i.e. 114 years; Table 1). MS and standard deviation were higher, and the first-order autocorrelation (AC-1) lower in *Betula utilis* than in *Abies spectabilis*. In both species, EPS was higher than the critical threshold of 0.85, underlying a strong common signal in the chronologies.

Climate–growth association revealed that *Abies spectabilis* was more responsive to summer temperatures, whereas *Betula utilis* was to pre-monsoon precipitation (Figure 3). *Abies spectabilis* had a significant positive correlation with mean temperature of the previous year's November ($r = 0.22$, $p < 0.05$) and current August ($r = 0.19$, $p < 0.05$). Instead, *Betula utilis* showed a significant negative correlation with mean temperature of the current year's April, whereas its correlation with total monthly precipitation was significantly positive in October of the previous year ($r = 0.19$, $p < 0.05$), April of the current year ($r = 0.20$, $p < 0.05$) and during winter (DJF, $r = 0.20$, $p < 0.05$), while we detected a significant negative response for total precipitation of July of the current year ($r = -0.22$, $p < 0.05$).

3.2 | Carbon isotopes

After correction of $\delta^{13}\text{C}$ in tree rings for the variation in atmospheric $\delta^{13}\text{C}$ ($\delta^{13}\text{C}_{\text{air}}$) due to anthropogenic fossil fuel emissions, we observed an increase in $\delta^{13}\text{C}_{\text{corr}}$ with time for both species and sites (Figure 4a), indicating a progressive enrichment of heavy carbon (^{13}C) in the wood. Values of $\delta^{13}\text{C}_{\text{corr}}$ were higher on average and its positive trend with time more pronounced in *Abies spectabilis* than *Betula utilis*. Moreover, *Abies spectabilis* trees showed on average higher values of $\delta^{13}\text{C}_{\text{corr}}$ (-21.2 ± 0.16) at Pangboch (PB, 4,200 m a.s.l.) than Deboche (DB, 3,850 m a.s.l.; $\delta^{13}\text{C}_{\text{corr}} = -22.3 \pm 0.29$), whereas differences between sites were not significant in *Betula utilis* (average $\delta^{13}\text{C}_{\text{corr}} = -24.57 \pm 0.35$; Figure 4a).

The observed changes in $\delta^{13}\text{C}_{\text{corr}}$ implied that the discrimination against ^{13}C ($\Delta^{13}\text{C}$) also changed with time, resulting in a decreasing trend in both species, more pronounced in *Abies spectabilis* than in *Betula utilis*. While for *Betula utilis* $\Delta^{13}\text{C}$ was similar

at both sites, for *Abies spectabilis* it was lower at the higher PB site (Figure 4b).

A reduction in $\Delta^{13}\text{C}$ implies that less water is lost for a given amount of carbon fixed with photosynthesis. Accordingly, we found that the intrinsic water-use efficiency (iWUE) increased with time in both species, with *Abies spectabilis* being more efficient in saving water (i.e., has a higher iWUE) than *Betula utilis* but also showing a significantly higher iWUE at PB ($104.98 \pm 1.57 \mu\text{mol/mol}$) than at DB ($102.84 \pm 2.85 \mu\text{mol/mol}$; Figure 4c).

3.3 | Oxygen isotopes

Tree-ring data of the ratio of stable oxygen isotopes ($\delta^{18}\text{O}$) revealed a long-term positive trend in both species and at both sites (Figure 4d),

indicating that during the last century and more there was a progressive increase in the relative content of heavy oxygen isotopes (^{18}O) in the wood biomass. $\delta^{18}\text{O}$ was clearly higher in *Abies spectabilis* than *Betula utilis*, and the latter species also showed some significant differences between sites, with $\delta^{18}\text{O}$ being significantly lower in some periods at the higher PB site.

3.4 | Climate association with $\delta^{13}\text{C}$, $\Delta^{13}\text{C}$, iWUE and $\delta^{18}\text{O}$

Overall, we found that all the isotope data of tree rings showed very similar correlations with temperature and precipitation. Specifically, $\delta^{13}\text{C}$, iWUE and $\delta^{18}\text{O}$ showed a positive correlation (negative for $\Delta^{13}\text{C}$) with mean temperature in all months ($r > 0.6$), except July

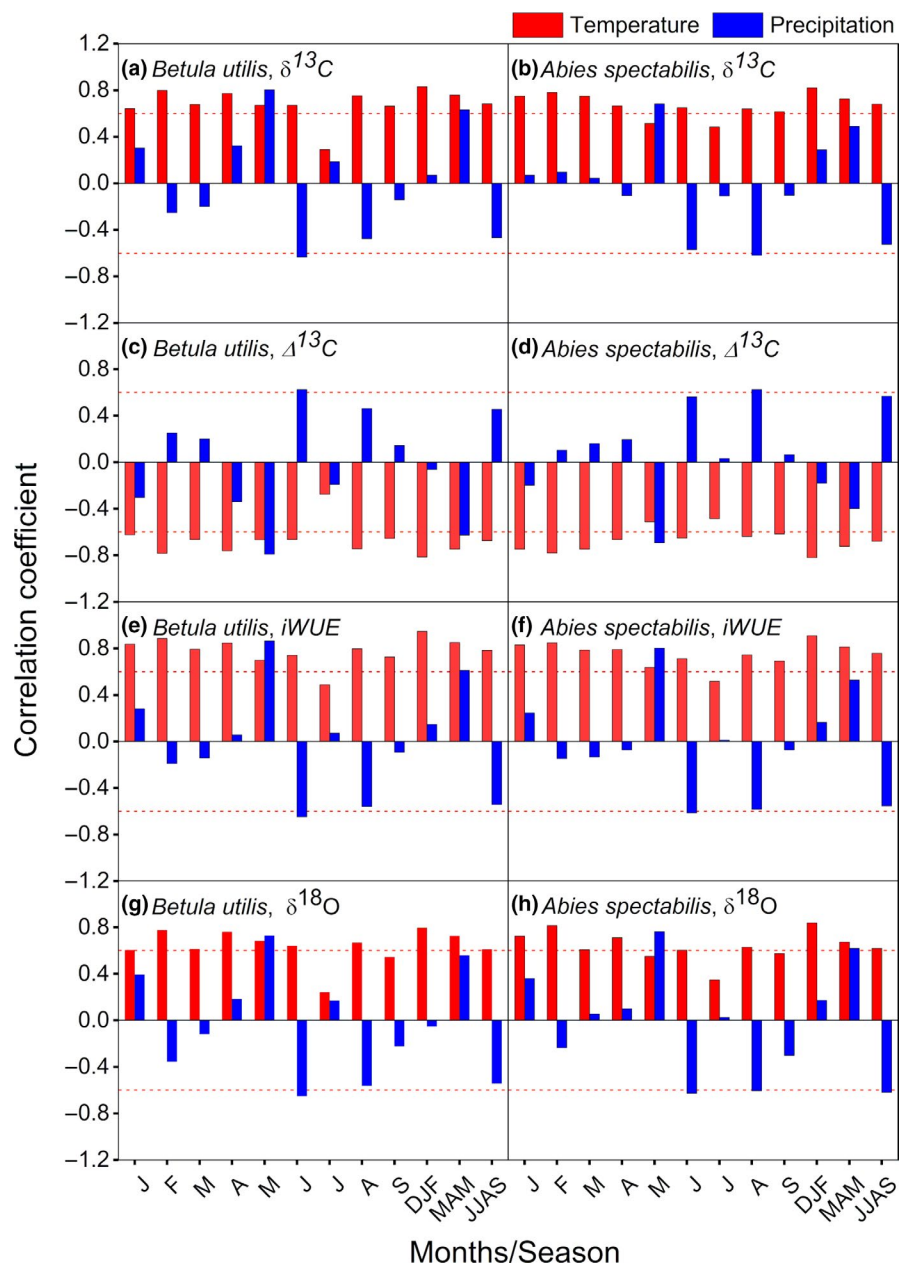


FIGURE 5 Correlations between $\delta^{13}\text{C}$ (isotopic fractionation of ^{13}C), $\Delta^{13}\text{C}$ (discrimination against ^{13}C), iWUE (intrinsic water-use efficiency) and $\delta^{18}\text{O}$ (isotopic fractionation of ^{18}O) in tree rings of *Abies spectabilis* and *Betula utilis* with mean monthly/seasonal temperature (T) and total monthly/seasonal precipitations (P). DJF (December to February), MAM (March to May), and JJAS (June to September) correspond to winter, spring and summer seasons, respectively. The horizontal dashed line indicates the 95% significance threshold [Colour figure can be viewed at wileyonlinelibrary.com]

(Figure 5). Instead, correlations with total monthly precipitation were significantly positive (negative for $\Delta^{13}\text{C}$) in May (and more in general in the pre-monsoon season) and significantly negative (positive for $\Delta^{13}\text{C}$) in June and August, i.e. towards the beginning and end of the monsoon season ($r < -0.6$; Figure 5).

4 | DISCUSSION

Our results confirmed that the physiology and growth of trees at the treeline in the Central Himalaya are primarily conditioned by both the pre-monsoon and summer monsoon season. A number of different factors can affect the performance and survival of trees in this harsh environment. Strong winds and avalanches, as well as ice blasting, can produce frequent mechanical damages. Moreover, the strong desiccation potential of the dry air (i.e. high vapour pressure deficit, VPD) at these elevations imposes biophysical limitations to water transport, the low atmospheric pressure negatively affects CO_2 assimilation, and the low temperatures slow down the enzymatic activity of fixing carbon into wood biomass (Körner, 2012).

Since treeline position worldwide coincides approximately with the average temperature of $\sim 6^\circ\text{C}$ during the growing season (Körner and Paulsen, 2004), climate warming is promoting the treeline's advance towards higher latitudes and altitudes in different regions (Harsch *et al.*, 2009). Nonetheless, this phenomenon can also depend on other more local conditions favouring tree establishment (Holtmeier and Broll, 2005). In addition, the increase in CO_2 concentration has been supposed to act as a sort of atmospheric fertilization for plants, especially at high elevations, where the CO_2 partial pressure gets lower, thus theoretically making it more difficult for Rubisco to fix CO_2 molecules (carbon limitation hypothesis: Körner, 2003; Handa and Körner, 2005). More generally, a higher CO_2 partial pressure relative to that of oxygen (O_2) is known to reduce the rate of O_2 fixation by Rubisco and its negative effect on the plant's carbon balance, which is partially compensated for by photorespiration (Peterhansel *et al.*, 2010). Moreover, a plant's metabolic activity is usually constrained by low temperatures, so climate warming in cold environments is not only able to extend the growing season by anticipating the onset and cessation of the vegetative period (Parmesan and Yohe, 2003; Körner and Basler, 2010), but also to increase the plant's metabolic rate, thus accelerating tree growth and forest dynamics (Petit, Anfodillo, Carraro, and Grani, 2011).

We performed tree RW measurements and analyses of stable C and O isotopes contained in tree rings in order to analyse the long-term physiological responses of treeline trees in the Central Himalaya, and in particular whether the higher temperatures at the beginning of the growing season induced stronger physiological limitations due to water shortage, or, on the contrary, provided more favourable growth conditions as a result of the effect of anticipation of the summer monsoon season (Crimmins and Crimmins, 2011).

Although the used CRU database has a wide spatial resolution incapable of capturing the wide climate variability between valleys and elevation in the Himalayan region (Schwab *et al.*, 2018), we

found that tree rings encoded a few significant signals of climate variability. *Betula utilis* was more sensitive than *Abies spectabilis* to precipitation during the driest months of the year (from October to April), i.e. before and during the reactivation of physiological activities. Instead, *Abies spectabilis* was more sensitive to summer temperatures. Moreover, late summer temperatures also had a positive effect on the growth of the following year, likely determined by the C accumulated as non-structural carbohydrates (NSC) in storage compartments, which is mostly significant towards the end of the season when the current year's allocation of xylem biomass is usually completed (Martínez-Vilalta *et al.*, 2016). The fact that *Abies spectabilis* is less sensitive to precipitations than *Betula utilis* was supported by our analyses of stable C isotopes, according to which a higher water-use efficiency in the conifer species compared to the broad-leaved one was evident. We could hypothesize that *Abies spectabilis* has a shallower rooting system than *Betula utilis* considering the observed higher cellulose's $\delta^{18}\text{O}$, which is compatible with a higher fractionation by evaporation of water towards the soil surface. Regarding species-specific differences in C isotopes, we observed a weaker discrimination against ^{13}C in *Abies spectabilis* than in *Betula utilis*. Most likely, the conifer species has a higher photosynthetic efficiency and tighter stomatal control, resulting in a higher water-use efficiency. Site-specific differences in discrimination against ^{13}C could be attributed to the effect of soil features on stomatal conductance, with stronger limitation to gas exchanges under shallower and drier soil condition (i.e., at Pangboche compared to Deboche).

The long-term trends assessed for $\delta^{13}\text{C}$ and its derived traits ($\Delta^{13}\text{C}$ and iWUE) indicated that over the last 150 years a progressive increase in water-use efficiency (WUE) has characterized the cost-benefit relationship of C assimilation by photosynthesis vs. water loss by transpiration in both species, consistent with a previous reports for other Himalayan timberline tree species (Huang *et al.*, 2017; Panthi, Fan, and van der Slepen, 2020). Mechanistically, plants can increase their WUE substantially in two different ways. In one case plants would respond to drier environmental conditions by reducing their stomatal conductance to prevent excessive water losses by leaf transpiration and the consequent development of high xylem tensions, which in turn would produce negative effects on the xylem conductance due to the occurrence of embolism (Vilagrosa, Chirino, Peguero-Pina, Barigah, and Cochard, 2013). On the contrary, WUE can also increase due to a higher enzymatic efficiency in photosynthetic CO_2 assimilation and in NSC fixation in biomass (Hartmann and Trumbore, 2016). In our trees, the long-term increase in $\delta^{18}\text{O}$ indicated that relatively heavier oxygen isotopes (^{18}O) remained increasingly fixed in the more recent xylem. This suggests that the increase in WUE was not due to an overall reduction in stomatal conductance and the consequent reduction in isotope fractionation during diffusion of transpired vapour through the stomata.

Early season precipitation was revealed to be important for the physiology of treeline trees. We found that $\delta^{13}\text{C}$, iWUE and $\delta^{18}\text{O}$ positively correlated with both total precipitation and mean temperature of May. The lack of an opposite association with precipitation and temperatures would discard the hypothesis that gas exchange

during the early growing season is limited by water shortage (Farquhar *et al.*, 1982; Farquhar *et al.*, 1989a) in this area of the Himalayas (Sigdel *et al.*, 2018). On the contrary, it provides further support for the hypothesis that more precipitation occurs around the onset of the growing season, likely due to the effect of large-scale changes in atmospheric circulation determining conditions for stronger evaporation from the ocean's surface and for the development of convective cells reaching higher elevations in the atmosphere (Sigdel and Ikeda, 2012; Liang *et al.*, 2019). In fact, the stronger isotopic fractionation during evaporation from a warmer ocean (i.e. resulting in higher $\delta^{18}\text{O}$) (Rahul, Prasanna, Ghosh, and Anilkumar, 2018) possibly coupled also with a reduced frequency of snowy precipitations (i.e. resulting in a higher $\delta^{18}\text{O}$ due to reduced fractionation during condensation; Viste and Sorteberg, 2015) most likely determined the observed long-term trend of cellulose's $\delta^{18}\text{O}$ increasing during the last 150 years.

In conclusion, climate change is affecting the physiological response of the two dominant tree species, *Abies spectabilis* and *Betula utilis*, at one of the world's highest treelines (at Sagarmatha – Mt. Everest – National Park, Central Himalaya). In particular, higher temperatures and higher water availability at the onset of the vegetative season are likely releasing growth limitations to both *Abies spectabilis* and *Betula utilis*, respectively.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

GP and PC conceived the research idea; GP and SP carried out field sampling; SP carried out sample preparation and measurements of ring width; SP and MS carried out isotope measurements; SP performed statistical analyses; SP, with contributions from GP, wrote the paper; all authors discussed the results and commented on the manuscript.

DATA AVAILABILITY STATEMENT

Original data used for this study are available at the public repository of the University of Padua (Research Data Unipd) (<https://doi.org/10.25430/researchdata.cab.unipd.it.00000344>; URI: <http://researchdata.cab.unipd.it/id/eprint/344>).

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