Lin, Yutong; Lai, Yuan; Tang, Songbo; [et al.]. «Environment-driven intraspecific variation shows coordination of functional traits of deciduous oaks among and within different biological levels». Journal of Forestry Research, Vol. 35 (April 2024), art. 83. DOI 10.1007/s11676-024-01721-x

Environment-driven intraspecific variation shows coordination of functional traits of deciduous oaks among and within different biological levels

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Abstract Deciduous oaks (*Quercus* spp.) are distributed from subalpine to tropical regions in the northern hemisphere and have important roles as carbon sinks and in climate change mitigation. Determining variations in plant functional traits at multiple biological levels and linking them to environmental variables across geographical ranges is important for forecasting range-shifts of broadly-distributed species under climate change. We sampled leaves of five deciduous *Quercus* spp. covering approximately 20 degrees of latitude (~ 21° N−41° N) and 20 longitude (~ 99° E−119° E) across China and measured 12 plant functional traits at different biological levels. The traits varied distinctively, either within each biological level or among different levels driven by climatic and edaphic variables. Traits at the organ level were significantly correlated with those at the cellular and tissue levels, while traits at the whole-plant level only correlated with those at the tissue level. The *Quercus* species responded to changing environments by regulating stomatal size, leaf thickness and the palisade mesophyll thickness to leaf thickness ratios with contrasting degree of effect to adjust the whole-plant functioning, i.e., intrinsic water use efficiency (iWUE), carbon supply and nitrogen availability. The results suggest that these deciduous *Quercus* spp. will maintain vigour by increasing iWUE when subjected to large temperature changes and insufficient moisture, and by accumulating leaf non-structural carbohydrates under drought conditions. The findings provide new insights into the inherent variation and trait coordination of widely distributed tree species in the context of climate change.

Keywords Climate gradient; Intraspecific variation; Plant functional traits; Deciduous *Quercus* species; Whole-plant function

Introduction

Climate change impacts water and energy cycles globally (Zeng et al 2017), with substantial consequences for plant morphology and physiology, including leaf functional traits of individual species (Peguero-Pina et al 2016; Teshera-Levye et al 2020). Plant processes and functions, including plant intrinsic water use efficiency (iWUE), carbon (C) assimilation and leaf $\delta^{15}N$ values, of forest ecosystems, are also affected (He et al 2020; Tarin et al 2020; Tang et al 2022a, 2022b).

Plant functional traits, which can be classified at different biological levels, i.e., cell, tissue, organ and whole-plant, are significantly informative for the responses and adaptations of individual plants to environmental changes, as they reflect aspects of C acquisition, water use and gaseous exchange (Lambers and Poorter 1992; Baillie and Fleming 2020; Lin et al 2021). For example, at the cellular level, the behavior of stomata directly regulates the balance of C gain (photosynthesis) and water loss (transpiration) (Ainsworth and Rogers 2007). Stomatal traits such as size (*SS*), density (*SD*) and pore index (*SPI*) control water vapor and gas exchange between the plant and the atmosphere (Lawson and Vialet-Chabrand 2019). At the tissue level, anatomical traits such as the ratio of palisade mesophyll thickness to leaf thickness (*PTR*) play important roles in maintaining photosynthesis (Baillie and Fleming 2020) and cold resistance in temperate

Project funding: This study was supported by the Key-Area Research and Development Program of Guangdong Province (2022B1111230001), the Science and Technology Foundation of Guangxi Zhuang Autonomous Region (Guike AD23026080), the National Natural Science Foundation of China (No. 42071065), and Natural Science Foundation of US (No. 2021898).

Corresponding editor: Shuxuan Li

Yutong Lin and Yuan Lai made equal contributions to this paper.

forests (Liu et al 2019). At organ level, specific leaf area (*SLA*) is a determinant that comprises or covaries with the leaf economic spectrum (Wright et al 1994), and is positively associated with the plant's resource acquisition strategies (Lambers and Poorter 1992). At the whole-plant level, traits like iWUE, which is calculated from leaf $\delta^{13}C$ values, plant N availability which is indicated by $\delta^{15}N$ values, and the concentration of non-structural carbohydrates (NSC) indicating the C supply levels of the whole plant (Pantin et al 2013), are critical variables linking to C, water and N cycles (Tarin et al 2020; Tang et al 2022b).

To some extent, plant species can respond positively to changing environments to survive and improve fitness (Azuma et al 2019; Struckman et al 2019). With water deficits, plants close stomata (cellular level) and thicken adaxial epidermis (organ level) to prevent water loss and enhance iWUE (whole-plant level) (David et al 2007; Lawson and Vialet-Chabrand 2019; Luo et al 2023). The closure of stomata results in the reduction of C assimilation, decreasing leaf NSC and C supply, which leads to C starvation (Pantin et al 2013). Increasing *SLA* (organ level) is one of the ways plants improve iWUE (whole-plant level) because thick epidermal layers or small leaf areas reduce transpiration (Wright et al 1994). In addition, plants can regulate different functional traits within the same biological level to adjust to environmental changes. For example, within the whole-plant level, tree species may increase iWUE but decrease leaf $\delta^{15}N$ values with increasing latitude, while tending not to change either iWUE or leaf $\delta^{15}N$ values with longitude (Tang et al 2021). However, the changing synchrony and the regulation of functional traits among different biological levels for single lineage species grown over wide geographic areas have been scarcely addressed.

Drivers of functional trait variations under changing environments have been frequently explored, but many concentrated on traits at only one specific level, e.g., the cellular level such as *SS*, *SD* of a single species (Zhou et al 2013; Yan et al 2017), or the whole-plant level such as iWUE and leaf $\delta^{15}N$ of multiple forest species (Tang et al 2022a, 2022b). A recent study concerning leaf functional traits of evergreen oaks (*Cyclobalanopsis*, Fagaceae) at multiple biological levels and along elevational gradients found that there were different drivers on trait variations, i.e., mean annual precipitation (*MAP*), aridity index (*AI*) and pH were drivers of both cellular and tissue traits, whereas pH, soil phosphorus concentrations and mean annual temperature (*MAT*) were the determinants of organ traits (Lin et al 2021). Up until the present, drivers of trait variation at multiple levels of some widely distributed species have been seldom studied over broad geographic ranges. Research on this aspect is especially critical to predicting range-shifts in species distributions (Martínez-Sancho et al 2018), changes in community composition (Blonder et al 2017) and functions of forest ecosystems (Cristiano et al 2020) under climate change.

To date, regional and/or global patterns of plant functional traits responding to environmental change have largely been explored across species or communities, sometimes based on meta-analyses of the literature (Poorter et al 2009; Wang and Ali 2021). However, broad geographic patterns of plant functional traits from the cellular to the whole-plant level and their association with environmental variables based on measurements within a species in a single lineage (e.g., genus) with wide geographic distribution have been scarcely explored (Sun et al 2017; Tian et al 2019). This type of research will be critical to understanding the underlying mechanisms for their distribution across multiple climate zones. In addition, this research can help predict the range-shifts of species as well as their possible adaptive strategies to climatic changes in different macroecological regions (Tang et al 2018; He et al 2019).

The wide geographic distribution of some tree species provides an opportunity to investigate the impacts of climate change on functional traits or range-shifts (Rigling et al 2013; Pecl et al 2017). Oaks (*Quercus* genus, Fagaceae) are widely distributed from subalpine to tropical forests in the northern hemisphere (Eustaquio et al 2017). The distribution of some *Quercus* species, affected by increasing frequencies of extreme climatic events, e.g., *Q. robur* and *Q. suber*, shrank significantly (Kremer et al 2012; Touhami et al 2020), possibly resulting in alterations in biodiversity and population dynamics of forest ecosystems. Despite occupying broad ranges from temperate to subtropical zones in China (Zhou 1992), deciduous *Quercus* species have received less attention of how functional traits respond to environmental change (Kuglitsch et al 2008; Peguero-Pina et al 2016) than evergreen *Quercus* species (David et al 2007; Koehler et al 2012; Niinemets 2015; Cavender-Bares and Ramírez-Valiente 2017). Further, most studies of *Quercus* have been carried out in Europe and North America (Ramírez-Valiente and Cavender-Bares 2017; Badano et al 2019; Ramírez-Preciado et al 2019). Similar research from Asia is lacking.

In this study, the geographical patterns and determinants of 12 functional traits were investigated from the cellular to the whole-plant level of five deciduous *Quercus* species. Traits at the cellular level were stomatal size, density and pore index; the tissue level were leaf thickness, ratios of palisade mesophyll thickness to leaf thickness and adaxial epidermis thickness. Traits examined at the organ level were specific leaf area, concentrations of leaf chlorophyll and carotenoids, and at the whole-plant level, they were intrinsic water-use efficiency (iWUE), leaf non-structural carbohydrates (NSC) and leaf $\delta^{15}N$ values. The following questions were addressed: (1) do the traits vary and converge among and within different biological levels with achanging environment? (2) are there different determinants of the geographic variation in traits at different levels? (3) how do deciduous *Quercus* species coordinate or regulate the traits at different levels under a changing environment? Answers to these questions will provide new insights into the regulation of plasticity, linkages between structure and functions, and the potential adaptative mechanisms of deciduous *Quercus* species in response to ongoing climate change.

Materials and methods

Sampling and measurement

Five deciduous *Quercus* species (*Q. variabilis* Blume, *Q. aliena* Blume, *Q. fabrei* Hance, *Q. acutissima* Carruth and *Q. serrata* Murray) were sampled from 22 locations covering approximately 20° N–40° N and 99° E–119° E across China (Fig. 1). The locations have a mean annual temperatures and mean annual precipitation ranging from 8.5 to 24.0 °C and from 463 to 1807 mm, respectively (Fig. S1).

Mature, healthy leaves of each *Quercus* species were sampled from at least three individual open grown trees per species in each site from natural forests during the mid- and late-growing seasons (from July to October). At least 50 fully expanded, sun-exposed leaves were collected from trees with similar diameters at breast height and height, stored in ice bags, and transported to the laboratory within 8 hrs. Soil samples at 20 cm depth beneath each tree were taken at the same time.

The 12 functional traits from the cellular to the whole-plant level were classified and determined according to their significance (Table 1). Six leaves (replicates) per tree from each location were used for stomatal trait measurements. Stomatal length (*SL*, μm) and guard cell thickness (*GCT*, μm) were determined using an optical microscope (B302, Chongqing Optical Instrument Co., Ltd., Chongqing, China) coupled with an electronic image analysis equipment (also from OPTPro software, Optical Instrument Co., Ltd.). The traits included *SS*, *SD*, and *SPI* were calculated accordingly (Eqs. 1−3):

$$SS(\mu m^2) = 2 \times GCT \times SL \quad (1)$$

$$SD(ind.\ mm^{-2}) = number\ of\ stomata/LA \quad (2)$$

$$SPI(\%) = SD \times SL^2 \times 10^{-4} \quad (3)$$

Three fresh leaves per tree from each location were cut into 1.0 cm×0.5 cm pieces along the main vein and used for anatomical measurements. Leaf thickness (*LT*, μm), palisade mesophyll thickness (*PT*, μm) and Leaf adaxial thickness (*AD*, μm) were measured using an optical microscope coupled with OPTPro software. *PTR* was calculated as *PT* to *LT* ratios (Wang et al 2016; Liang et al 2019).

Twenty flesh leaves per species from each site were measured for leaf area, dried at 80 °C for 48 h and weighed. *SLA* was the ratio of leaf area to oven-dried weight. Frozen leaves, approximately 0.2 g were cut and dipped into 5 mL 80% acetone in the dark to determine the concentrations of chlorophyll a (Chl_a), chlorophyll b (Chl_b), and carotenoid (Car) (μg mg^{-1}, fresh weight) by absorption at 663, 645 and 440 nm, respectively, using a spectrophotometer (Unico, Shanghai, China). Chl_a and Chl_b were summed as the total chlorophyll (Chl) (Dai et al 2009).

Table 1 Classification of measured plant functional traits of the deciduous *Quercus* species at different biological levels

Biological levels	Functional traits and abbreviation	Significance	Unit
Cellular	Stomatal size (*SS*)	Related to the sensitivity of stomatal response (Liu et al 2018)	μm^2
	Stomatal density (*SD*)	Describes the number of stomata within a specific area (Liu et al 2018)	ind. mm^{-2}
	Stomatal pore index (*SPI*)	Comprehensive parameters reflecting the stomatal conductance (Sack et al 2003)	%
Tissue	Leaf thickness (*LT*)	Thick leaves help protect from mechanical damage (Lohbeck et al 2013)	μm
	Leaf adaxial thickness (*AD*)	Protects the internal structure of the leaves and prevents excessive water loss (Lambers and Poorter 1992)	μm
	Palisade mesophyll thickness to leaf thickness ratio (*PTR*)	Larger *PTR* contains more chloroplasts than those contained, favoring light absorption (Tian et al 2016)	-
Organ	Specific leaf area (*SLA*)	A comprehensive indicator reflecting the photosynthetic capacity of plants (Lohbeck et al 2013)	$cm^2\ g^{-1}$
	Concentrations of leaf chlorophyll (Chl)	Directly related to photosynthesis (Gitelson et al 2003)	$\mu g \cdot mg^{-1}$
	Concentrations of leaf carotenoids (Car)	It captures light energy and delivers it to chlorophyll (Lichtenthaler 1987)	$\mu g \cdot mg^{-1}$
Whole-plant	Intrinsic water use efficiency (iWUE)	Reflects the plant's ability to use water (Zhang et al 2018)	$\mu mol \cdot mol^{-1}$
	Leaf $\delta^{15}N$ value	Indicates the N availability of plants (McLauchlan et al 2017)	‰
	Leaf concentration of non-structural carbohydrates (NSC)	Indicates carbon supply levels of plant and physiological state (Mitchell et al 2013)	$mg \cdot g^{-1}$

A second leaf sample was dried to a constant weight at 65 °C for 72 h and ground for stable C isotopes, expressed as $\delta^{13}C$, and N isotopes, expressed as $\delta^{15}N$ values using a mass spectrometer (Thermo Finnigan, North Pod Waltham, MA. USA). Plant iWUE was calculated from $\delta^{13}C$ values according to Farquhar et al (1982) and Ehleringer and Cerling (1995) (Eqs. 4−6):

$$\Delta^{13}C = (\delta_a - \delta_p)/(1000 + \delta_p) \times 1000 \quad (4)$$

$$\Delta^{13}C = a + (b - a) \times (C_i/C_a) \quad (5)$$

$$iWUE = (C_a - C_i)/1.6 \quad (6)$$

where, $\Delta^{13}C$ (‰) is C isotope discrimination; δ_a, the $\delta^{13}C$ value for source atmospheric CO_2 (−8.4 ‰); δ_p, the $\delta^{13}C$ value of the leaves; a = 4.4 ‰, b = 27 ‰; C_i is the intracellular CO_2 concentration in cells; C_a is atmospheric CO_2 concentration (411 mg L^{-1}).

The subsample was freeze-dried and ground for non-structural carbohydrate (NSC) determination. Approximately 0.05 g of powder was extracted in 4 mL of 80% (v/v) aqueous ethanol, bathed for 30 minutes at 85 °C, and centrifuged for 5 min at 3000 rpm. After extraction twice, the supernatants were combined to determine soluble sugars and the residue dissolved in 30% (v/v) perchloric acid to determine leaf starch (Mitchell et al 2013). NSC was the sum of soluble sugars and starch.

Environmental variables

The means of climatic variables over 1991−2020 at the sampling sites were extracted from version 4 of the Climatic Research Unit gridded Time Series (https://data.ceda.ac.uk/badc/cru/data/cru_ts/cru_ts_4.05) (Harris et al 2020). Temperature-related factors included the frequency of frost days per year (F_{frost}), monthly average temperature (*MAT*), monthly average daily maximum temperature (T_{max}), monthly average daily minimum temperature (T_{min}), and diurnal temperature range (T_{range}). Precipitation-related factors included mean annual precipitation (*MAP*), aridity index (*AI*), frequency of wet days per year (F_{wet}), and potential evapotranspiration (*PET*). All the variables were extracted using the function in "raster" R packages according to the latitude and longitude of sampling locations. In this study, all climatic factors except for *PET* changed significantly with latitude, while F_{frost}, T_{min}, *AI*, *MAP*, and *PET* changed with longitude (Fig. S1).

Soil samples were air-dried, ground, and sieved and concentrations of organic carbon (*SOC*), total N (*SN*), total phosphorus (*SP*), and soil water content (*SWC*) were determined according to Liu (1996). *SOC* and *SN* were identified using the $K_2Cr_2O_7$ titration method and micro-Kjeldahl method, respectively; *SP* was determined using the molybdenum antimony colorimetric method after digestion with H_2SO_4-$HClO_4$; *SWC* levels were found by oven drying 20 g of fresh soil at 105 °C for 48 h; pH was measured in a 1: 2.5 of soil: water (w/v) mixture using a glass-electrode meter (FiveEasyPlusTM FE28, Mettler Toledo, Switzerland). Variations of the edaphic properties along the latitudinal and the longitudinal gradients can be found in Fig. S1.

Statistical analysis

To detect the influence of phylogenetic development on the traits at different biological levels, an ultrametric phylogenetic tree (scenario 1) was pruned using phylo.maker function in V.PhyloMaker R package (Qian and Jin 2016). Phylogenetic signals of the traits based on Pagel's lambda (λ) is recommended by researchers (Hao et al 2015) and were calculated by phylosig function in phytools R package, in which a value of λ close to 1 ($P < 0.05$) suggests a strong phylogenetic signal. In this study, significant phylogenetic signals were not found ($\lambda < 1$, $P > 0.05$, Fig. S2), implying that the functional traits were mainly associated with changes in environmental factors, as further identified by the variance of sampling sites explaining the variation of traits (Fig. S3). Thus, the interpretation of geographical gradients on the variation of functional traits at each level was done with the data of the five species together.

Prior to analysis, the functional traits at different biological levels and the climatic factors and edaphic properties were tested for approximate normality with the Shapiro-Wilk test and by zero-mean normalization to achieve normality. Two-way ANOVAs were used to assess the effects of sampling sites and species on the variation of leaf traits. The relationships between latitude or longitude and the functional traits were carried out by generalized linear mixed models (GLMMs) in which the species was considered the random independent variable, i.e., the function formula is "lmer (traits ~ latitude/longitude + (1 | Species)", by step and lmer function in lmerTest R package (Kuznetsova et al 2017).

The environmental variables were examined for multicollinearity and those with high collinearity. i.e., the variance inflation factor > 5, were eliminated (Hovenden et al 2019). Finally, *MAT*, T_{range}, *MAP*, *PET*, *SOC*, *SP*, *SWC*, and pH were used to explore their effects on leaf traits along the latitudinal gradient. Along the longitudinal gradient, the environmental factors were T_{min}, T_{range}, *MAP*, F_{wet}, *PET*, *SN*, *SP*, pH and *SWC*. The linear model selected was then used to explore the effects of climatic and edaphic factors on leaf traits through the glmulti R package (Anderson 2008). The function glmulti was used to select the optimal model, among all linear models, based on Akaike information criterion (AIC). The coordination of the different biological levels was assessed by the relationships of PC1 among the traits after principal components analysis (PCA) on the functional traits at each level. Finally, to test the relationship between the stomatal, anatomical traits and the whole-plant level traits, structural equation models (SEMs) were utilized to explore the pathway of climatic and edaphic variables via stomatal and anatomical traits that influence plant iWUE, C supply (leaf NSC) and leaf $\delta^{15}N$ values. According to multiple goodness-of-fit criteria, including the probability level (*P*), comparative fit index (*CFI*), the ratio of χ^2 to degrees of freedom (χ^2 /Df), and root mean squared error of approximation (RMSEA), the model was chosen when $P > 0.05$, χ^2 / Df ≤ 2, CFI ≥ 0.95, and RMSEA had the lowest value (Schermelleh-Engel et al 2003). All statistical analyses were performed using the R v.3.6.3 software (R Core Team 2019). Significance was set at $P < 0.05$.

Results

Variation of functional traits along latitudes and longitudes

The sampling location accounted for the largest variance of the functional traits across the geographic range (Fig. S3). All traits at the whole-plant level exhibited significant changes along latitudes and longitudes. Within each biological level, the functional traits did not always converge along either the latitudinal (Fig. 2) or the longitudinal (Fig. 3) gradients. At the cellular level, only the stomatal pore index (*SPI*) increased with increasing latitude (Fig. 2c), but none of the three stomatal traits varied with increasing longitude (Fig. 3a−c); at the tissue level, leaf thickness (*LT*) and adaxial thickness (*AD*) increased with latitude (Fig. 2d, e), while *LT* and the ratio of palisade mesophyll thickess/leaf thickness (*PTR*) decreased with increasing longitude (Fig. 3d, f); at the organ level, chlorophyll (Chl) and caroteniods (Car) increased with latitude (Fig. 2h, i); specific leaf area (*SLA*) increased but chlorophyll levels decreased with increasing longitude (Fig. 3g, h); at the whole-plant level, intrinsic water use efficiency (iWUE) and leaf non-structural carbohydrates (NSC) increased and leaf δ^{15}N decreased with latitude (Fig. 2j−l), while both iWUE and leaf δ^{15}N decreased with increasing longitude (Fig. 3j, l).

The functional traits at different biological levels varied diversely with changing climatic and edaphic variables. Across the four levels, all traits changed significantly with increasing mean annual temperatures (Fig. S4a), whereas *SD*, *SPI*, *LT*, iWUE, and leaf NSC increased but δ^{15}N decreased significantly with increasing T_{range} (Fig. S4b). SD and SLA did not change with mean annual precipitation (*MAP*), but *SPI*, *LT*, *AD*, Chl, Car, iWUE, and leaf NSC did decrease. *PTR* and leaf δ^{15}N increased significantly with the increasing *MAP* (Fig. S4c). *SD*, *PTR* and *SLA* did not vary significantly with increasing aridity (Fig. S4d). Soil pH had positive effects on the traits except for *SPI*, *PTR*, *SLA* and leaf δ^{15}N (Fig. S4e), while *SP* had positive effects except for stomatal density (Fig. S4f).

Driving factors of geographic variation in functional traits

The driving factors differed among biological levels. Along the latitudes at the cellular level (Figs. 4a−c), the three stomatal traits were positively affected by mean annual precipitation; *SD* and *SPI* were positively affected by T_{range}, and negatively by *SOC* and *PET*. In addition, stomatal density was positively correlated with mean annual precipitation but negatively with pH. At the tissue level (Figs. 4d−f), both *LT* and *AD* were positively correlated with *PET* and pH, but negatively with *MAT* and T_{range}; *PTR* was negatively affected by pH. At the organ level (Figs. 4g−i), *MAT*, T_{range}, *SP* and *PET* were the determinants of specific leaf area, pH and *SOC*; Chl and Car were correlated positively with but negatively with mean annual temperatures. At the whole-plant level (Figs. 4j−l), T_{range}, *MAT*, *SOC* and *MAP* were the determinants of iWUE; *MAP*, pH and *MAT* were the determinants of leaf δ^{15}N, while *PET*, pH, *SOC*, *MAT* and T_{range} were the determinants of leaf NSC.

Along the longitudinal gradient, stomatal size was influenced by soil F_{wet} and pH (Fig. 4m), stomatal density was affected by all factors except for SN (Fig. 4n), stomatal pore index was controlled by *SP*, T_{min}, *SWC*, T_{range}, and *MAP* (Fig. 4o). At the tissue level (Figs. 4p−r), *LT* was affected by T_{range}, *PET* and F_{wet}; both *AD* and *PTR* were affected by F_{wet}; in addition, *AD* was significantly affected by pH, *SWC*, *SN*, and *PTR* by *PET* and T_{min}. At the organ level (Figs. 4s−u), *SLA* was affected by T_{min}, soil pH and *SP*; Chl was controlled by climatic and edaphic variables and Car by T_{min}, pH, *SN*, F_{wet} and *PET*. At the whole-plant level (Figs. 4v−x), *MAP*, T_{range}, *SWC*, *PET*, T_{min} and F_{wet} were the determinants of leaf δ^{15}N, while *SWC*, soil pH, and T_{range} were the determinants of leaf NSC.

Coordination of traits among and within different biological levels across geographic ranges

Along the latitudinal gradient, PC1 accounted for 59%, 62%, 64% and 56% of functional traits at the cellular, tissue, organ, and whole-plant level, respectively. Along the longitudinal gradient, the values were 60%, 54%, 62% and 39%, respectively (Fig. S5). Therefore, the coordination of traits among the different biological levels across the geographic ranges were assessed based on the correlations of the functional traits of PC1 (Fig. 5).

Overall, traits at the organ level were significantly correlated with those at the cellular and the tissue levels, while traits at the whole-plant level were correlated with those at the tissue level. Notably, correlations among the traits were recognized across the biological levels, e.g., *SPI* was positively correlated with *SS* and *SD* at the cell level, *AD* was positively correlated with *LT* but negatively with *PTR* at the tissue level. At the organ level, Chl was correlated only with Car; δ^{15}N was correlated negatively with both leaf NSC and iWUE, between which there were positive correlations (Fig. 5).

Pathways of whole-plant trait regulation across geographic range

Given the significant correlations among the different biological levels and among iWUE, leaf NSC and δ^{15}N values at the whole-plant level (Fig. 5, $P < 0.05$), which environmental variables and how regulate iWUE, leaf NSC and δ^{15}N were determined (Figs. 6a−c). *MAP* and T_{range} showed direct and indirect effects on iWUE, with the indirect effects achieved via regulating *SS* (Fig. 6a). Besides the direct negative effects of *MAP* and positive F_{wet} on δ^{15}N values, *MAP*, F_{wet} and pH had indirect effects on δ^{15}N via regulating *PTR* and iWUE

(Fig. 6b). Similarly, changes in mean annual precipitation directly and indirectly affected leaf NSC, with the indirect effects achieved via regulating *PTR* (negative) and *LT* (positive). Changes in pH had both direct and indirect effects on leaf NSC through the regulation of *PTR* (Fig. 6c). Overall, the *Quercus* species in this study responded to changing environments (*MAP*, F_{wet}, T_{range} and pH) by regulating *SS* (−0.17), *PTR* (0.13) and *PTR*(−0.09), *LT* (0.23) to different degreesto adjust plant iWUE, leaf δ^{15}N and leaf NSC, respectively (Fig. 6d).

Discussion

Intraspecific variations were determined for 12 functional traits of deciduous *Quercus* species across climatic and edaphic gradients. There were significant effects of sampling location on variance of the traits (Fig. S3). A lack of consistent patterns in functional traits among and within different biological levels along different axes of environmental change were not consistent with previous research in other regions (Cavender-Bares et al 2007; Cavender-Bares and Ramírez-Valiente 2017; Ramírez-Valiente and Cavender-Bares 2017). Geographic variation in traits at different biological levels was driven by multiple climatic and edaphic variables (Fig. 4), which was inconsistent with Liang et al (2019) who found that precipitation alone was the determinant of the spatial variation in hydraulic traits of *Castanopsis fargesii* Franch. in subtropical China.

Variations in cellular traits with climatic and edaphic variables (Fig. S4) and the significant effects of *MAP* and T_{range} on *SS*, *SD* and *SPI* (Fig. 4) are possibly due to the sensitivity of stomata to temperature and moisture changes and to the flexibility of pores adjusting stomatal behavior to changing temperature (Woolfenden et al 2018; Lawson and Vialet-Chabrand 2019). These effects imply that the deciduous *Quercus* species in this regulate stomatal conductance response to changing temperatures and rainfall. The significant changes in *LT*, *AD* and *PTR* with increasing temperature (Fig. S4) are in line with the fundamental principle that plants thicken leaves and enlarge epidermal layers in arid and cold environments (Wang et al 2011; Münchinger et al 2023). Low *PTR* benefit the cold resistance of plants at high latitudes (Tian et al 2016; Liu et al 2019) and light absorption (Terashima et al 2011; He et al 2019). The decline of ratios of palisade mesophyll thickness to leaf thickness along a longitudinal gradient (Fig. 3f) implies that deciduous *Quercus* species enhance their defensive strategy and photosynthetic capacity where precipitation is low. The effects of *MAT* and *SWC* on the spatial patterns of *PTR* (Fig. 4) implies that deciduous *Quercus* species regulate anatomical traits to adapt to changes in temperature and soil humidity. *SLA*, Chl and Car play important roles in photosynthesis and transpiration (Poorter et al 2009), leaf lifespan and growth rate (Wang et al 2020). The increase in specific leaf area with increasing longitude (Fig. 3) suggests that deciduous *Quercus* species have thicker and denser leaves in arid zones which is benificial to resisting long-term external disturbances and improving adaptability (Sardans et al 2006; Ramírez-Valiente et al 2017). The significant effects of *MAT*, T_{range}, *PET* and *SP* on variations in *SLA* (Fig. 4) indicate the importance of temperature, precipitation, and soil nutrient status for plant growth. Modification of specific leaf area may be a possible adaptive strategy of deciduous oaks at the organ level in response to changes in temperature and edaphic conditions.

Plant iWUE plays an important role in the carbon and water cycles of ecosystems (Soh et al 2019; Adams et al 2020). Our results indicate that these *Quercus* species exhibit high iWUE at high latitudes and low longitudes, which are consistent with the results of Teshera-Levye et al (2020). The increase in iWUE from south to north and from east to west (Fig. 3) and the positive effects of *MAT* and T_{range} on iWUE are not consistent with the spatial change in multiple forest species across China (Tang et al 2021, 2022a), which might be attributed to plant-dependent differences in response to environmental changes at ecosystem and species levels (Niu et al 2011). The negative effects of mean annual precipitation on iWUE (Fig. 4) suggest that deciduous *Quercus* species enhance intrinisic water use efficiency under a decrease in water availability across China (Tang et al 2022a).

Given the indication of leaf δ^{15}N to plant N availability (Mason et al 2022; Tang et al 2022b), the geographical patterns of leaf δ^{15}N observed here indicate the influence of temperature, precipitation, and pH on N availability of deciduous *Quercus*, which might due to their interactive and independent effects (Table 1, Fig. 4) on N demand of plants and/or N supply of ecosystems (McLauchlan et al 2017; Mason et al 2022). Leaf NSC indicates the status of C supply (O'Brien et al 2014) to assure growth when encountering environmental stress (He et al 2020; Mo et al 2020). The increased leaf NSC with latitudes (Fig. 3) coincides with the spatial patterns of *Q. variabilis* along a 1500- km transect in China (Liu et al 2018). There were positive effects of pH, potential evapotranspiration and negative effects of T_{range}, *MAT* on leaf NSC along the latitudinal gradient (Fig. 4), which suggests that deciduous *Quercus* species might enhance C supply by accumulating leaf NSC to endure non-productive seasons in the temperate zone.

Carbon, N and the water cycle are closely related to the demand-supply relationships between plants and the environment (Yu et al 2014), and their correlations bridge plant iWUE, N availability, and C supply (Soh et al 2019; Adams et al 2020). Plants reduce stomatal conductance to improve intrinsic water use efficiency as an adaptive plastic response to drought (Liu et al 2018; Lawson and Vialet-Chabrand 2019). Shifts in correlations across functional traits among different or within the same biological level indicate the coordination of deciduous oaks faced with environmental change. The synergies and trade-offs among different biological traits provide evidence that these *Quercus* species maintain a balance in water utilization, N uptake and C supply, e.g., having lower iWUE and less accumulation of leaf non-structural carbohyrates grown on sites with more water available. Our results suggest that deciduous *Quercus* will maintain vigor by increasing iWUE when subjected to large temperature changes and insufficient moisture, and by accumulating non-structural carbohyrates under drought and soil acidity conditions. The findings confirm the different effects and importance of stomatal and tissue traits on the physiological function of whole-plant traits.

Conclusion

This study revealed spatial patterns and driving factors of functional trait variation in five, widely-distributed deciduous oaks across a broad geographic range. Traits at the cellular, tissue, organ, and whole-plant levels varied with the changing environment and were driven by different climatic and edaphic variables. Whole-plant traits of the deciduous *Quercus* species are regulated by different cellular and tissue traits that are closely coordinated under a changing environment. Research on plant functional traits at multiple levels is informative in predicting shifts in distribution and in providing the basis for further exploration of *Quercus* species under global change.

Acknowledgements We thank Caichuan Huang, Chaodong Yang, Chenhua Lei, Chunyu Zhou, Jiang Liu, Jinfu Liu, Liqin Gao, Meihua Liu, Rongxun Xu, Xiaofei Lu, Xiaoming Fang, Xinchun Lin, Xinghao Tang, Yihua Xiao and Yongxing Li for their generous help in samplings.

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