Global Ecology and Conservation 53 (2024) e03023

Contents lists available at ScienceDirect

Global Ecology and Conservation

journal homepage: www.elsevier.com/locate/gecco

Original research article

Site-specific climate sensitivity of tree-ring width and vessel anatomical features of *Juglans regia* L. in Bhutan Himalaya

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ARTICLE INFO

Keywords:
Bhutan Himalaya
Climate sensitivity
Intra-annual variation
Juglans regia
Quantitative wood anatomy
Tree rings
Vessel anatomical features

ABSTRACT

Climate change significantly impacts tree growth in the Himalayas, however, there is little information on how broadleaved tree species in Himalayan regions respond to climate change. *Juglans regia* L. (Common or Persian walnut) plays a vital economic and ecological role across the Himalayas, but little is known about how environmental changes influence the growth and wood anatomical traits of this species. In this study, we developed chronologies of tree-ring width (TRW) and six quantitative vessel anatomical features of Persian walnut trees from two sites in the Bhutan Himalayas. We evaluated how TRW and vessel anatomical features respond to climatic conditions and extreme climate events for the period 1960–2020. We found the climate sensitivity of tree growth differed at the two study sites, with the southwest-facing Dodeyna site (DOD) exhibiting higher moisture sensitivity than the north-facing Chimithangka site (CHI). The climate sensitivity of vessel traits was much stronger than that of tree-ring widths, particularly at the DOD. Hydraulic efficiency-related vessel traits (Mean vessel area, MVA; theoretical hydraulic conductivity, K_h; and hydraulic diameter, D_h) were positively sensitive to moisture availability, whereas the hydraulic safety-related vessel traits (vessel density, VD; and vessel grouping index, RVGI) were negatively sensitive to moisture availability at the DOD site. We further observed that MVA and K_h were significantly higher during extreme dry events at CHI site but displayed opposite directions at DOD site. Notably higher RVGI at the DOD site during dry years indicated an enhancement of hydraulic safety against drought. These findings highlight that the climate sensitivity of tree-ring width and vessel traits of walnuts were site-specific and mediated by aspects with the drier site (south-facing slope) being more sensitive. Whilst consideration of the aspects and site-specific climate sensitivity of tree species is crucial to developing robust forest conservation strategies at a larger spatial scale in the Himalayan region which is more vulnerable to climate change.

1. Introduction

The global surface temperature has been increasing at an alarming rate in recent decades which is 0.99 °C higher in the past two

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https://doi.org/10.1016/j.gecco.2024.e03023
Received 1 April 2024; Received in revised form 30 May 2024; Accepted 1 June 2024
Available online 3 June 2024
2351-9894/

decades of the 21st century (2001–2020) than that of 1850–1900 (IPCC, 2023). Concomitantly, frequent extreme climate and weather events such as heatwaves, and persistent droughts have been intensified (Hansen et al., 2012; IPCC, 2023). Global climate change and associated extreme weather events alter the characteristics of ecosystem function (Bonan, 2008). Climate change has affected tree growth and forest productivity globally, including tropical regions, Europe, and the Himalayas (Allen et al., 2010; Debel et al., 2021; Panthi et al., 2020; Zuidema et al., 2022). The Himalayan region is one of the world's major climate change hotspots (Sabin et al., 2020; Chauhan et al., 2023), where climate change has detrimental impacts on ecosystem services and biodiversity loss (Rani et al., 2022). Due to complex topography, the Himalayas host the world's highest snowcapped mountains as well as the lowland tropical/subtropical forests (Chauhan et al., 2023), thus offering a natural experimental setup for studying climate change impacts on tree growth and forest productivity (Panthi et al., 2020). As climatic conditions are decisive factors for tree growth, temperature rise and droughts can affect the physiology of tree growth (Panthi et al., 2020), and ultimately the productivity of forest ecosystems. Hence, it is vital to understand how vegetation response evolves to changing environmental conditions as well as to study the climatic influence on the structure and functions of forest ecosystems (Song and Zeng, 2017).

Having unique characteristics to record extensive ecological information, tree rings and quantitative wood anatomical features offer valuable insights into contemporary and historical perspectives of environmental changes (Panthi et al., 2020; Björklund et al., 2023). Tree rings provide continuous yearly past climatic records for regions or periods with no instrumental climate data (García-Suárez et al., 2009). Wood anatomical variation is an important source of information on the tree adaptation to climate (Tarelkin et al., 2016), with quantitative wood anatomy (QWA) analysis offering more climatic signals than ring width data due to higher intra-annual resolution and close relation to water transport efficiency (Chave et al., 2009; Fonti et al., 2010; Zimmermann et al., 2021). Climatic and environmental processes drive a large part of inter- and intra-annual variability, in which trees respond by adjusting their xylem anatomical structure (Wimmer, 2002; Fonti and Jansen, 2012), which directly reflects the interaction of physiological and environmental factors (Zhang et al., 2020; Zhu et al., 2021). Inter- and intra-specific differences in xylem hydraulic architecture provide information about the plasticity of a species under changing environmental conditions (Fonti et al., 2010), such as precipitation, temperature, and periods of flooding (Islam, 2019). Climatic signals of vessel features in the diffuse-porous (Sass and Eckstein, 1995) and semi-ring porous to ring-porous wood types (Pumijumnong and Park, 1999; Campelo et al., 2010) differed from the tree-ring width (Pumijumnong and Park, 1999; Campelo et al., 2010). Earlywood vessel area of ring-porous winter deciduous oak (*Quercus faginea*) showed a negative correlation with winter temperature (Alla and Camarero, 2012). Combining tree rings and wood anatomy can be a powerful tool for climate change studies since climatic and environmental information is carried by intra-annual anatomical features related to hydraulic efficiency (Olano et al., 2012; Guada et al., 2021). There has been limited attention to combined research involving tree rings and wood anatomical analysis in the Himalayas, despite being a highly climate-sensitive region (Panthi et al, 2017; Islam et al., 2021). While previous studies in the Himalayas mainly focused on conifer species (Bhattacharyya and Chaudhary, 2003; Buckley et al., 2005; Panthi et al., 2017, 2020, Zheng et al., 2021a,b), studies investigating the growth response of broadleaved tree species to climate change are still limited (Pandey, 2018; Panthi et al., 2021; Dhyani et al., 2023).

Intra-annual vessel characteristics of a species can significantly change in response to adverse weather conditions which leads to notable variations in survival and growth rates among individuals of the same species (Gričar et al., 2024). Dry weather conditions affect the wood structure of European beech, reflecting a change from a diffuse-porous to a semi-ring porous structure (Arnič et al., 2021). The Mongolian pine significantly reduced the xylem theoretical hydraulic conductivity by forming a narrower annual ring width attributed to smaller earlywood tracheids during extreme drought (Han et al., 2023). The water-conducting system in broadleaved trees is primarily determined by the size, number, and spatial arrangement of the vessels (Carlquist, 2001). These vessel anatomical features are important ecological and physical traits as they primarily control the conductive capacity, the risk of embolism as well as the mechanical strength of the wood (Diaconu et al., 2016).

Juglans regia L (family Juglandaceae), commonly known as "common walnut", "English walnut" or "Persian walnut" is a "Tertiary relict species" (Beer et al., 2008), and natively distributed across the mountain ranges of Central Asia, South Asia, and Southwest China (Najafi et al., 2014; Yan et al., 2024). *J. regia* is a deciduous species having a semi-ring porous wood structure (Yaman, 2008). Having high economic value for its fruit and timber, the species is widely cultivated in montane regions across the world (Shigaeva and Darr, 2020; Xu et al., 2022). Walnut forests provide various important environmental benefits, such as the equalization of seasonal variations of water flows, slope stabilization and soil protection, and carbon sequestration (Rao et al., 1999; Vahdati et al., 2018). Despite being an important natural resource almost throughout the temperate regions of the world, relatively little is known about the inter-annual variability of its wood anatomical characteristics of this species (Wani and Khan, 2013). Trygov et al. (2024) showed that the growth of walnuts was mainly limited by temperature across elevational gradients in Kyrgyzstan. Meanwhile, model-based predictions show the possible range contraction of *J. regia* populations under warming climates across wider regions in Europe (Paź-Dyderski et al., 2021). However, our understanding of climate sensitivity of tree-growth and vessel anatomical features of walnuts, and their hydraulic adjustments during extreme dry and wet years are still limited in the Himalayan regions. In this study, we used *Juglans regia* as a model species to uncover the general insights on the sensitivity of temperate broad-leaved forests to climate shifts in the Himalayan region. We developed new tree-ring width and vessel anatomical chronologies of *J. regia* from the Bhutan Himalaya to investigate the climate sensitivity of tree growth and vessel anatomical traits, and to assess variations in plasticity of vessel hydraulic features in response to extreme climate events (dry and wet years). Our main research questions were: 1) what are the dominating climatic factors impacting the growth and wood anatomical characteristics of *J. regia* and do these responses differ between south and north-facing aspects? 2) do hydraulic vessel traits show variations in patterns during dry and wet years? We hypothesized that: 1) the growth of *J. regia* is primarily limited by moisture availability, while wood anatomical traits exhibit higher climate sensitivity than ring-width index. The climate sensitivity of tree-growth and vessel anatomical features would differ between north and south-facing aspects since the south aspect receives direct solar radiation thus leading to stronger evapotranspiration, and 2) vessel anatomical features respond differently to dry and wet conditions, forming smaller vessels with more vessel groupings during dry years and larger vessels with fewer vessel groupings during wet years.

2. Materials and methods

2.1. Study area and climate

This study was carried out in the cool temperate forests of Dodeyna (DOD; 27.59°N, 89.63°E) and Chimithangka (CHI; 27.44°N, 89.53°E) in Thimphu District, Bhutan (Fig. 1). According to climate data of Simtokha (Thimphu) meteorological station (27.4 °N, 89.7 °E, 2310 m a.s.l., period: 1996–2020), the study region receives 582 mm total annual precipitation, while the average mean annual temperature is 14.9 °C (Fig. S1). Since available station data are short-spanned (1996–2020) to infer the climate sensitivity of tree growth and vessel features, we used gridded monthly climate datasets for further analyses. The gridded data revealed average annual temperature for the study area is about 6.2 °C, and the annual precipitation is about 1594 mm (Fig. S1). A significant increasing trend was observed for minimum temperature (0.0021 °C per year), mean (0.0018 °C per year), and maximum temperature (0.0014 °C per year), while no significant trend was observed for the precipitation (Fig. S2).

The two sites differ in their aspects where tree-ring samples collected from DOD are situated on the southwest (SW) while samples from CHI are on the northeast (NE). The elevation ranged from 2535 m a.s.l. to 2680 m a.s.l. with an average slope of 6° for DOD. The forest was dominated by blue pine (*Pinus wallichiana*) and Oak (*Quercus semicarpifolia*) along with walnut (*Juglans regia*). Samples collected from CHI ranged from 2449 m a.s.l. to 2539 m a.s.l. with an average slope of 30° and primarily dominated by blue pine. The soil water content was slightly lower for DOD site (20.71 %) than for CHI site (23.32 %), according to the measurement of the gravimetric method in February 2020. However, the differences between the two sites were not statistically significant (Fig. S3).

2.2. Tree-ring sampling, measurement, and chronology development

A total of 30 trees (60 cores) from the DOD site and 31 trees (62 cores) from the CHI site were sampled in March and April of 2020. Two increment cores were extracted from each tree at their breast height, using an increment borer with an inner diameter of 5.15 mm (Haglöf, Sweden). Standard dendrochronological techniques were followed for the sample preparation and tree-ring measurements (Stokes and Smiley, 1996). The cores were air-dried, fixed, and carefully sanded progressively using different grades of sandpapers (220–3000 grits) until the cells and annual ring boundaries were visible. Tree-ring widths (TRW) were measured with the resolution of 0.001 mm under the stereomicroscope (× 40 magnification) connected to a LINTAB digital positioning table (LINTAB™ 6, Rinntech, Germany). Growth patterns for the measured series were visually matched (Stokes and Smiley, 1996) and crossdated using TSAPwin

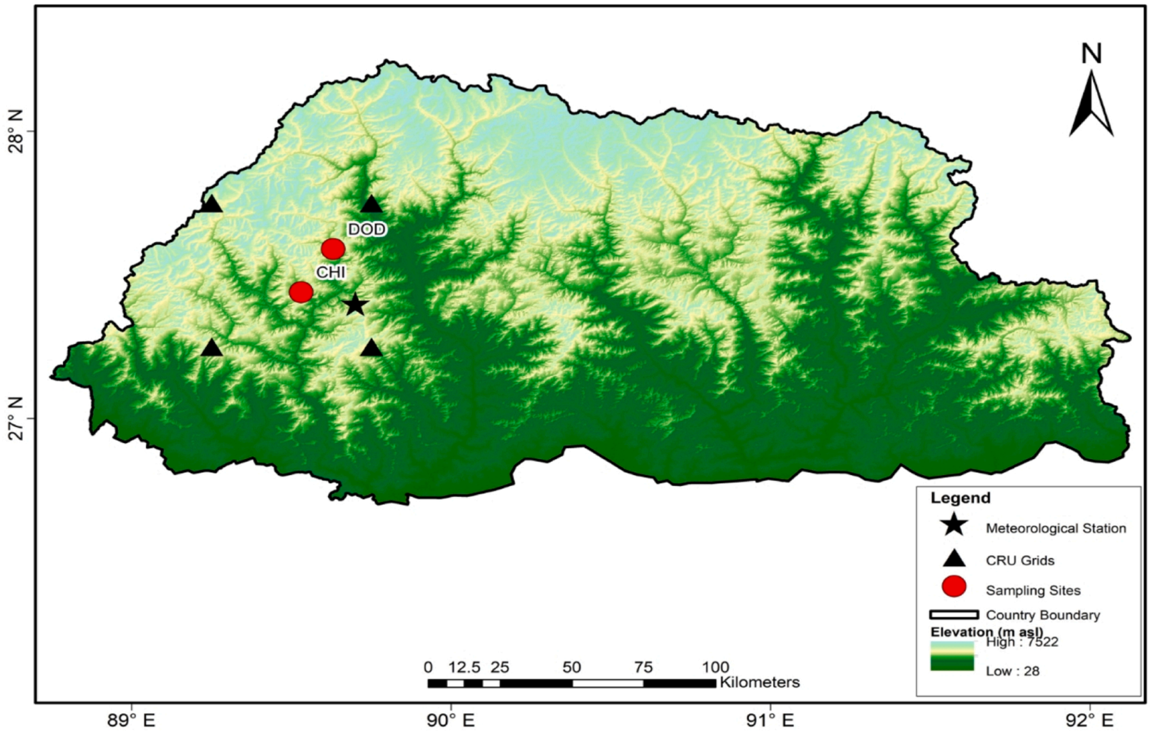

Fig. 1. Map of Bhutan showing the location of walnut tree-ring sampling sites, meteorological station, and the CRU grid points.

software (Rinn, 2003). The quality of cross-dating was further checked using COFECHA software (Holmes, 1983). Finally, 28 trees (50 cores) from the CHI site and 26 trees (45 cores) from the DOD site were successfully crossdated to their calendar year of formation (Table 1); while trees showing individual growth patterns were excluded from further analyses.

All the crossdated raw ring-width series were standardized to develop ring-width chronology by using the "*dplR*" package (Bunn, 2008) in R (R Core Team, 2023). All the raw ring width series were detrended using a negative exponential curve or fitting a linear regression (straight line) to minimize the noise related to the effects of biological growth trends including tree cambial age and to maximize the climate signals. Raw TRW data were power-transformed before detrending (Cook and Peters, 1997) to stabilize the variance of each raw series with a decreasing number of samples with time (Frank et al., 2007). The detrended series were then averaged to develop mean function chronology by computing a bi-weight robust mean (Briffa and Jones, 1990). The residual chronology, which preserves only high-frequency climate signals but is also free of the time-series autocorrelation, was used for further analysis. The statistical quality of the chronologies was evaluated by using the coefficients commonly applied in dendrochronology: expressed population signal (EPS, Wigley et al., 1984), mean inter-series correlation (rbar), mean sensitivity (MS) (Fritts and Shatz, 1975; Schulman, 1956), signal to noise ratio (SNR), and first-order auto-correlation (AC1) (Fritts, 1976), and average growth rate (AGR).

2.3. Measurement of vessel anatomical features

Ten well crossdated and undamaged tree cores with high inter-series correlations were selected from each site for quantitative wood anatomy analysis (QWA). The finely smoothed wood surfaces were rubbed with white chalk to improve the contrast of each wood vessel from other wood traits (Campelo et al., 2010). High-resolution digital images (2.20 μm/pixel at 100× magnification) of wood surfaces for each tree core were obtained by using a Zeiss Smartzoom 5 microscope (Carl Zeiss Microscopy GmbH, Germany) (Fig. S4). To prevent distorted images caused by uneven or curved core surfaces, images were captured in 2-inch sections along the core length. Adobe Photoshop CS3 (Adobe Systems Incorporated, USA) was used to simplify the measurements, further increase the contrast between the vessels and other wood elements, and we also manually edited unclear cases of vessel lumen area due to preparation artifacts.

Semi-automatic image analysis tool, ROXAS (von Arx et al., 2013; Wegner et al., 2013) was used for the measurement of vessel anatomical features, which relies on the software Image-Pro Plus (v7.0, Media Cybernetics, Silver Spring, Maryland, USA). Six vessel parameters were used for further analysis including mean vessel lumen area per ring (MVA), theoretical xylem hydraulic conductivity (K_h), percentage of conductive area (RCTA), mean hydraulic diameter (D_h), vessel density (VD), and vessel grouping index (RVGI). For accurate measurements, the configuration file for the walnut species was created to detect different sizes based on the high-resolution digital images taken. Misidentified annual ring boundaries, and missing vessels during the measurements were corrected using the manual function of ROXAS until all vessels were detected. All the six vessel anatomical parameters were calculated by using the program ROXAS as:

$$\text{Hydraulic diameter } (D_h) = \left(\frac{\Sigma d^4}{n}\right)^{0.25} \tag{1}$$

where d is the diameter of the vessel (Tyree and Zimmerman, 2002).

$$\text{Mean vessel lumen area (MVA)} \quad = \frac{1}{\mathrm{n}}\sum\nolimits_{i=1}^{n} \mathrm{A}_i \tag{2}$$

where A is the vessel area (μm^2), and n is the number of vessels per ring.

$$\text{Theoretical xylem hydraulic conductivity } (K_h) = \frac{\pi \mathrm{D}^2}{128 * \eta} \tag{3}$$

where D is the diameter, and η is the viscosity index of water (1.002×10^{-9} MPa s at 20°C). K_h is the hydraulic conductivity [Kg m MPa^{-1} s^{-1}] based on the Hagen–Poiseuille law (Scholz et al., 2013).

$$\text{Vessel density (VD)} \quad = \frac{\text{Number of vessel in each ring}}{\text{Ring area } (\mathrm{mm}^2)} \tag{4}$$

Table 1

Chronology statistics of walnuts at Chimithangka (CHI) and Dodeyna (DOD) in Thimphu, Bhutan Himalaya. MS: mean sensitivity; MSL, mean segment length; AGR, average growth rate; SNR: signal-to-noise ratio; Rbar: mean inter-series correlation; EPS: expressed population signal. AGR was calculated from raw series, whereas MS, SNR, Rbar, and EPS were calculated from detrended series. Rbar, EPS, and SNR were calculated for the common period 1980–2020, while others were calculated for the whole period.

Site	Time span	Trees/Cores	Trees/Cores for common period analysis	AGR (mm/year)	MS	Rbar	SNR	EPS
CHI	1920–2020	28/50	15/26	2.875	0.304	0.164	5.646	0.850
DOD	1903–2020	26/45	16/24	3.506	0.212	0.183	5.838	0.854

$$\mathrm{RCTA} = \frac{\mathrm{CTA}}{\mathrm{RA}} \tag{5}$$

where CTA is the cumulative area of all counted vessels (mm^2), and RA is the ring area (mm^2).

$$\text{Vessel grouping index (RVGI)} = \frac{\text{Total number of vessels}}{\text{Total number of vessel groupings}} \tag{6}$$

RVGI is a vessel grouping index with 1 indicating exclusively solitary vessels, and the higher the index, the greater the degree of vessel grouping (Scholz et al., 2013).

To develop chronologies of xylem vessel parameters, measurements were standardized to reduce the possible influence of biological age effects by fitting a two-third cubic smoothing spline curve using the "*dplR*" package (Bunn, 2008) in the R program (R Core Team, 2023). A robust bi-weight mean was computed to remove the influence of outliers on the chronologies (Briffa and Jones, 1990). Gini coefficient (Gini), first-order autocorrelation (AC1), and mean inter-series correlation (Rbar) were used to test the robustness and signal strength of the chronologies. The Gini coefficient (Gini, 1921) measures distribution inequality as a function of the sum of absolute differences between all pairs of individuals.

2.4. Climate data

To analyze the growth-climate correlation relationships, the gridded monthly climate datasets (Temperature, Precipitation, and scPDSI) for the period 1960–2020 from climatic research unit (CRU) grid-points at 0.5° × 0.5° spatial resolution (CRU TS v4.07, Harris, et al., 2020 https://crudata.uea.ac.uk/cru/data/hrg) via KNMI Climate Explorer (Trouet and Oldenborgh, 2013; https://climexp.knmi.nl/start.cgi) were used. Besides, gridded SPEI (Standardized Precipitation Evapotranspiration Index) datasets from the Global SPEI database (https://www.spei.csic.es/, SPEIbase v.2.9) were extracted. SPEI is a widely used meteorological drought index which is a measure of the standardized difference between water supply (precipitation) and water demand (potential evapotranspiration) (Vicente-Serrano et al., 2010). The climate responses based on three-month integrations of SPEI (SPEI03) were evaluated as it is more strongly correlated with soil moisture contents than other timescales (Wang et al., 2024).

2.5. Climate sensitivity and statistical analyses

To determine the climate sensitivity of tree radial growth of *J. regia*, the bootstrapped Pearson's correlation coefficients were computed between tree ring-width chronologies and monthly climate datasets for the period 1960–2020. Since the climate of the previous year's growing season may affect tree growth in the current year (Fritts, 1976), the climate of the previous year's late growing season months for climate sensitivity analysis was also considered. Accordingly, the climate sensitivity analysis was performed on a "15-month" dendroclimatic window spanning from the previous year August to October of the current growth year. Besides, correlation coefficients for dry (previous November to current April) and wet (May to September) seasons were also calculated. Climate sensitivity analysis was done using the 'dcc' function in 'treeclim' package (Zang and Biondi, 2015) in R (R Core Team, 2023). Furthermore, the climate sensitivity of six vessel anatomical features (RCTA, K_h, VD, MVA, D_h, and RVGI) was also computed for the period 1960–2020.

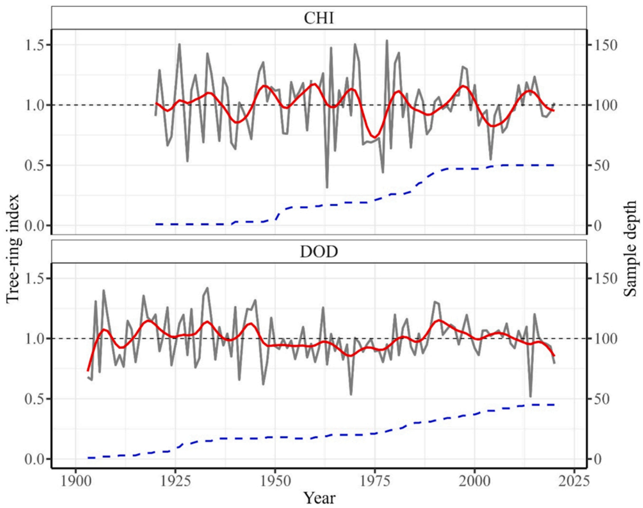

Fig. 2. Ring-width chronology (grey line) of Juglans regia with 10-year low pass filter (red line) and numbers of sample used (sample depth, blue dashed line) at Chimithangka (CHI) and Dodyena (DOD) sites.

Three extreme dry years (1965, 1982, and 2009) and wet years (1974, 1978, and 2000) were selected (Fig. S5) based on annual three-month SPEI values for the period 1960–2020 to assess the plasticity of tree-ring structures during extreme weather conditions (Schume et al., 2004). Furthermore, each annual growth ring was arbitrarily divided into four radial tree-ring sectors (referred to as quarters Q1-Q4) of equal width based on its center coordinate to assess the intra-annual variability of xylem vessel features. The porosity ratio and Gini coefficients of vessel area in tree rings were also calculated to quantitively estimate ring porosity. The Gini coefficient was calculated using the R-package "*reldist*" (Handcock, 2016) while the porosity ratio (PoRa) was computed as the ratio between the lumen area of the widest vessel in the first third of the ring and the lumen area of the widest vessel in the following part (Castagneri et al., 2020). Lower PoRa indicates that the area of vessels in the ring is similar, as was expected for diffuse-porous wood, whereas higher values correspond to greater inequality, as expected for ring-porous wood (Castagneri et al., 2020). All the analyses were performed using R version 2023.09.1 (R Development Core Team, 2023).

3. Results

3.1. Tree-ring and vessel anatomical features

The DOD tree-ring chronology spanned 118 years from 1903 to 2020, and the tree-ring chronology of the CHI site spanned 101 years from 1920 to 2020. The site chronologies showed moderate to high values of MS, Rbar, SNR, and EPS demonstrating their dendroclimatic potential (Table 1, Fig. 2). The average annual growth rate of walnuts in DOD (3.506 mm/year) was higher than in CHI site (2.875 mm/year). Chronologies of quantitative vessel anatomical features of walnuts were constructed for ten well cross-dated series with high inter-series correlations. The standard chronologies of six vessel anatomical features (RCTA, K_h, VD, MVA, D_h, and RVGI) were developed for the period 1960–2020 (Fig. S6). MVA was significantly negatively correlated with RGVI and VD for both CHI and DOD sites (Fig. S7). However, D_h was significantly negatively correlated with VD only at DOD site. Significant differences in the tree ring width and anatomical features between the two study sites were also observed, with CHI site showing significantly lower TRW, D_h, VD, RVGI, and K_h, and a higher RCTA as compared with the DOD site (Fig. S8).

3.2. Climate sensitivity of tree-growth and vessel anatomical features

The correlation analysis between monthly climate data and the tree-ring index indicated that moisture availability was the primary

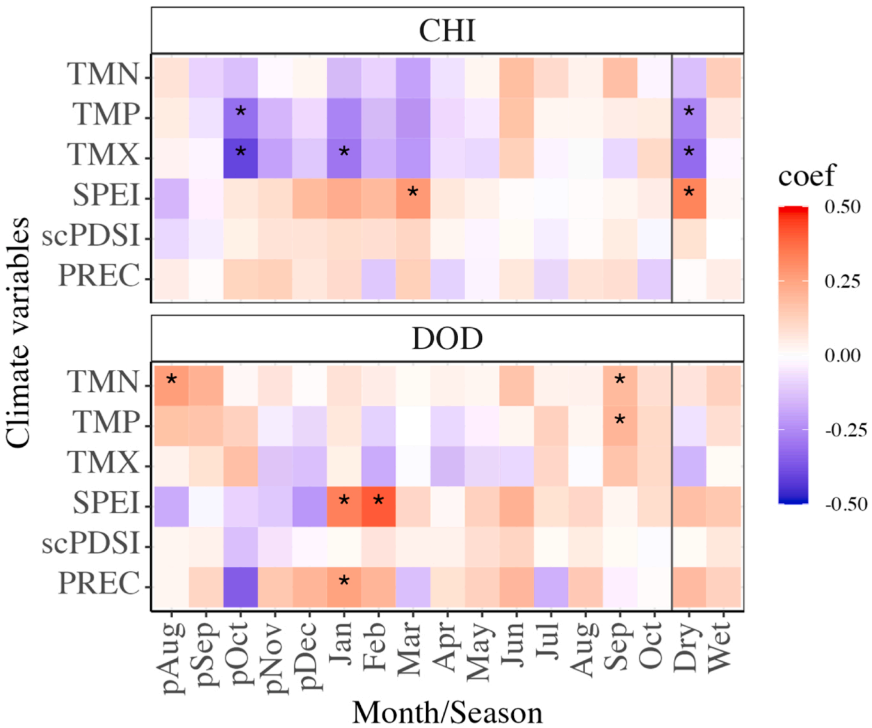

Fig. 3. Correlation coefficients between tree-ring chronologies of two sites and climatic variables. Climate variables included mean (TMP), maximum (TMX), and minimum (TMN) temperature, precipitation (PREC), Standardized Precipitation Evapotranspiration Index (SPEI), and self-calibrating Palmer drought severity index (scPDSI). Correlation coefficients were calculated for a 15-month window from the previous August (pAug) to the current October (Oct) and for two seasons: dry (pNov-April) and wet (May-Sept) for the period 1960–2020 for both CHI and DOD sites. The color bar shows the range of correlation coefficients. Significance level: *, $p < 0.05$.

limiting factor for the growth of walnuts in both sites. Ring-width chronology at the CHI site showed a significant positive correlation with SPEI for the current March and dry season, but a negative correlation with mean temperature of the previous October and dry season. The negative relationship was much stronger with the maximum temperature of the previous October, current January, and dry season. Similarly, the tree growth at the DOD site showed significantly positive correlations with SPEI in January and February, precipitation (PREC) in January, minimum temperature (TMN) in previous August, and mean and minimum temperatures in

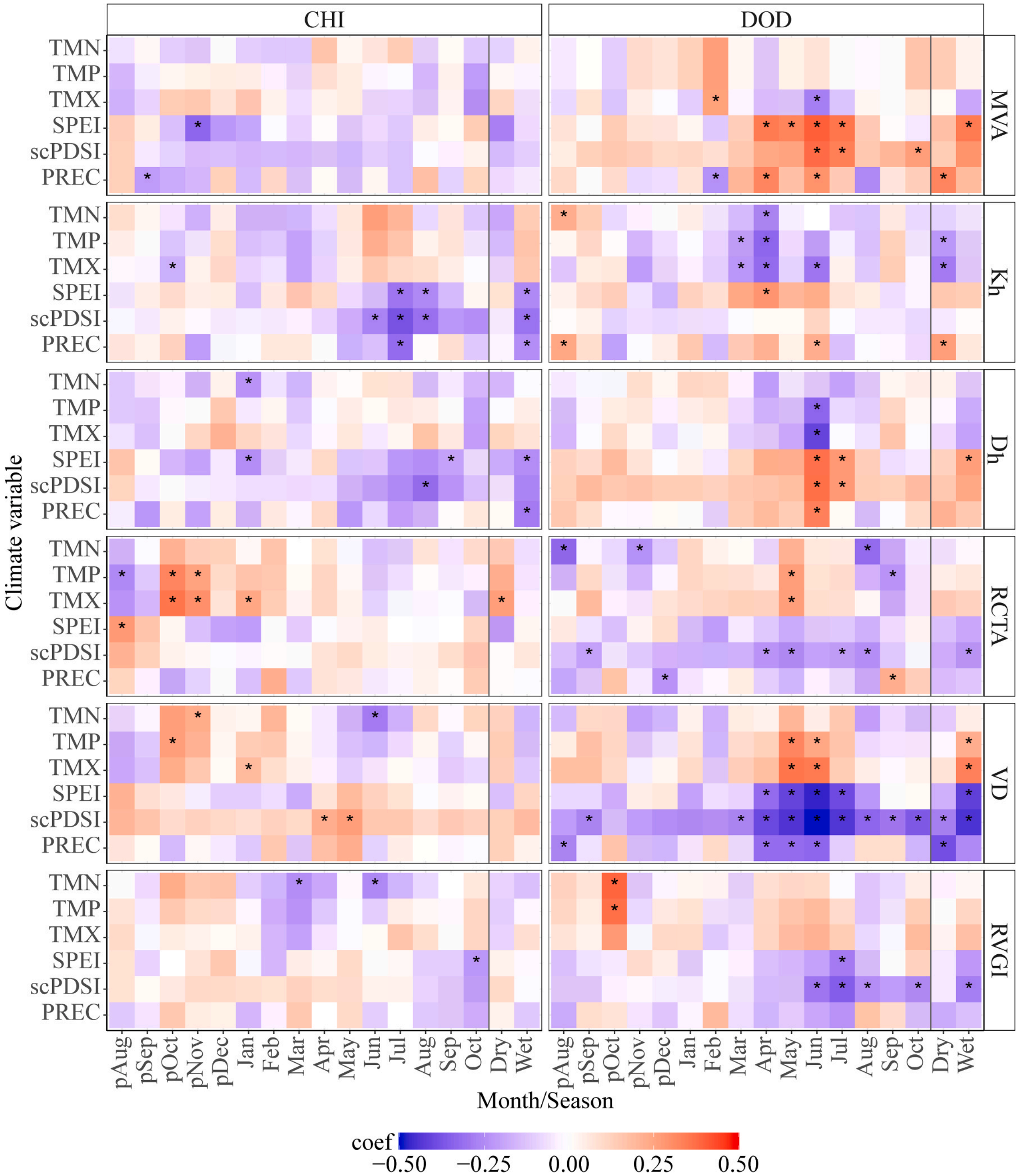

Fig. 4. Climate sensitivity of six wood anatomical features at CHI and DOD sites. Wood anatomical features: MVA, Mean vessel area; K_h, Theoretical hydraulic conductivity; D_h, mean hydraulic diameter; RCTA, Percentage of conductive area within xylem; VD, Vessel density; RVGI, Vessel grouping index. Climatic variables: TMP, Mean temperature; TMX, Maximum temperature; TMN, Minimum temperature; PREC, Precipitation; SPEI, Standardized Precipitation Evapotranspiration Index; scPDSI self-calibrating Palmer drought severity index. Correlation coefficients were calculated for a 15-month window from the previous August (pAug) to the current October (Oct) and for two seasons: dry (pNov-April) and wet (May-Sept) for the period 1960–2020. The color bar shows the range of correlation coefficients. Significance level: *, $p < 0.05$.

September (Fig. 3).

The response of vessel anatomical features to climate variables also differed between the two sites, with DOD site showing a stronger climate signal than that of CHI site. Hydraulic efficiency-related vessel traits (MVA, K_h, D_h) showed significant positive moisture sensitivity while the hydraulic safety-related vessel traits (VD, RVGI) revealed significant negative temperature sensitivity at the DOD site. The phenomenon was alike for vessel traits at CHI site but the relationship was either non-significant or opposite to that of the DOD site (Fig. 4).

Although the sensitivity of hydraulic efficiency-related annual vessel traits (VA, $K_{h,}$ and D_h) to temperature was not statistically significant at CHI site, the sensitivity of intra-annual VA, K_h, and D_h (during the Q3 and Q4 of tree-ring) showed significant positive relationships with temperatures of spring months and/or early growing season (Fig. S9). On the contrary, the negative moisture sensitivity of K_h and D_h in CHI site was weaker in intra-annual resolution compared to annual resolution (Fig. 4; Fig. S9). Similarly, positive moisture sensitivity of K_h, $D_{h,}$ and MVA at DOD site was more evident for Q2-Q4 than that of annual ring. Intra-annual negative temperature sensitivity of D_h at DOD site remained consistent during spring months. Furthermore, the negative temperature sensitivity of K_h during the spring months/early growing season weakened for the second quarter. Meanwhile, the negative temperature sensitivity of MVA at DOD site becomes more evident for Q1, Q3, and Q4 of tree rings (Fig. S9).

3.3. *Vessel anatomical feature's response to extreme climates*

The vessel anatomical features of *J. regia* significantly differed between dry and wet years. During the dry years, D_h, MVA, and K_h

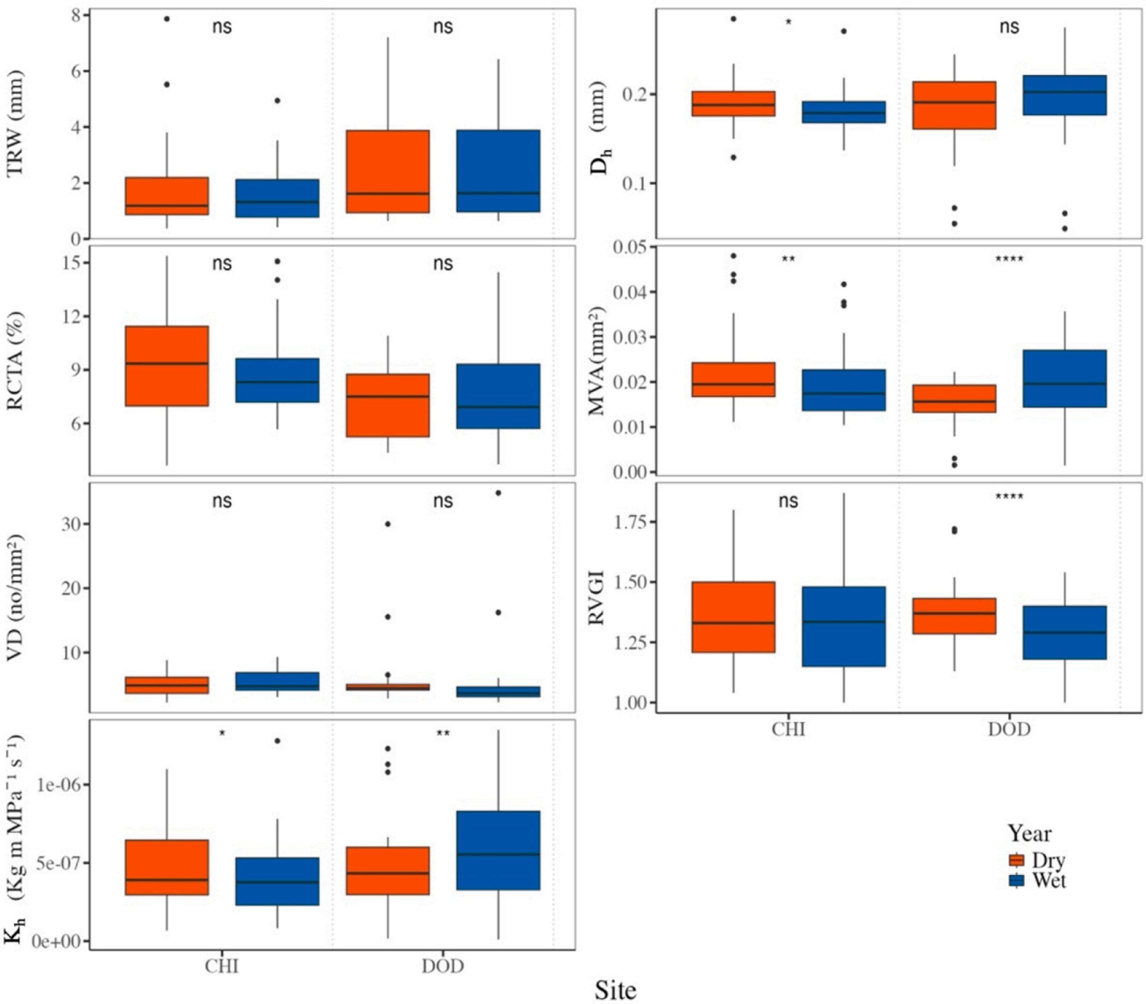

Fig. 5. Tree-ring width (TRW) and mean vessel anatomical features; D_h, mean hydraulic diameter; RCTA, Percentage of conductive area within xylem; MVA; Mean vessel area; VD, Vessel density; RVGI, Vessel grouping index; K_h, Theoretical hydraulic conductivity during extreme dry (red) and wet (blue) years. The mean between dry and wet years was compared by commuting student's T-test. Significance level: ns, statistically non-significant; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; and ****, $p < 0.0001$.

significantly decreased for CHI site, meanwhile, MVA and K_h significantly increased, and the VD decreased for DOD site. RVGI in dry years was significantly higher for DOD site (Fig. 5). A higher porosity ratio (PoRa) was observed for dry years for CHI site, whereas DOD site displayed a higher porosity ratio during wet years. However, walnut trees maintained semi-ring porosity of their vessel traits at both sites (PoRa > 1). Despite slight differences in porosity ratio, the Gini coefficient for both sites showed similar responses with comparable levels of porosity (Fig. 6). Furthermore, MVA, D_h, and K_h showed variation between extremely dry and wet years, especially for the first two quarters but with different patterns between CHI and DOD sites, indicating the intra-annual variation in hydraulic efficiency-related wood traits is site-specific (Fig. S10).

4. Discussion

4.1. Climate sensitivity of radial growth and vessel anatomical features

In line with the hypothesis of walnut tree growth in Bhutan Himalaya being limited by moisture availability, the climate-growth response analysis revealed negative temperature sensitivity but positive moisture sensitivity during early spring or dry period (Fig. 3). Previous studies across the Himalayas and nearby Mountain regions also showed positive (negative) moisture (temperature) sensitivity of growth of broadleaved tree species (Dawadi et al., 2013; Liang et al., 2014, 2019) and conifers species (Fan et al., 2009; Gaire et al., 2017; Panthi et al., 2017; Tiwari et al., 2017; Yang et al., 2022). Contrary to our results, some other studies revealed a positive effect of winter temperature on the radial growth of some broad-leaved species in the Himalayas (Panthi et al., 2021; Shah and Mehrotra, 2017). More recently, Tyrgotov et al. (2024) showed that the growth of walnuts along an elevational gradient in Kyrgyzstan was mainly limited by temperature. The non-consistent results between the present study and Tyrgotov et al. (2024) highlight that more dendroecological studies of walnut trees across different sites and regions could help understand how this tree species will respond to climate change.

As Zhu et al. (2021) stated, the sampling sites should also be considered, with temperature often limiting the vessel features in mesic or high-elevation areas, while precipitation or drought stress limiting these features in xeric or low-altitude areas. Similar to our results, the growth of walnuts from Southern Kyrgyzstan was positively correlated with precipitation during the previous summer and previous winter and negatively correlated with temperatures of the previous year's autumn (Winter et al., 2009). Our study revealed much stronger climate signals in the vessel features than in tree rings, which could be because ring width was determined by the integrating radial growth throughout the growing season. At the same time, measurements of wood-anatomical variables yield much more information from different parts of the tree ring, which is highly variable along radial (time) position within a tree ring. The intra-annual vessel features supplement the findings of the annual vessel features (Fig. 4, Fig. S9). The intra-annual xylem formation can be used to better understand how short-term climate conditions during the growing season affect vessel formation, and the influence of intra-seasonal climate variability can be evaluated on other vessel features such as vessel grouping (Arnič et al., 2021).

Generally, the size of vessels (D_h or MVA) in angiosperms is an indicator of hydraulic efficiency or safety (Fonti et al., 2010). The most pronounced difference in the response of the vessel parameters to the climate suggests a trade-off between hydraulic safety and efficiency, which is widely reported in the studies of wood anatomy (Arnič et al., 2021; Han et al., 2023). The K_h, D_h, and MVA showed a significant positive correlation with moisture variables (SPEI, precipitation, scPDSI), while the moisture variables were negatively correlated with RCTA, RVGI, and VD in DOD and vice versa in CHI (Fig. 4).

Climate sensitivity of radial growth and inter- and intra-annual vessel features were more pronounced in the DOD site on the south aspect than in the CHI site on the north aspect (Fig. 3, Fig. 4, Fig. S9). South-facing slopes receive more direct solar radiation, leading to higher temperatures, and increased evapotranspiration rates, and drier soil conditions as compared to the north-facing slopes (Bilir et al., 2021; Quadri et al., 2021). Since the south-facing slope is typically drier than the north-facing slope, tree species in the DOD site could be faced with higher demand for evapotranspiration making them more susceptible to moisture conditions and a significant soil

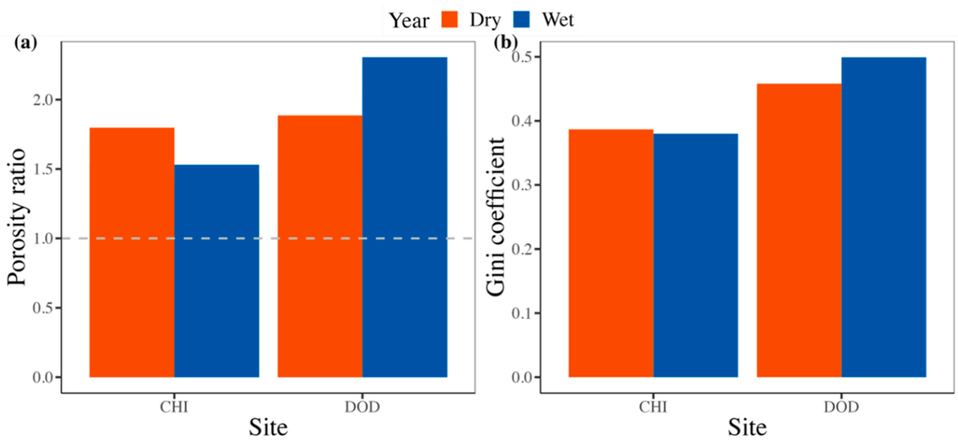

Fig. 6. Gini coefficient and porosity ratio during extreme climates (dry and wet years) in DOD and CHI sites.

water deficit (Martinez del Castillo et al., 2018; Arnič et al., 2021). Our results showed that soil water content was slightly lower at DOD site compared to the CHI site (Fig. S3). However, other factors, such as soil texture, porosity, and bulk density would also affect soil water availability for these two sites (Zhang et al., 2021), which were unfortunately not measured in the present study.

Similar to our findings, conifer tree species (genera *Picea*, *Juniperus*, *Cedrus*, and *Abies*) from the northwest Himalayas on south-facing slopes tend to be more moisture-limited, while trees on north-facing slopes are less moisture-stressed and their growth is more strongly influenced by temperature variations, especially during the growing season (Sohar et al., 2017). The climate-growth interactions of trees show a great regional variation due to different topography and climatic gradients throughout the Himalayan regions, thus, growth limitation by moisture or temperature would depend on aspect, elevation, and local precipitation patterns (Pandey et al., 2018; Liang et al., 2019; Panthi et al., 2020; Zheng et al., 2021). With climate warming and accompanied atmospheric drought (Yuan et al., 2019), tree growth in the south-facing slopes would be more likely to decline than that of the north-facing slopes in the Himalayan regions.

4.2. *Vessel hydraulic response to extreme climates*

The vessel features of *J. regia* significantly differed between the extreme dry and wet years (Fig. S9). Gričar et al. (2024) also reported variations among sites for dry and wet years, where the wet year promoted tree-ring widths and vessel sizes, and the dry year negatively affected both traits. Besides the Gini coefficient being quite similar for both sites, the porosity ratio (PoRa) was different (Fig. 6). Porosity ratio was slightly higher in CHI site for dry years, while it was the opposite in DOD site (Fig. 6), indicating that diffuse porosity was marginally affected by site conditions (Castagneri et al., 2020). However, *J. regia* maintained its porosity and displayed a semi-ring porous pattern in both dry and wet years (Yaman, 2008).

MVA significantly differed between dry and wet years. The trees on the SW-facing slope on higher elevation had wider rings for both dry and wet years as compared to those on NE-facing slopes. RVGI was significantly higher in the dry years than the wet years at the DOD site (Fig. 5; Fig. S11). Similar results were also reported by Zhu et al. (2021), indicating that the number and size of vessels increase to maximize ring width during favorable growth conditions and vice-versa during unfavorable growth conditions. Yaman (2008) reported an increasing number of vessels per group of *J. regia* wood with increasing altitude in the western Black Sea region, which might be explained as an enhancement of hydraulic safety. The vessel size (D_h or MVA) in the rings is generally considered as an indicator of hydraulic efficiency (Han et al., 2023) whereas vessel density and RVGI are related to hydraulic safety (Argüelles-Marrón et al., 2023). In the Bhutan Himalaya, *J. regia* reduced the MVA and increased RVGI during dry years at the DOD site for hydraulic safety against embolism due to direct solar insolation in the south aspect. Furthermore, higher hydraulic conductivity during wet years, indicates trees are physiologically more efficient in conducting a higher amount of water during favorable conditions. In contrast, trees maintained significantly higher hydraulic conductivity, MVA, and vessel diameters at CHI site in dry year in the north aspect, indicating trees adjust higher hydraulic efficiency during dry years if moisture availability is sufficient and solar insolation is relatively lower. Trees growing in arid areas and/or during drier environmental conditions improve their hydraulic safety by producing smaller and more vessels (Fonti et al., 2010). In dry years, diffuse-porous species (such as genera *Quercus*, *Fraxinus*, and *Fagus*) displayed an increase in vessel density and a decrease in vessel diameter aimed to preserve hydraulic conductivity (Zimmermann et al., 2021). Contrary to the findings of Zimmermann et al. (2021), we didn't observe a significant variation in vessel density during dry and wet years at both sites, indicating *J. regia* maintains consistent vessel density in their annual growth rings, which is probably associated with maintaining the vessel porosity of this species. Quantifying wood cell anatomical features provides a valuable approach to monitoring changes in tree growth, xylem functioning, and xylem plasticity in response to climate variability, which will improve our understanding of how trees adapt their hydraulic systems to cope with climate change (Castagneri et al., 2020; Arnič et al., 2021; Rita et al., 2022). The results thus provide new insight into the relationships of tree rings and vessel anatomical features of *J. regia* to the climate variability in Bhutan Himalaya.

5. Conclusions

This study established two new tree-ring width and quantitative wood anatomical features chronologies of *Juglans regia* from in the Bhutan Himalayas. This study is the first attempt to combine tree-ring width and quantitative wood anatomical features to evaluate climate influence on tree growth and hydraulic adjustment of walnut trees in Bhutan. Site-specific climate responses were observed, notably stronger moisture sensitivity in the DOD site in the south-facing mountain slopes. Variations in wood anatomy responses were also observed during dry and wet years, which underscore the trees' adaptability to changing climatic conditions. Our findings further highlight the climate sensitivity of vessel traits can even be evident for intra-annual resolution. Reduced mean vessel and increased vessel grouping during dry years in the DOD site highlight the tree's attempt to balance hydraulic safety and efficiency. This study will help extend the tree-ring network of broad-leaved species from the Himalayas and highlight the great potential of wood anatomical features of Persian walnuts for climate reconstruction. This study underscores climate impacts on the plasticity of tree growth and hydraulic adjustment of *Juglans regia* of this economically important species in Bhutan Himalaya. Our findings highlight an application of vessel anatomical features is a much stronger tool than tree rings to evaluate the sensitivity of hydraulic adjustment during extreme drought events. Moreover, our results also indicated that due to greater moisture sensitivity, tree growth in the south-facing slopes would be more likely to decline than that of the north-facing slopes in the Himalayan region.

Funding

This study was carried out in support of a research fund by the National Natural Science Foundation of China (NSFC, 31870591, 32150410353), and the Sci-Tech Department of Yunnan Province (202301AT070307, 202301AT070334). JD was supported by "The Alliance of International Science Organizations (ANSO)" scholarship for Master's study. SP was supported by the "2022 Zhihui Yunnan project" (202203AM140020) and the "Funds for Foreign Young Talents" from the Department of Resource Allocation and Management, Ministry of Science and Technology, China (RC-2022–035-BN). P.-L.F. and Z-X. F. were supported by the Ten Thousand Talent Project of Yunnan Province (YNWR-QNBJ-2019–190, YNWRQNBJ-2020–095) and West Light Talent Program of the Chinese Academy of Sciences (xbzg-zdsys-202218).

CRediT authorship contribution statement

Jambay Dema: Writing – original draft, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Pei-Li Fu:** Writing – review & editing, Supervision, Software, Resources, Methodology, Funding acquisition, Formal analysis, Conceptualization. **Shankar Panthi:** Writing – review & editing, Supervision, Software, Resources, Methodology, Investigation, Formal analysis, Conceptualization. **Ze-Xin Fan:** Writing – review & editing. **Zaw Zaw:** Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgments

We thank the Department of Forest and Park Services for approving the clearance to carry out this research. We acknowledge Mr. Dorji Drukpa and Ugyen Wangchuck Institute for Conservation and Environment for allowing us to use the laboratory. We thank the Divisional Forest Office Thimphu for helping us with the fieldwork. We thank the National Center for Hydrology and Meteorology for providing the meteorological data of Simtokha (Thimphu) station. Xishuangbanna Station for Tropical Rain Forest Ecosystem Studies and Public Technology Services Center, Xishuangbanna Tropical Botanical Garden, CAS, provided the Zeiss Smartzoom 5 microscope for scanning digital images of the wood surface of walnut trees.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.gecco.2024.e03023.

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