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

Sudip Pandey¹, Paolo Cherubini², Matthias Saurer², Marco Carrer¹ & Gaii Petit^{1,3}

¹ Dip. Territorio e Sistemi Agro-Forestali, Università degli Studi di Padova, Viale dell'Università 16,
 35020 Legnaro (PD), Italy

² Swiss Federal Research Institute WSL, CH-8903 Birmensdorf, Switzerland.

³ Corresponding author

Corresponding author:

Gaii Petit

Dept. Territorio e Sistemi Agro-Forestali, Università degli Studi di Padova

Address: Viale dell'Università 16, 35020 Legnaro (PD), Italy

email: gaii.petit@uniupd.it

Word count:

○ Abstract 189

○ Main body 3931

Number of tables: 1

Number of figures: 5

Keywords: stable isotopes; *Abies spectabilis*; tree growth; *Betula utilis*; climate change; treeline.

Running title

$\delta^{13}\text{C}$ - $\delta^{18}\text{O}$ in Himalayan treeline trees

1   **Abstract**

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

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

10   Growth of *A. spectabilis* strongly depended on summer temperatures, whereas that of *B. utilis* on
11   spring precipitation. $\delta^{13}C$ and $iWUE$ increased with time in both species, especially in *A. spectabilis*.

12   The long-term decrease in $\Delta^{13}C$ was accompanied by an increase in $\delta^{18}O$ in both species, thus
13   suggesting an increase in photosynthetic efficiency rather than a stronger stomatal control of
14   transpiration.

15   Climate change is progressively reducing the physiological limitations due to low temperatures and
16   low spring precipitations at the Central Himalayan treeline, thus potentially facilitating a further
17   altitudinal forest advance.

18

1   **Introduction**

2   Tree growth towards the forest limit at high altitude and latitude coincides approximately to the
3   average temperature of ~6 °C during the growing season (Körner & Paulsen 2004). In such
4   environments, increasing air temperature can have an important influence on plant metabolism,
5   biomass production and natural forest dynamics. Because of climate warming, the length of winter
6   dormancy is commonly becoming shorter and the vegetative period longer (Chmielewski & Rötzer
7   2001), determining a global trend of current treeline shifts towards higher latitudes and altitudes
8   (Kullman, 1998; Körner and Paulsen, 2004; Harsch et al., 2009). However, increasing air
9   temperatures may also become limiting for plant survival and recruitment in cold environments with
10   reduced water availability, as increasing air dryness determines higher evaporation rates from soil
11   and leaf evapotranspiration, as recently observed in the southern Tibetan Plateau (Lyu et al. 2019).

12   Himalayan regions are one of the most critical hotspots of climate change, with annual temperatures
13   increasing more than three times the average rate of warming at a global scale, i.e., +0.4 °C per decade
14   (Xu et al. 2009). As a consequence, ice caps and mountain glaciers are receding (Xu et al. 2009), and
15   the growing season is becoming longer (Shrestha et al. 2012). However, tree growth at the Himalayan
16   treelines is not only limited by cold air temperature, but also strongly influenced by the East Asian
17   and Southwest Monsoons (Liang et al. 2014), which determines arid conditions during the cold winter
18   and abundant precipitations in summer (Qi et al. 2013). Consequently, the treeline shifts towards
19   higher elevations can be limited by precipitations during the beginning of the vegetative season
20   (Sigdel et al. 2018). Recent dendrochronological analyses on dominant tree species at the Central
21   Himalayan treelines, reported a positive effect of summer temperatures on tree growth, especially in
22   *Abies spectabilis* (D. Don) Mirb., but also a certain degree of growth sensitivity to the amount of
23   precipitation falling at the beginning of the growing season, especially in *Betula utilis* D. Don (Gaire
24   et al. 2014; Pandey et al. 2018). Consequently, these studies raised the question of whether conditions
25   of pronounced air and soil dryness due to the low precipitation amounts during these periods of the
26   year can potentially expose plants to drought stress, with negative effects on growth and seedlings

1 establishment (Liang et al. 2014; Pandey et al. 2018). On the contrary, it could also be questioned
2 whether warmer oceans due to climate change anticipate the monsoon season (Loo et al. 2015), thus
3 determining conditions of overall better water availability at the beginning of the growing season,
4 with a positive stimulus for tree physiology and growth.

5 The analyses of stable C and O isotopes in tree rings are consolidated methods to retrospectively
6 investigate the tree physiological responses to climate variability (McCarroll & Loader 2004; Gessler
7 et al. 2014; Barbour & Song 2014). The ratio of heavier to lighter C stable isotopes ($\delta^{13}\text{C} = ^{13}\text{C}/^{12}\text{C}$)
8 in the plant biomass is lower compared to the atmosphere due to the isotope fractionation by air
9 diffusion through the stomata (i.e., ^{12}C in CO_2 molecules diffuses more easily than CO_2 with ^{13}C) and
10 by the enzymatic discrimination of Rubisco against ^{13}C during CO_2 fixation (Farquhar, Ehleringer, et
11 al. 1989). Dry environmental conditions can induce a reduction in stomatal conductance (g_s) to
12 prevent excessive water losses with transpiration and/or the development of excessive xylem tension
13 potentially causing the deterioration of the whole-tree hydraulic system (Liu et al. 2015; Wolf et al.
14 2016). Consequently, CO_2 diffusion is limited, $\delta^{13}\text{C}$ of the air contained in the leaves increases
15 progressively due to CO_2 fixation, but Rubisco's discrimination against ^{13}C becomes less effective,
16 thus maximising the C fixed in the plant biomass relatively enriched in ^{13}C . Alternatively, $\delta^{13}\text{C}$ of the
17 plant biomass can increase under conditions favouring the enzymatic activity of Rubisco and relative
18 increase in the rate of CO_2 fixation, like nitrogen fertilization (Makino 2003; Balster et al. 2009), or
19 an increase in temperature not associated with drought (Gessler et al. 2014; Altieri et al. 2015). Due
20 to the impact of physiological processes on carbon isotope fractionation, there is also a clear link
21 between $\delta^{13}\text{C}$ and the water-use efficiency, i.e., the ratio between loss of water per carbon gained by
22 the plant (Farquhar, Hubick, et al. 1989).

23 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
24 whether a variation in $\delta^{13}\text{C}$ in the biomass produced by a plant is due either to hydraulic limitations
25 imposing an overall reduction in g_s or to an increase in C assimilation efficiency with photosynthesis
26 (Saurer et al. 2002; Battipaglia et al. 2008; Leonelli et al. 2017). Fractionation of 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 condensing comparatively more easily. Therefore, assuming no changes in the isotopic composition of source water, conditions favouring high water losses with transpiration rates (particularly dry air) will leave the produced plant biomass depleted in ^{16}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 precipitations 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.

Materials and 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 $\times$ 100 m) were established close to the altitudinal treeline at Deboche (DB, 27.83° N, 86.77° E, 3850 m a.s.l) and Pangboche (PB, 27.85°N, 86.79°E, 4200 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 (Fig. 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 J.D. Hooker) Wenzig, *Acer campbellii* Hook.f. & Thomson ex Hiern and an increasing presence of dwarf *Rhododendron campanulatum* D. Don towards the limit with the alpine meadows (Singh & Singh 1987). *Betula utilis* has a high

freezing tolerance (Zobel & Singh 1997), and in Central Himalaya is mainly diffused on the moist areas of north-facing slopes from approximately 2700 m a.s.l. up to the treeline (approximately 4300 m a.s.l.). Instead, *A. spectabilis* develops on mesic north-facing slopes between 2800 up to more than 4000 m a.s.l (Shrestha et al. 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 *A. spectabilis* and 113 *B. 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.

3.1 – 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 & Smiley 1968; Holmes 1983). To assess inter-annual variability of ring-width we i) removed size/age trends commonly observed in ring-width (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 & Kalriukstis 1990) and iii) averaged the detrended series from the trees by bi-weight robust (Cook 1985) to obtain species-specific indexed chronologies of ring width using the R-package dpIR (Bunn 2008). Pearson's correlation was then assessed for the relationships of bi-weight robust mean of ring width (*iRW*) with meteorological data, i.e., total monthly precipitation and average monthly temperatures.

3.2 – Analyses of carbon and oxygen stable isotopes

Analyses of stable isotopes were conducted for a subset of cores (5 per species per site) having the highest correlation between their *RW* chronologies. Cores were split into segments of 10-years 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 of <0.05mm in diameter. Ground samples (approximately 10 mg) were packed in porous fiber 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 h at 60 °C followed by a wash with 7% sodium chloride (NaClO₂) for 30 h 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, Greifensee, Switzerland) and then enclosed in a sliver capsule (3.3x5mm). The abundance of stable isotopes of carbon ($\delta^{13}C$, i.e., the relative deviation of $^{13}C/^{12}C$ of the sample from the international standard Vienna Pee Dee Belemnite) and oxygen ($\delta^{18}O$, i.e., the relative deviation of $^{18}O/^{16}O$ of the sample from the international standard Vienna Standard Mean Ocean water) was measured using a Continuous Flow Isotopic Ratio Mass Spectrometer (CF-IRMS) based on pyrolysis at 1450 °C (PYRO-cube, Elementar, Hanau, Germany), coupled with a mass spectrometer (IRMS Delta V Advantage, Thermo Fisher Scientific, Waltham, MA, USA) via a variable open split interface (Conflo III, Finnigan, Germany) (Woodley et al. 2012). The analytical errors (standard deviations) of the isotope measurements were less than 0.2‰ and 0.3‰ for $\delta^{13}C$ and $\delta^{18}O$, respectively.

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

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

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

25

26 3.3 – Estimate of carbon discrimination ($\Delta^{13}C$) 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:

$$\Delta^{13}\text{C} = \frac{\delta^{13}\text{C}_{\text{air}} - \delta^{13}\text{C}}{1 + \frac{\delta^{13}\text{C}}{1000}} \quad \text{eq. 2}$$

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 et al. (1982) as:

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

where C_i and C_a are the CO_2 concentration in the leaf mesophyll and free atmosphere, respectively. Combining eq. 2 and eq. 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, Hubick, et al. 1989), respectively, it follows that the C_i can be calculated as:

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

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:

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

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 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.

3.4 – Climatic data

Data of mean monthly temperatures and total monthly precipitations were obtained from the Climatic Research Unit (CRU) TS 4.01 dataset with a spatial resolution of $0.5^\circ \times 0.5^\circ$ grid (Harris et al. 2014). These CRU meteorological data have been reported to correlate well with data of mean monthly

temperatures and total monthly precipitations recorded at the Pyramid Research Station (at 5050 m a.s.l. in the Kumbhu 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).

3.5 – Statistical analysis

Quality of the *iRW* chronologies was statistically checked. Mean sensitivity (*MS*), expressed population signal (*EPS*), first-order autocorrelation (*AC-I*) and mean inter-series correlation (*Rbar*) were calculated for each species (Fritts 1976; Holmes 1983; Wigley et al. 1984). Pearson's correlation coefficient was assessed between the *iRW* series and monthly data of average temperature and total precipitations from July of the previous year to September of the current year, and for the seasonal temperature and total precipitations calculated for the winter (DJF), pre-monsoon (MAM) and monsoon (JJAS) periods. Pearson's correlation coefficient was also computed between the anomaly data ($\delta^{13}C$, $\Delta^{13}C$, *iWUE* and $\delta^{18}O$) and monthly and seasonal data of mean monthly temperature and total monthly precipitations averaged for the same time interval (i.e., 10 years).

Results

Association of iRW chronologies with climate

The detrended ring width (*iRW*) chronologies extended from 1858 to 2015 for *A. spectabilis* and from 1805 to 2015 for *B. utilis* (Fig 2). Statistical parameters were calculated for the maximum period common to all cores (1901-2015; 114 years) (Table 1). The mean sensitivity (*MS*) and standard deviation were higher, and the first-order auto-correlation (*AC-I*) lower in *B. utilis* than *A. 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 *A. spectabilis* was more responsive to summer temperatures, whereas *B. utilis* to pre-monsoon precipitation (Fig. 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, *B. utilis* showed a significant negative correlation with mean temperature of the current year's April, whereas its correlation with total monthly precipitations was significantly positive for the previous year's October ($r=0.19$, $p<0.05$), current year's April ($r=0.20$, $p<0.05$) and during winter (DJF, $r=0.20$, $p<0.05$), while we detected a significant negative response for total precipitations of the current year's July ($r=-0.22$, $p<0.05$).

Carbon isotopes

After correction of $\delta^{13}C$ in tree rings for the variation in atmospheric $\delta^{13}C$ ($\delta^{13}C_{air}$) due to anthropogenic fossil fuel emissions, we observed an increase in $\delta^{13}C_{corr}$ with time for both species and sites (Fig. 4a), indicating a progressive enrichment of heavy carbon (^{13}C) in the wood. Values of $\delta^{13}C_{corr}$ were higher on average and its positive trend with time more pronounced in *A. spectabilis* than *B. utilis*. Moreover, *A. spectabilis* trees showed on average higher values of $\delta^{13}C_{corr}$ (-21.2 ± 0.16) at Pangboch (PB, 4200 m a.s.l.) than Deboche (DB, 3850 m a.s.l.; $\delta^{13}C_{corr}=-22.3 \pm 0.29$), whereas differences between sites were not significant in *B. utilis* (average $\delta^{13}C_{corr}=-24.57 \pm 0.35$) (Fig. 4a).

The observed changes in $\delta^{13}C_{corr}$ implied that the discrimination against ^{13}C ($\Delta^{13}C$) also changed with time, resulting in a decreasing trend in both species, more pronounced in *A. spectabilis* than *B. utilis*. While for *B. utilis* $\Delta^{13}C$ was similar at both sites, for *A. spectabilis* it was lower at the higher PB site (Fig. 4b).

A reduction in $\Delta^{13}C$ 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 *A. spectabilis* being more efficient in saving water (i.e., has a higher *iWUE*)

than *B. utilis* but also showing a significantly higher *iWUE* at PB ($104.98 \pm 1.57 \mu\text{mol mol}^{-1}$) than DB ($102.84 \pm 2.85 \mu\text{mol mol}^{-1}$) (Fig. 4c).

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 (Fig. 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 *A. spectabilis* than *B. 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.

*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 temperatures and precipitations. Specifically, $\delta^{13}\text{C}$, *iWUE* and $\delta^{18}\text{O}$ showed a positive correlation (negative for $\Delta^{13}\text{C}$) with mean temperature of all months ($r > 0.6$), except July (Fig. 5). Instead, correlations with total monthly precipitations 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 ($r < -0.6$) (Fig. 5).

Discussion

Our results confirmed that the physiology and growth of trees at the treeline in Central Himalaya is 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

1 CO₂ assimilation, and the low temperatures slow down the enzymatic activity of fixing carbon into
2 wood biomass (Körner 2012).

3 Since worldwide treeline position coincides approximately to the average temperature of ~6 °C
4 during the growing season (Körner & Paulsen 2004), climate warming is promoting the treeline
5 advance towards higher latitudes and altitudes in different regions (Harsch et al. 2009). Nonetheless,
6 this phenomenon can also depend on other more local conditions favouring tree establishment
7 (Holtmeier & Broll 2005). In addition, the increase in CO₂ concentration has been supposed to act as
8 a sort of atmospheric fertilization on plants, especially at high elevations, where the CO₂ partial
9 pressure gets lower, thus theoretically making it more difficult for the Rubisco to fix CO₂ molecules
10 (carbon limitation hypothesis: Handa et al., 2005; Körner, 2003). More generally, a higher CO₂ partial
11 pressure relative to that of oxygen (O₂) is known to reduce the rate of O₂ fixation by Rubisco and its
12 negative effect on the plant's carbon balance, which is partially compensated for by photorespiration
13 (Peterhansel et al. 2010). Moreover, a plant's metabolic activity is usually constrained by low
14 temperatures, so climate warming in cold environments is not only able to extend the growing season
15 by anticipating the onset and cessation of the vegetative period (Parmesan & Yohe 2003; Körner &
16 Basler 2010), but also to increase the plant's metabolic rate, thus accelerating tree growth and forest
17 dynamics (Petit et al. 2011).

18 We performed tree-ring width measurements and analyses of stable C and O isotopes contained in
19 tree rings in order to analyse the long term physiological responses of treeline trees in the Central
20 Himalaya, and in particular whether the higher temperatures at the beginning of the growing season
21 induced stronger physiological limitations due to water shortage, or, on the contrary, provided more
22 favourable growth conditions under the effect of the anticipation of the summer monsoon season
23 (Crimmins et al. 2011).

24 Although the used CRU database has a wide spatial resolution incapable of capturing the wide climate
25 variability between valleys and elevation in the Himalayan region (Schwab et al. 2018), we found
26 that tree rings encoded a few significant signals of climate variability. *B. utilis* was more sensitive

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

18 The long-term trends assessed for $\delta^{13}C$ and its derived traits ($\Delta^{13}C$ and *iWUE*) indicated that over the
19 last 150 years a progressive increase in water use efficiency (*WUE*) has characterized the cost-benefit
20 relationship of C assimilation with photosynthesis vs. water loss with transpiration in both species,
21 consistent with a previous reports for other Himalayan timberline tree species (Huang et al. 2017;
22 Panthi et al. 2020). Mechanistically, plants can increase their *WUE* substantially in two different
23 ways. In one case plants would respond to the drier environmental condition by reducing stomatal
24 conductance to prevent excessive water losses with leaf transpiration and the consequent development
25 of high xylem tensions, which in turn would produce negative effects on the xylem conductance due
26 to embolism formation (Vilagrosa et al. 2013). On the contrary, *WUE* can also increase due to a higher

1 enzymatic efficiency in photosynthetic CO₂ assimilation and in *NSC* fixation in biomass (Hartmann
2 & Trumbore 2016). In our trees, the long-term increase in $\delta^{18}O$ indicated that relatively heavier
3 oxygen isotopes (¹⁸O) remained increasingly fixed in the more recent xylem. This suggests that the
4 increase in *WUE* was not due to an overall reduction in stomatal conductance and the consequent
5 reduction in isotope fractionation during diffusion of transpired vapour through the stomata.

6 Early season precipitations were revealed to be important for the physiology of treeline trees. We
7 found that $\delta^{13}C$, *iWUE* and $\delta^{18}O$ positively correlated with both total precipitations and mean
8 temperature of May. The lack of an opposite association with precipitations and temperatures would
9 discard the hypothesis that gas exchanges during the early growing season are limited by water
10 shortage (Farquhar et al. 1982; Farquhar, Ehleringer, et al. 1989) in this area of the Himalayas (Sigdel
11 et al. 2018). On the contrary, it provides further support to the hypothesis that more precipitations are
12 occurring around the onset of the growing season, likely due to the effect of large-scale changes in
13 atmospheric circulation determining conditions for stronger evaporation from the ocean's surface and
14 the development of convective cells reaching higher elevations in the atmosphere (Sigdel & Ikeda
15 2012; Liang et al. 2019). In fact, the stronger isotopic fractionation during evaporation from the
16 warmer ocean (i.e., resulting in higher $\delta^{18}O$) (Rahul et al. 2018) possibly coupled also with a reduced
17 frequency of snowy precipitations (i.e., resulting in a higher $\delta^{18}O$ due to a reduced fractionation
18 during condensation) (Viste & Sorteberg 2015) most likely determined the observed long-term trend
19 of increasing cellulose's $\delta^{18}O$ during the last 150 years.

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

1   **Author contributions**

2   G.P. and P.C. conceived the research idea; G.P. and S.P. carried out field sampling; S.P. carried out
3   sample preparation and measurements of ring width; S.P. and M.S. carried out isotope measurements;
4   S.P. performed statistical analyses; S.P., with contributions from G.P., wrote the paper; all authors
5   discussed the results and commented on the manuscript.

7   **Data accessibility statementy**

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

12   **Conflict of interest**

13   The authors declare no conflict of interest.

15   **Acknowledgements**

16   SP, MS and GP were supported by the Fondazione CariPARO, by SNF-grant no. 200020_182092
17   and by the Università degli Studi di Padova (DOR1877180/18), respectively.

19   **References**

- 20   Altieri, S., Mereu, S., Cherubini, P., Castaldi, S., Sirignano, C., Lubritto, C., & Battipaglia, G. 2015.
21   Tree-ring carbon and oxygen isotopes indicate different water use strategies in three
22   Mediterranean shrubs at Capo Caccia (Sardinia, Italy). *Trees - Structure and Function* 29: 1593–
23   1603.
- 24   Balster, N.J., Marshall, J.D., & Clayton, M. 2009. Coupling tree-ring $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ to test the effect

of fertilization on mature Douglas-fir (*Pseudotsuga menziesii* var. *glauca*) stands across the Interior northwest, USA. *Tree Physiology* 29: 1491–1501.

Barbour, M.M., & Song, X. 2014. Do tree-ring stable isotope compositions faithfully record tree carbon/water dynamics? *Tree Physiology* 34: 792–795.

Battipaglia, G., Jäggi, M., Saurer, M., Siegwolf, R.T.W., & Cotrufo, M.F. 2008. Climatic sensitivity of $\delta^{18}\text{O}$ in the wood and cellulose of tree rings: Results from a mixed stand of *Acer pseudoplatanus* L. and *Fagus sylvatica* L. *Palaeogeography, Palaeoclimatology, Palaeoecology* 261: 193–202.

Bunn, A.G. 2008. A dendrochronology program library in R (dplR). *Dendrochronologia* 26: 115–124.

Carrer, M., Von Arx, G., Castagneri, D., & Petit, G. 2015. Distilling allometric and environmental information from time series of conduit size: The standardization issue and its relationship to tree hydraulic architecture. *Tree Physiology* 35: 27–33.

Chmielewski, F.-M., & Rötzer, T. 2001. Response of tree phenology to climate change across Europe. *Agricultural and Forest Meteorology* 108: 101–112.

Cook, E.R. 1985. A Time Series Analysis Approach to Tree Ring Standardization.

Cook, E.R., & Kalriukstis, L. a. 1990. *Methods of Dendrochronology -Applications in the Environmental Sciences* (E. R. Cook & L. A. Kairiukstis, Eds.). Springer Netherlands, Dordrecht, Netherlands.

Crimmins, T.M., Crimmins, M.A., & Bertelsen, C.D. 2011. Onset of summer flowering in a “Sky Island” is driven by monsoon moisture. *New Phytologist* 191: 468–479.

Farquhar, G.D., Ehleringer, J.R., & Hubick, K.T. 1989. Carbon Isotope Discrimination and Photosynthesis. *Annual Review of Plant Physiology and Plant Molecular Biology* 40: 503–537.

Farquhar, G.D., Hubick, K.T., Condon, A.G., & Richards, R.A. 1989. Carbon Isotope Fractionation and Plant Water-Use Efficiency. In Rundell, P.W., Ehleringer, J.R., & Nagy, K.A. (eds.), *Stable Isotopes in Ecological Research*, pp. 21–40. Springer, New York, NY.

1 Farquhar, G., O’Leary, M., & Berry, J. 1982. On the Relationship Between Carbon Isotope
2 Discrimination and the Intercellular Carbon Dioxide Concentration in Leaves. *Australian*
3 *Journal of Plant Physiology* 9: 121.

4 Francey, R.J., Allison, C.E., Etheridge, D.M., Trudinger, C.M., Enting, I.G., Leuenberger, M.,
5 Langenfelds, R.L., Michel, E., & Steele, L.P. 1999. A 1000-year high precision record of δ^{13}
6 C in atmospheric CO₂. *Tellus B: Chemical and Physical Meteorology* 51: 170–193.

7 Fritts, H.. 1976. *Tree Rings and Climate*. The Blackburn Press, Caldwell., London.

8 Gaire, N.P., Koirala, M., Bhujju, D.R., & Borgaonkar, H.P. 2014. Treeline dynamics with climate
9 change at the central Nepal Himalaya. *Clim. Past* 10: 1277–1290.

10 Gessler, A., Ferrio, J.P., Hommel, R., Treydte, K., Werner, R.A., & Monson, R.K. 2014. Stable
11 isotopes in tree rings: towards a mechanistic understanding of isotope fractionation and mixing
12 processes from the leaves to the wood. *Tree Physiology* 34: 796–818.

13 Handa, I.T., Körner, C., & Hättenschwiler, S. 2005. A test of the treeline carbon limitation hypothesis
14 by in situ CO₂ enrichment and defoliation. *Ecology* 86: 1288–1300.

15 Harris, I., Jones, P.D., Osborn, T.J., & Lister, D.H. 2014. Updated high-resolution grids of monthly
16 climatic observations - the CRU TS3.10 Dataset. *International Journal of Climatology* 34: 623–
17 642.

18 Harsch, M.A., Hulme, P.E., McGlone, M.S., & Duncan, R.P. 2009. Are treelines advancing? A global
19 meta-analysis of treeline response to climate warming. *Ecology Letters* 12: 1040–1049.

20 Hartmann, H., & Trumbore, S. 2016. Understanding the roles of nonstructural carbohydrates in forest
21 trees - from what we can measure to what we want to know. *The New phytologist* 211: 386–403.

22 Holmes, R.L. 1983. Computer-assisted quality control in tree- ring dating and measurement. *Tree-*
23 *ring Bulletin* 43: 69–78.

24 Holtmeier, F.-K., & Broll, G. 2005. Sensitivity and response of northern hemisphere altitudinal and
25 polar treelines to environmental change at landscape and local scales. *Global Ecology and*
26 *Biogeography* 14: 395–410.

1 Huang, R., Zhu, H., Liu, X., Liang, E., Griebinger, J., Wu, G., Li, X., & Bräuning, A. 2017. Does
2 increasing intrinsic water use efficiency (iWUE) stimulate tree growth at natural alpine
3 timberline on the southeastern Tibetan Plateau? *Global and Planetary Change* 148: 217–226.

4 Keeling, C.D., Mook, W.G., & Tans, P.P. 1979. Recent trends in the $^{13}\text{C}/^{12}\text{C}$ ratio of atmospheric
5 carbon dioxide. *Nature* 277: 121–123.

6 Körner, C. 2012. *Alpine Treelines: Functional Ecology of the Global High Elevation Tree Limits*.
7 Springer, Basel.

8 Körner, C. 2003. Carbon limitation in trees. *Journal of Ecology* 91: 4–17.

9 Körner, C., & Basler, D. 2010. Phenology under global warming. *Science* 327: 1461–1462.

10 Körner, C., & Paulsen, J. 2004. A world-wide study of high altitude treeline temperatures. *Journal of*
11 *Biogeography* 31: 713–732.

12 Kullman, L. 1998. Tree-limits and montane forests in the Swedish Scandes: Sensitive biomonitors of
13 climate change and variability. *Ambio* 27: 312–321.

14 Leonelli, G., Battipaglia, G., Cherubini, P., Saurer, M., Siegwolf, R.T.W., Maugeri, M., Stenni, B.,
15 Fusco, S., Maggi, V., & Pelfini, M. 2017. *Larix decidua* $\delta^{18}\text{O}$ tree-ring cellulose mainly reflects
16 the isotopic signature of winter snow in a high-altitude glacial valley of the European Alps.
17 *Science of the Total Environment* 579: 230–237.

18 Liang, E., Dawadi, B., Pederson, N., & Eckstein, D. 2014. Is the growth of birch at the upper
19 timberline in the Himalayas limited by moisture or by temperature? *Ecology* 95: 2453–2465.

20 Liang, E., Dawadi, B., Pederson, N., Piao, S., Zhu, H., Sigdel, S.R., & Chen, D. 2019. Strong link
21 between large tropical volcanic eruptions and severe droughts prior to monsoon in the central
22 Himalayas revealed by tree-ring records. *Science Bulletin* 64: 1018–1023.

23 Liu, Y.Y., Song, J., Wang, M., Li, N., Niu, C.Y., & Hao, G.Y. 2015. Coordination of xylem
24 hydraulics and stomatal regulation in keeping the integrity of xylem water transport in shoots of
25 two compound-leaved tree species. *Tree Physiology* 35: 1333–1342.

26 Loo, Y.Y., Billa, L., & Singh, A. 2015. Effect of climate change on seasonal monsoon in Asia and

its impact on the variability of monsoon rainfall in Southeast Asia. *Geoscience Frontiers* 6: 817–823.

Lyu, L., Zhang, Q.-B., Pellatt, M.G., Büntgen, U., Li, M.-H., & Cherubini, P. 2019. Drought limitation on tree growth at the Northern Hemisphere's highest tree line. *Dendrochronologia* 53: 40–47.

Makino, A. 2003. Rubisco and nitrogen relationships in rice: Leaf photosynthesis and plant growth. *Soil Science and Plant Nutrition* 49: 319–327.

Martínez-Vilalta, J., Sala, A., Asensio, D., Galiano, L., Hoch, G., Palacio, S., Piper, F.I., & Lloret, F. 2016. Dynamics of non-structural carbohydrates in terrestrial plants: a global synthesis. *Ecological Monographs* 86: 495–516.

McCarroll, D., & Loader, N.J. 2004. Stable isotopes in tree rings. *Quaternary Science Reviews* 23: 771–801.

Pandey, S., Carrer, M., Castagneri, D., & Petit, G. 2018. Xylem anatomical responses to climate variability in Himalayan birch trees at one of the world's highest forest limit. *Perspectives in Plant Ecology, Evolution and Systematics* 33: 34–41.

Panthi, S., Fan, Z.-X., van der Sleen, P., & Zuidema, P.A. 2020. Long-term physiological and growth responses of Himalayan fir to environmental change are mediated by mean climate. *Global Change Biology* 26: 1778–1794.

Parnesan, C., & Yohe, G. 2003. A globally coherent fingerprint of climate change impacts across natural systems. *Nature* 421: 37–42.

Peterhansel, C., Horst, I., Niessen, M., Blume, C., Kebeish, R., Kürkcüoglu, S., & Kreuzaler, F. 2010. Photorespiration. *The Arabidopsis Book* 8: 2–24.

Petit, G., Anfodillo, T., Carraro, V., Grani, F., & Carrer, M. 2011. Hydraulic constraints limit height growth in trees at high altitude. *New Phytologist* 189: 241–252.

Qi, W., Zhang, Y., Gao, J., Yang, X., Liu, L., & Khanal, N.R. 2013. Climate change on the southern slope of Mt. Qomolangma (Everest) Region in Nepal since 1971. *Journal of Geographical*

1 *Sciences* 23: 595–611.

2 Rahul, P., Prasanna, K., Ghosh, P., Anilkumar, N., & Yoshimura, K. 2018. Stable isotopes in water
3 vapor and rainwater over Indian sector of Southern Ocean and estimation of fraction of recycled
4 moisture /704/47/4112 /704/106 /134 article. *Scientific Reports* 8: 7552.

5 Saurer, M., Aellen, K., & Siegwolf, R. 1997. Correlating $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ in cellulose of trees. *Plant,*
6 *Cell and Environment* 20: 1543–1550.

7 Saurer, M., Schweingruber, F., Vaganov, E.A., Shiyatov, S.G., & Siegwolf, R. 2002. Spatial and
8 temporal oxygen isotope trends at the northern tree-line in Eurasia. *Geophysical Research*
9 *Letters* 29: 10.1-10.4.

10 Schwab, N., Kaczka, R.J., Janecka, K., Böhner, J., Chaudhary, R.P., Scholten, T., & Schickhoff, U.
11 2018. Climate change-induced shift of tree growth sensitivity at a Central Himalayan treeline
12 ecotone. *Forests* 9: 267.

13 Shrestha, K.B., Chhetri, P.K., & Bista, R. 2017. Growth responses of *Abies spectabilis* to climate
14 variations along an elevational gradient in Langtang National Park in the central Himalaya,
15 Nepal. *Journal of Forest Research* 22: 274–281.

16 Shrestha, U.B., Gautam, S., & Bawa, K.S. 2012. Widespread climate change in the Himalayas and
17 associated changes in local ecosystems. *Plos One* 7: e36741.

18 Sigdel, M., & Ikeda, M. 2012. Summer monsoon rainfall over Nepal related with large-scale
19 atmospheric circulations. *Journal of Earth Science & Climatic Change* 03: 1–5.

20 Sigdel, S.R., Wang, Y., Julio Camarero, J., Zhu, H., Liang, E., & Peñuelas, J. 2018. Moisture-
21 mediated responsiveness of treeline shifts to global warming in the Himalayas. *Global Change*
22 *Biology* 24: 5549–5559.

23 Singh, J.S., & Singh, S.P. 1987. Forest vegetation of the Himalaya. *Botanical Review* 53: 80–192.

24 Stokes, M.A., & Smiley, T.L. 1968. *An Introduction to Tree Ring Dating*. University of Chicago
25 Press, Chicago.

26 Tiwari, A., Fan, Z.X., Jump, A.S., Li, S.F., & Zhou, Z.K. 2017. Gradual expansion of moisture

sensitive *Abies spectabilis* forest in the Trans-Himalayan zone of central Nepal associated with climate change. *Dendrochronologia* 41: 34–43.

Vilagrosa, A., Chirino, E., Peguero-Pina, J.J., Barigah, T.S., Cochard, H., & Gil-Pelegrín, E. 2013. Xylem cavitation and embolism in plants living in water-limited ecosystems. In *Plant Responses to Drought Stress: From Morphological to Molecular Features*, pp. 63–109.

Viste, E., & Sorteberg, A. 2015. Snowfall in the Himalayas: An uncertain future from a little-known past. *Cryosphere* 9: 1147–1167.

Wigley, T.M.L., Briffa, K.R., & Jones, P.D. 1984. On the average value of correlated time series, with applications in dendroclimatology and hydrometeorology. *Journal of Climate and Applied Meteorology* 23: 201–213.

Wolf, A., Anderegg, W.R.L., & Pacala, S.W. 2016. Optimal stomatal behavior with competition for water and risk of hydraulic impairment. *Proceedings of the National Academy of Sciences* 113: E7222–E7230.

Woodley, E.J., Loader, N.J., McCarroll, D., Young, G.H.F., Robertson, I., Heaton, T.H.E., Gagen, M.H., & Warham, J.O. 2012. High-temperature pyrolysis/gas chromatography/isotope ratio mass spectrometry: Simultaneous measurement of the stable isotopes of oxygen and carbon in cellulose. *Rapid Communications in Mass Spectrometry* 26: 109–114.

Xu, J., Grumbine, R.E., Shrestha, A., Eriksson, M., Yang, X., Wang, Y., & Wilkes, A. 2009. The melting Himalayas: Cascading effects of climate change on water, biodiversity, and livelihoods. *Conservation Biology* 23: 520–530.

Zobel, D.B., & Singh, S.P. 1997. Himalayan forests and ecological generalizations. *BioScience* 47: 735–745.

1 **Tables**

2

3 **Table 1:** Chronology statistics of ring-width chronologies for *Abies spectabilis* and *Betula utilis*,
 4 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 (<i>MS</i>)	0.15	0.12
Expressed Population signals (<i>EPS</i>)	0.94	0.94
First order correlation (<i>AC-I</i>)	0.59	0.46
Series inter-correlation (<i>Rbar</i>)	0.18	0.17

5

6

Figure captions

Fig. 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.

Fig. 2. Tree ring-width chronologies of *Abies spectabilis* and *Betula utilis* from the study area.

Fig 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.

Fig. 4. Long-term trend of $\delta^{13}C$ (isotopic fractionation of ^{13}C), $\Delta^{13}C$ (discrimination against ^{13}C), *iWUE* (intrinsic water use efficiency) and $\delta^{18}O$ (isotopic fractionation of ^{18}O) for *A. spectabilis* and *B. utilis* tree ring cellulose from 1866 to 2015 from Deboche and Pangboche. Error bars represent standard errors.

Fig. 5. Correlations between $\delta^{13}C$ (isotopic fractionation of ^{13}C), $\Delta^{13}C$ (discrimination against ^{13}C), *iWUE* (intrinsic water use efficiency) and $\delta^{18}O$ (isotopic fractionation of ^{18}O) in tree rings of *A. spectabilis* and *B. 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.

1   Figure 1

3

1   Figure 2

1   Figure 3

3

1   Figure 4

3

1 Figure 5