

Sampling strategy and climatic implication of tree-ring cellulose oxygen isotopes of *Hippophae tibetana* and *Abies georgei* on the southeastern Tibetan Plateau

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Abstract The tree-ring cellulose oxygen isotopes ($\delta^{18}\text{O}$) for four trees of *Hippophae tibetana* and four trees of *Abies georgei* growing in different locations around the terminal moraine in Xincuo from 1951 to 2010 were measured to explore its potential for reconstructing climatic variations in the southeastern Tibetan Plateau. The mean and standard deviation of tree-ring $\delta^{18}\text{O}$ at different heights do not have significant differences, and there are no significant differences in the mean and standard deviation of tree-ring $\delta^{18}\text{O}$ between trees near the brook and trees at the top of moraine, indicating that we can collect samples for tree-ring $\delta^{18}\text{O}$ analysis regardless of sampling heights and that the micro-environment does not affect tree-ring $\delta^{18}\text{O}$ significantly. The mean inter-series correlations of cellulose $\delta^{18}\text{O}$ for *A. georgei*/*H. tibetana* are 0.84/0.93, and the correlation between $\delta^{18}\text{O}$ for *A. georgei* and *H. tibetana* is 0.92. The good coherence between inter-tree and inter-species cellulose $\delta^{18}\text{O}$ demonstrates the possibility of using different species to develop a long chronology. Correlation analysis between tree-ring $\delta^{18}\text{O}$ and climate

parameters revealed that $\delta^{18}\text{O}$ for *A. georgei*/*H. tibetana* had negative correlations ($r = -0.62/r = -0.69$) with relative humidity in July–August, and spatial correlation revealed that $\delta^{18}\text{O}$ for *A. georgei*/*H. tibetana* reflected the regional Standardized Precipitation Evapotranspiration Index (29°–32° N, 88°–98° E). In addition, tree-ring $\delta^{18}\text{O}$ in Xincuo has a significant correlation with tree-ring $\delta^{18}\text{O}$ in Bhutan. The results indicate that cellulose $\delta^{18}\text{O}$ for *A. georgei* and *H. tibetana* in Xincuo is a good proxy for the regional hydroclimate.

Keywords Tree-ring cellulose oxygen isotopes · Relative humidity · Sampling design · *Abies georgei* · *Hippophae tibetana*

Introduction

The southeastern Tibetan Plateau (TP) is a combined area of the Himalayas, Nyainqentanglha and Hengduan Shan that surround the valley of Yaluzangbu River. Throughout this valley, the Indian summer monsoon brings rich mass that develops into numerous concentrated mountain glaciers in the southeastern TP (Yao et al. 2012; Bolch et al. 2012). Both glaciers and monsoon precipitation from the southeastern TP provide water resources for the Yaluzangbu River which people access in the Indian continent. Accordingly, it is crucial to understand the monsoon variability in this area. However, most meteorological records are less than 60 years in length, which hampers our study on variability of multi-decadal to centennial timescales.

Although tree-ring $\delta^{18}\text{O}$ is helpful for improving our understanding of regional hydroclimate variability in the southeastern TP, existing tree-ring $\delta^{18}\text{O}$ studies are still limited. Until now, tree species such as Tibetan juniper (*Juniperus*

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tibetica) (Grießinger et al. 2011, 2016), Linzhi spruce (*Picea likiangensis* var. *linzhiensis*) (Shi et al. 2011), and Balfour spruce (*P. likiangensis* var. *balfouriana*) (Liu et al. 2013) have been investigated for potential long-term paleoclimate reconstruction based on tree-ring $\delta^{18}\text{O}$. Shi et al. (2011) and Liu et al. (2013) showed that tree-ring $\delta^{18}\text{O}$ values of both Linzhi spruce and Balfour spruce in the southeastern TP are negatively correlated with cloud cover. Tibetan junipers have been shown to record signals of relative humidity (Wernicke et al. 2015; Grießinger et al. 2016) and precipitation (Grießinger et al. 2011). Despite their differences in specific climate signals, $\delta^{18}\text{O}$ of all of these species showed an ability to capture regional hydroclimate variability, allowing for a multi-species tree-ring oxygen isotope network.

Additionally, most of the existing $\delta^{18}\text{O}$ series have used the pooling method for oxygen isotope analysis (Grießinger et al. 2011; Liu et al. 2013). Linan et al. (2011) noted, “In most of the cases, pooled chronologies show high synchronicity with averaged individual samples at inter-annual scale but some differences also show up especially when comparing $\delta^{18}\text{O}$ decadal to multi-decadal variations.” In addition, trees with similar ages may show different trends (Xu et al. 2013), and differences in trends among individuals may be obscured by pooling strategies. Moreover, isotope data that are derived from individual trees show higher climate signals than data from a pooled chronology (Liu et al. 2014). In the southern TP, Wernicke et al. (2015) employed the individual method and determined a high correlation between tree-ring $\delta^{18}\text{O}$ with relative humidity in July–August ($r = -0.71$).

Although there is no evidence of an age effect regarding $\delta^{18}\text{O}$ for Linzhi spruce in the southern TP (Shi et al. 2011), some questions should still be investigated regarding sampling strategy and tree-ring $\delta^{18}\text{O}$ research, for example, whether the sampling height or micro-environment where trees grow affect the tree-ring $\delta^{18}\text{O}$. Leonelli et al. (2013) found that the $\delta^{18}\text{O}$ of trees fed by glacial melt waters is significantly more depleted than at a control site fed only by precipitation. To develop long-term chronologies, samples from relict are usually used, and we cannot guarantee the same sampling height for samples from living trees and from relict. Besides, sampling heights for samples that grow on steep slopes are usually not identical. It is important to know if the sampling heights affect tree-ring $\delta^{18}\text{O}$.

Therefore, this study aims to clarify the climatic implications of tree-ring $\delta^{18}\text{O}$ for two new tree species, *Hippophae tibetana* and *Abies georgei*, in the southeastern TP. We analyzed inter-annual tree-ring $\delta^{18}\text{O}$ for *A. georgei* and *H. tibetana* growing on both the outer and the inner sides of the terminal moraine using individual tree analysis to investigate the effects of (1) sampling heights, (2) micro-environment, and (3) tree species on tree-ring cellulose $\delta^{18}\text{O}$. We posit that the absolute values of tree-ring $\delta^{18}\text{O}$ might not be affected by sampling heights and micro-environment, and the climatic

implication of tree-ring $\delta^{18}\text{O}$ for different species in the same sampling site should be similar. Therefore, we can build up a long tree-ring $\delta^{18}\text{O}$ chronology based on multi-trees and multi-species in a local area for paleoclimate reconstruction.

Materials and methods

Sampling site

Tree-ring samples were taken from *H. tibetana* (Fig. 1, yellow spots) and *A. georgei* (Fig. 1, green spots) growing on both the outer and the inner sides of the terminal moraine (30.09° N, 94.27° E, 3930 m a.s.l.). Although usually considered as a type of shrub, the *H. tibetana* that grows in the study area is taller than 3 m and has a distinct trunk, so *H. tibetana* in the study area should be considered as a tree rather than a shrub.

Core samples of each tree were collected using a 5-mm borer. The cores were air-dried and polished to make the tree-ring borders clearly visible. The ring widths in the samples were then measured, at a resolution of 0.01 mm using a binocular microscope with a linear stage interfaced by a computer (Lintab5). Cross-dating was performed in the laboratory by matching variations in ring width from all cores to determine the absolute year of each ring. Quality control was completed using the program COFECHA (Holmes 1983).

Cellulose extraction and isotope measurements

Eight trees were selected for the isotopic analysis. Three (nos. XC12, XC14, XC15) and five (nos. XC02, XC05, XC06, XC07, XC08) trees are located in the inner and the outer sides of the terminal moraine, respectively, and sample no. XC15 grows near the brook (Fig. 1d).

The modified plate method (Xu et al. 2011, 2013) that follows the chemical treatment procedure of the Jayme-Wise method (Green 1963; Loader et al. 1997) was used to extract α -cellulose. This method was used because it can extract α -cellulose directly from the wood plate, thereby increasing efficiency and saving time. We wrapped the cellulose samples (sample weight, 80–260 μg) in silver foil and measured tree-ring cellulose oxygen isotope ratios ($^{18}\text{O}/^{16}\text{O}$) using an isotope ratio mass spectrometer (Delta V Advantage, Thermo Scientific) interfaced with a pyrolysis-type high-temperature conversion elemental analyzer (TC/EA, Thermo Scientific) at the Graduate School of Environmental Studies, Nagoya University and Research Institute for Humanity and Nature, Japan. Cellulose $\delta^{18}\text{O}$ values were calculated by comparison with Merck cellulose (laboratory working standard), which was inserted at every eight tree-ring cellulose samples during the measurements. Oxygen isotope results are presented in δ notation as the per mil (‰) deviation from Vienna Standard Mean Ocean Water (VSMOW): $\delta^{18}\text{O} = [(R_{\text{sample}}/$

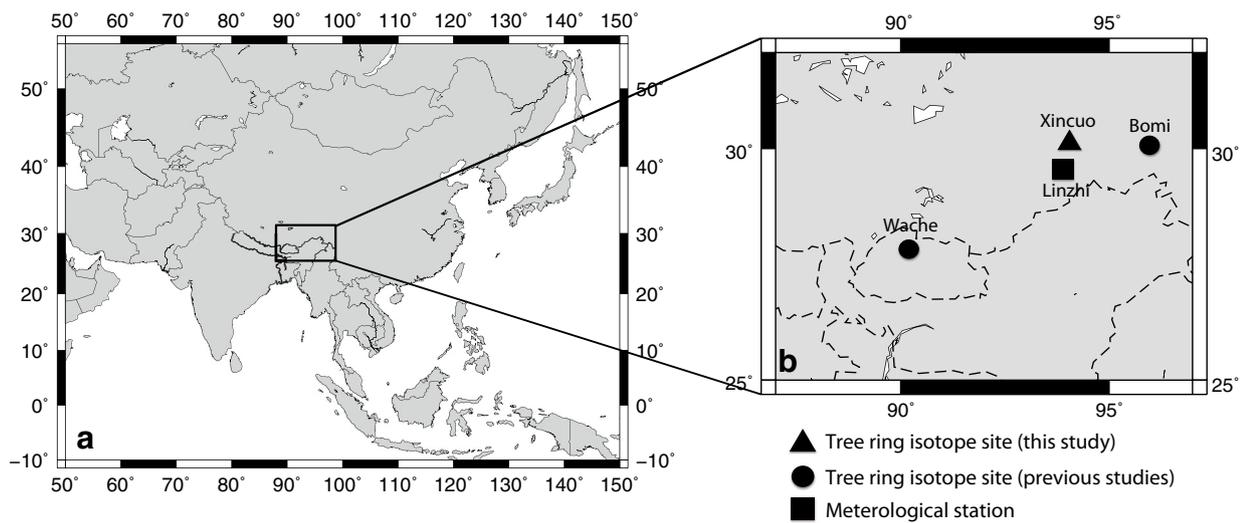


Fig. 1 Map of the study area and individual trees in the sampling site. The study site (Xincuo) is shown as a *black triangle*, the locations of previous data from Bomi (Shi et al. 2011) and Bhutan (Sano et al. 2013) are shown as *black spots*, and the meteorological station at

Linzhi is marked as a *black square* (a, b); sampling locations for *A. georgei* and *H. tibetana* are marked as *green and yellow spots*, respectively (c, d)

$R_{\text{standard}} - 1] \times 1000$, where R_{sample} and R_{standard} are the $^{18}\text{O}/^{16}\text{O}$ ratios of the sample and standard, respectively. The analytical uncertainties for repeated measurements of Merck cellulose (working standard samples) and cellulose samples were approximately $\pm 0.1\text{‰}$ ($n = 100$) and $\pm 0.3\text{‰}$ ($n = 10$), respectively.

Local climate and statistical analyses

According to climate data from the Linzhi meteorological station (Fig. 2), which is the station closest to the study site, monsoon rainfall (from June to September) brought by the Indian summer monsoon provides 71.5% of the region’s annual precipitation (Fig. 2). A previous study on the growth of *A. georgei* var. *smithii* in the southeastern TP indicated that cell enlargement began on May 31 and cell wall thickening ended between late September and early October in 2008 (Li et al. 2013), suggesting that the monsoon season is the main growing season for *A. georgei*. In addition, we used the Royal

Netherlands Meteorological Institute Climate Explorer (<http://www.knmi.nl/>) to examine spatial correlations between tree-ring cellulose $\delta^{18}\text{O}$ and the Standardized Precipitation

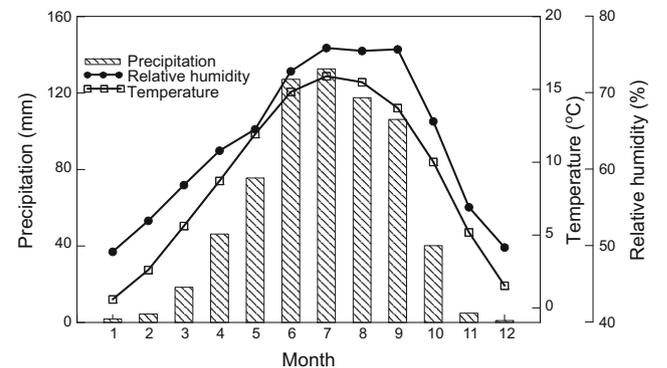


Fig. 2 Monthly mean temperature (*square*), precipitation (*gray bar*), and relative humidity (*black spots*) (1954–2013) at the Linzhi instrumental station

Evapotranspiration Index (SPEI) (Vicente-serrano et al. 2010).

Results and discussion

Sampling heights and micro-environment effects on cellulose $\delta^{18}\text{O}$

To develop long-term chronologies using samples from different sampling heights, it is important to know if the sampling heights affect tree-ring $\delta^{18}\text{O}$ changes. We measured tree-ring $\delta^{18}\text{O}$ at different sampling heights for *A. georgei* (27 and 171 cm, Fig. 3a) and *H. tibetana* (20 and 117 cm, Fig. 3b). Some data are missing in the tree-ring $\delta^{18}\text{O}$ time series (Fig. 3 and 4), relating mostly to either the loss of several rings during the chemical treatment or the loss of cellulose samples during the oxygen isotopic measurement. Tree-ring $\delta^{18}\text{O}$ at different heights for *A. georgei* and *H. tibetana* show high synchronicity ($r = 0.96$, $n = 58$, for *A. georgei*; $r = 0.96$, $n = 49$, for *H. tibetana*), and there are no significant differences in mean and standard deviation between tree-ring $\delta^{18}\text{O}$ values at different heights (Table 1).

Usually, for purposes of climate reconstruction, dendroclimatologists collect samples within sites controlled by the same factors. However, the micro-environment

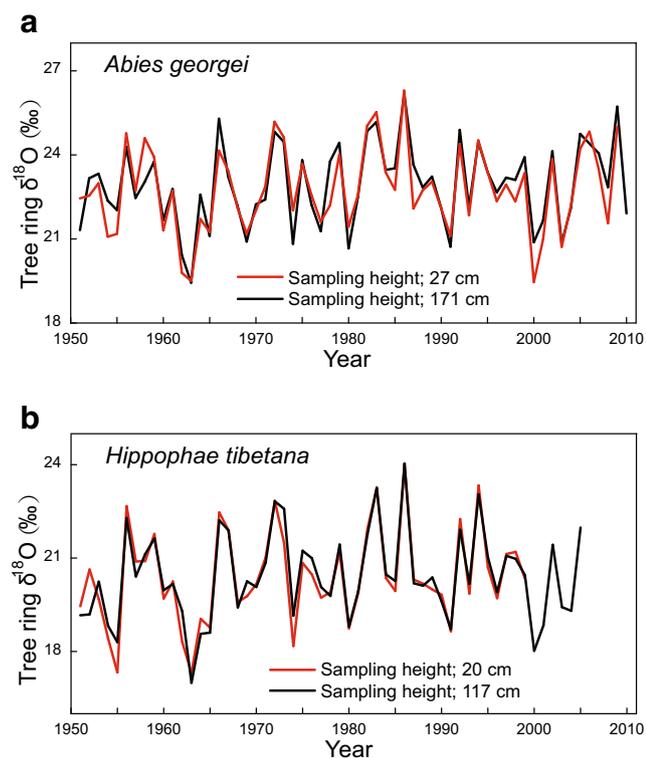


Fig. 3 Tree-ring $\delta^{18}\text{O}$ values from different sampling heights for *A. georgei* (a) and *H. tibetana* (b)

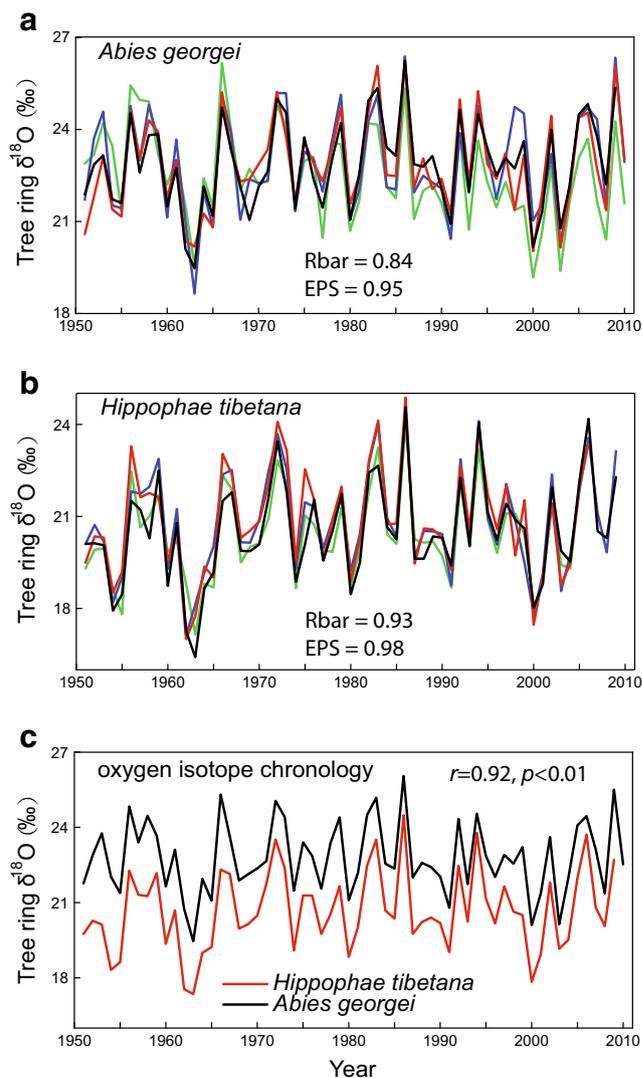


Fig. 4 Tree-ring $\delta^{18}\text{O}$ series from the four trees analyzed for *A. georgei* (a) and *H. tibetana* (b) in this study. c The $\delta^{18}\text{O}$ chronology for *A. georgei* and *H. tibetana*

in which trees live may be different. We compared cellulose $\delta^{18}\text{O}$ for a tree (XC15, in Fig. 1d) that grows near a brook with a tree that grows on top of moraine (XC08, in Fig. 1d). Cellulose $\delta^{18}\text{O}$ values for these two trees are highly correlated ($r = 0.943$, Table 2), and there are no significant differences for mean and standard deviation of tree-ring $\delta^{18}\text{O}$ between them (Table 1). In addition, tree-cellulose $\delta^{18}\text{O}$ growing on both the outer and inner sides of the terminal moraine shows significant positive correlations (Table 2). The average range for cellulose $\delta^{18}\text{O}$ among four trees of *H. tibetana* and *A. georgei* are approximately 0.5 and 0.2‰, respectively, both of which are smaller than that found in previous studies (1–4‰) (Leavitt 2010). If we consider the analytical uncertainties for oxygen isotopes (0.3‰), the differences in mean values among different trees within the same species are not significant. Therefore, we can build up long tree-ring $\delta^{18}\text{O}$

Table 1 Mean and standard deviation of each sample and species

Sample number	XC05a 117 cm	XC05b 20 cm	XC07a	XC08a	XC15b	<i>Hippophae tibetana</i>	XC2a 27 cm	XC2c 171 cm	XC6a	XC12b	XC14c	<i>Abies georgei</i>
Mean(‰)	20.48	20.41	20.49	20.89	20.96	20.70	22.80	22.90	22.87	22.90	22.69	22.83
STD (‰)	1.42	1.50	1.61	1.61	1.69	1.55	1.43	1.43	1.46	1.61	1.46	1.38
Autocorrelation	0.152	0.107	0.181	0.151	0.186	0.125	0.137	N/A	0.007	N/A	0.11	−0.008

chronology using samples from different heights and from the local area.

Inter-annual variations of tree-ring $\delta^{18}\text{O}$ for *A. georgei* and *H. tibetana*

Individual tree-ring cellulose $\delta^{18}\text{O}$ time series from the *A. georgei* and *H. tibetana* measured in this study are illustrated in Fig. 4. The mean values, standard deviations, and first-order autocorrelation of the ten $\delta^{18}\text{O}$ time series for *A. georgei* and *H. tibetana* are shown in Table 1. The inter-tree differences in $\delta^{18}\text{O}$ values within the same species were small. The first-order autocorrelation for each core and species was lower than 0.2. Four trees within the same species showed consistent variations (Fig. 4a, b, Table 2), and the mean inter-series correlations (R_{bar}) for *A. georgei* and *H. tibetana* were 0.84 and 0.93, respectively; the expressed population signals (EPS) for *A. georgei* and *H. tibetana* were 0.95 and 0.98, respectively. It should be noted that the R_{bar} of tree-ring cellulose oxygen isotopes for *H. tibetana* is higher than that reported in previous published results.

The $\delta^{18}\text{O}$ variations between *A. georgei* and *H. tibetana* are also similar. The two $\delta^{18}\text{O}$ time series are significantly correlated ($r = 0.92$, $n = 59$, $p < 0.01$, Fig. 4c). The average value of *A. georgei* is higher ($\sim 2\text{‰}$) than that of *H. tibetana* (Table 1). Because the two species were sampled near one another, their water source should be identical. One possible reason for the $\sim 2\text{‰}$ difference between them could be the species' characteristics, such as leaf morphology. The leaves of *H. tibetana* are larger than those of *A. georgei*, which may result in a longer effective length when water is transported from the

vein to the evaporating surface; therefore, water used for photosynthesis would contain more of a signal from xylem water, leading to depleted oxygen isotopes in carbohydrate and finally in cellulose (Barbour et al. 2004). Another reason for the difference may be the proportions of isotopic exchange between xylem water and carbohydrate oxygen during cellulose formation, which exhibited species-specific variations and caused the difference between average $\delta^{18}\text{O}$ in different species (Li et al. 2011; Song et al. 2014). A previous study in Japan also revealed that the average $\delta^{18}\text{O}$ of trees with needle leaf is higher than in broadleaf trees ($\sim 1.7\text{‰}$) (Li et al. 2015).

Climatic implication of tree-ring $\delta^{18}\text{O}$ for *A. georgei* and *H. tibetana*

Correlations between tree-ring $\delta^{18}\text{O}$ for the two species *A. georgei* and *H. tibetana* and their climatic parameters are shown in Fig. 5. The annual $\delta^{18}\text{O}$ values of *A. georgei* and *H. tibetana* show negative correlations with relative humidity in July–August ($r_{\delta^{18}\text{O of } A. georgei\text{-rh}} = -0.62$, $p < 0.001$, $n = 55$; $r_{\delta^{18}\text{O of } H. tibetana\text{-rh}} = -0.69$, $p < 0.001$, $n = 56$) and precipitation in July–August ($r_{\delta^{18}\text{O of } A. georgei\text{-precipitation}} = -0.50$, $p < 0.01$, $n = 55$; $r_{\delta^{18}\text{O of } H. tibetana\text{-precipitation}} = -0.52$, $p < 0.01$, $n = 56$) and positive correlations with temperature in July–August ($r_{\delta^{18}\text{O of } A. georgei\text{-temperature}} = 0.44$, $p < 0.01$, $n = 55$; $r_{\delta^{18}\text{O of } H. tibetana\text{-temperature}} = 0.43$, $p < 0.01$, $n = 56$, Fig. 5). Previous studies revealed that the correlation between tree-ring $\delta^{18}\text{O}$ of *P. likiangensis* var. *balfouriana* in southeast Bomi and relative humidity in July–August of Linzhi is -0.39 (Liu et al. 2013), and the correlation between tree-ring $\delta^{18}\text{O}$ of *P. likiangensis* var. *linzhiensis* near Bomi with relative humidity in June–August in Bomi is -0.46 (Shi et al. 2011). Tree-ring $\delta^{18}\text{O}$ for *A. georgei* and *H. tibetana* in Xincuo show a stronger climate signal (Fig. 5).

Based on the tree-ring oxygen isotope fractionation model, relative humidity is often the main controlling factor affecting tree-ring cellulose oxygen isotopes (Roden et al. 2000). Lower relative humidity enhances transpiration, which results in enriched leaf-water oxygen isotopes (Dongmann et al. 1974), and signal of leaf-water oxygen isotopes is finally incorporated into cellulose oxygen isotopes. On the other hand, lower relative humidity resulted in enriched soil water oxygen isotopes near the evaporating front (Allison et al. 1983). In addition, precipitation $\delta^{18}\text{O}$ as another controlling factor of

Table 2 Correlation coefficients among time series of $\delta^{18}\text{O}$

<i>r</i>	07a	15b	08a	05	14c	06a	12b
15b	0.916*						
08a	0.940*	0.943*					
05	0.937*	0.936*	0.928*				
14c	0.676*	0.778*	0.746*	0.746*			
06a	0.863*	0.939*	0.907*	0.913*	0.784*		
12b	0.821*	0.842*	0.810*	0.871*	0.806*	0.854*	
02	0.880*	0.922*	0.885*	0.917*	0.785*	0.921*	0.900*

* $p < 0.01$

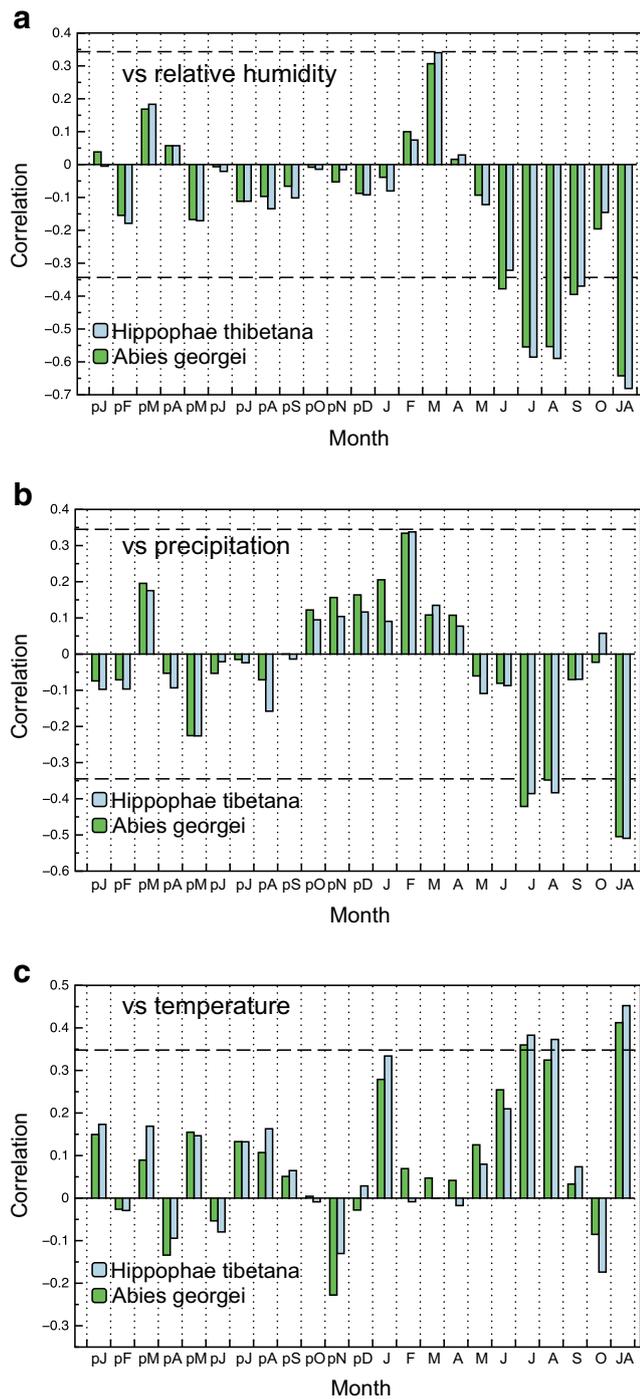


Fig. 5 Correlations between tree-ring $\delta^{18}\text{O}$ chronologies for *A. georgei* and *H. tibetana* and relative humidity (a), precipitation (b), and temperature (c) obtained from the Linzhi instrumental station during the period of 1954–2010

tree-ring $\delta^{18}\text{O}$ is related to relative humidity during convective processes (Risi et al. 2008) and re-evaporation and the isotopic exchange process between water vapor and precipitation $\delta^{18}\text{O}$ (Zhang et al. 2005). All of the above processes explain the close relationship between tree-ring $\delta^{18}\text{O}$ and relative humidity. The correlations between tree-ring $\delta^{18}\text{O}$ and temperature/

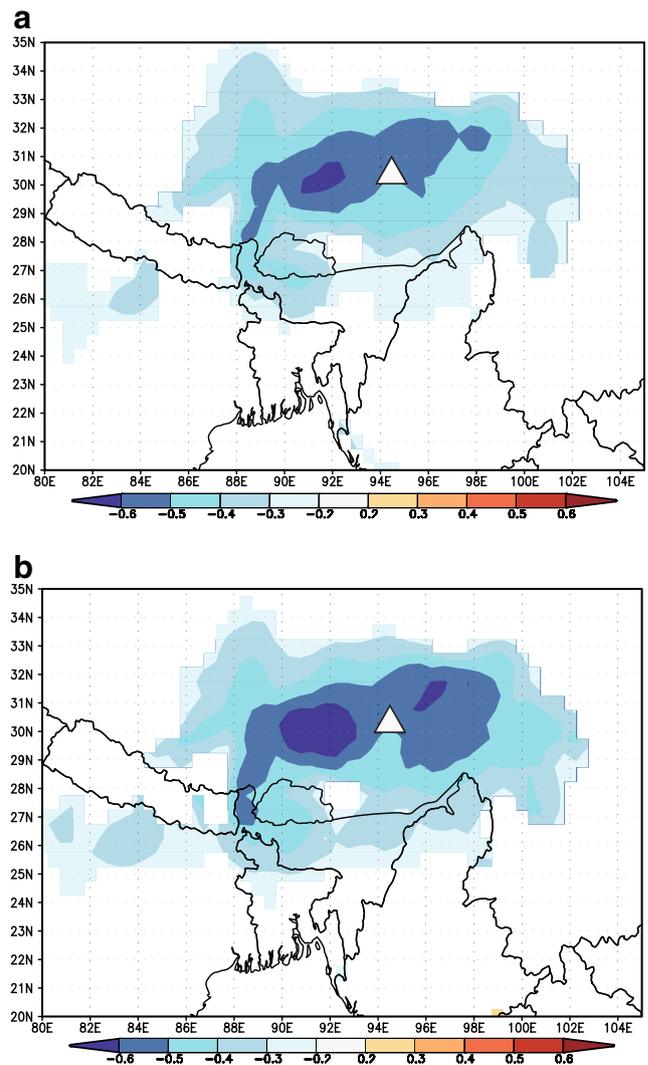


Fig. 6 Spatial correlations between tree-ring $\delta^{18}\text{O}$ for *A. georgei* (a) and *H. tibetana* (b) and June–September SPEI during the period of 1951–2010

precipitation can be explained by the fact that temperature and precipitation are significantly correlated with relative humidity. For example, the correlation coefficient between relative humidity in July–August and temperature/precipitation is -0.50 and 0.80 , respectively.

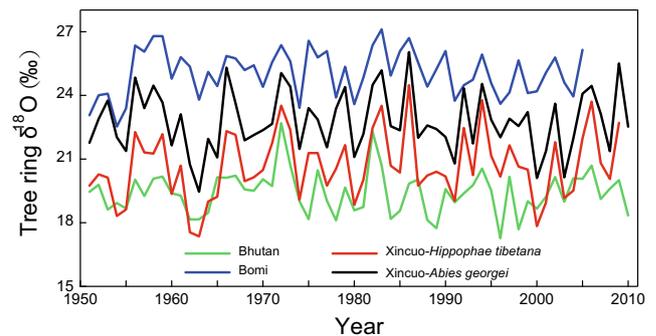


Fig. 7 Tree-ring $\delta^{18}\text{O}$ time series from Xincuo, Bomi, and Bhutan

Spatial correlation analysis was used to evaluate the spatial representativeness of tree-ring cellulose $\delta^{18}\text{O}$ (Fig. 6). Gridded relative humidity data are not available in the study area, so the Standardized Precipitation Evapotranspiration Index (SPEI, Vicente-serrano et al. 2010) was used instead. The results suggest that tree-ring $\delta^{18}\text{O}$ for *A. georgei* and *H. tibetana* represents the regional hydroclimate (29°–32° N, 88°–98° E) in the southeastern TP (Fig. 6). In addition, tree-ring $\delta^{18}\text{O}$ for *A. georgei* and *H. tibetana* in Xincuo shows consistent variations with tree-ring $\delta^{18}\text{O}$ in Bomi and Bhutan (Fig. 7). It should be noted that the tree-ring $\delta^{18}\text{O}$ values for *A. georgei* and *H. tibetana* in Xincuo show significant correlations ($r_{\delta^{18}\text{O}}$ of *A. georgei*-Bhutan = 0.53, $p < 0.01$, $n = 60$; $r_{\delta^{18}\text{O}}$ of *H. tibetana*-Bhutan = 0.61, $p < 0.01$, $n = 59$) with tree-ring $\delta^{18}\text{O}$ values in Bhutan, although Xincuo is approximately 600 km apart from Wache in Bhutan (Fig. 1b).

Conclusion

In this study, we analyzed cellulose $\delta^{18}\text{O}$ for four trees of *H. tibetana* and four trees of *A. georgei* growing on both the outer and inner sides of the terminal moraine from 1951 to 2010, and found that the micro-environment where trees grow does not significantly affect tree-ring $\delta^{18}\text{O}$. In addition, the mean and standard deviation of tree-ring $\delta^{18}\text{O}$ at different heights does not show significant differences. These findings indicate that we can collect samples for tree-ring $\delta^{18}\text{O}$ analysis regardless of sampling heights, and normal sampling design is sufficient to develop a local oxygen isotope chronology.

The mean inter-series correlations of cellulose $\delta^{18}\text{O}$ for *A. georgei*/*H. tibetana* were 0.84/0.93, and the correlation between $\delta^{18}\text{O}$ for *A. georgei* and *H. tibetana* was 0.92. The inter-trees and inter-species cellulose $\delta^{18}\text{O}$ show extremely good coherence. Correlation analysis between tree-ring $\delta^{18}\text{O}$ and climate parameters revealed that $\delta^{18}\text{O}$ for both *A. georgei* and *H. tibetana* have negative correlations ($r = -0.62$, and $r = -0.69$) with relative humidity in July–August and reflected the regional hydroclimate. The results indicate that cellulose $\delta^{18}\text{O}$ for *A. georgei* and *H. tibetana* in Xincuo is a good proxy for regional relative humidity in July–August. All results reveal that we can develop a long tree-ring $\delta^{18}\text{O}$ chronology using multi-trees and multi-species in a local area for paleoclimate reconstruction.

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