

# Variation of stomatal traits from cold temperate to tropical forests and association with water use efficiency

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## Abstract

1. Stomata control carbon and water vapour exchange between leaves and the atmosphere, thus it can influence water use efficiency (WUE) and reflect plant adaptation to climate. However, the spatial patterns of leaf stomatal traits and relationships between stomatal trait and WUE across natural communities remain unclear.
2. We measured stomatal density, stomatal size and stomatal area fraction for 737 plant species from nine forests ranging from tropical to cold temperate forests.
3. Stomatal density, stomatal size and stomatal area fraction were all log-normally distributed, and different across species, plant functional groups (trees, shrubs, and herbs), and communities. At the regional scale, variation in stomatal traits was primarily related to species, followed by climate and soil types.
4. The community-weighted mean of stomatal size increased linearly with latitude, whereas those of stomatal density and stomatal area fraction showed humpbacked relationship. The community-weighted mean of stomatal area fraction was correlated with climatic aridity, consistent with the adaptation strategies of plant species to achieve high maximum rates of gas exchange in arid regions when water is available. Further, community-weighted mean of stomatal area fraction was positively correlated with WUE in natural forest communities, indicating that plants have lower stomatal conductance in order to adapt greater aridity conditions.
5. These findings highlight the strong associations of stomatal traits with plant functional group and climate at a regional scale, representing the adaptation strategies of stomatal traits across natural forest communities to climate.

## KEYWORDS

community, phylogeny, plant functional group, stomata, water use efficiency

## 1 | INTRODUCTION

Stomata control the exchange of gases between leaves and the atmosphere, and influence plant adaptation to climate as well as the global carbon and water cycles (Hetherington & Woodward, 2003). Across species and communities, stomatal density ( $d$ , pores per  $\text{mm}^2$ ) and stomatal length (SL,  $\mu\text{m}$ ) vary with latitude (Wang et al., 2015). However,

the relationships between stomatal traits, which directly related to maximum gas exchange and water use efficiency (WUE) at regional scales remains unclear.

Stomata respond to environmental changes on time scales ranging from minutes to millennia (Hetherington & Woodward, 2003), including short-term behaviours (opening or closing) and long-term adaptation of stomatal morphological traits to climate change (Casson &

Hetherington, 2010; Haworth, Elliott-Kingston, & McElwain, 2011). Of the stomatal traits, stomatal size ( $s$ ,  $\mu\text{m}^2$ ) and  $d$  are the most commonly measured, and are negatively related (Franks, Drake, & Beerling, 2009; Hetherington & Woodward, 2003), though this relationship is not sufficiently compensatory to equalise stomatal area fraction ( $f$ , the product of  $d$  and  $s$ ) across species. Thus, the  $f$  represents an index of the anatomical constraint on the maximum stomatal gas exchange ( $g_{\text{max}}$ ), which is often calculated, using assumptions for additional stomatal dimensions such that  $g_{\text{max}}$  is tightly related to  $f$  (Franks et al., 2009). Thus,  $f$ ,  $g_{\text{max}}$  or other closely associated indices (e.g. stomatal pore area index;  $\text{SPI} = d \times \text{stomatal length}^2$ ) have been linked across diverse species with operating stomatal conductance (Lawson, James, & Weyers, 1998; McElwain, Yiotis, & Lawson, 2016), other structural determinants of  $g_{\text{max}}$  (such as leaf hydraulic conductance, Sack, Cowan, Jaikumar, & Holbrook, 2003) and their anatomical determinants (such as vein density; Murphy, Jordan, & Brodribb, 2014; Scoffoni et al., 2015). Theoretically, a higher  $g_{\text{max}}$  should benefit species under low  $\text{CO}_2$ , higher irradiance or nutrient supply, or under selection for high productivity or competition (Franks & Beerling, 2009; Taylor et al., 2012). Consistent with these ideas, in controlled experiments on given species,  $d$  and  $s$  tend to shift plastically, so that  $g_{\text{max}}$  is higher with lower  $\text{CO}_2$  concentration (Beerling & Chaloner, 1993; Engineer et al., 2014), greater temperature (Luomala, Laitinen, Sutinen, Kellomaki, & Vapaavuori, 2005; Reddy, Robana, Hodges, Liu, & McKinion, 1998), and higher light intensity (Al Afas, Marron, & Ceulemans, 2007; Oberbauer & Strain, 1986). Counterintuitively,  $g_{\text{max}}$  can be higher for plants grown under lower water status (Fraser, Greenall, Carlyle, Turkington, & Friedman, 2009; Xu & Zhou, 2008), which has been considered to reflect the advantage in such plants of achieving higher rates of gas exchange in the shorter period in which water is available (Grubb, 1998; Maximov, 1931). Further, within a given community, trees tend to have smaller  $s$  and higher  $d$  (Salisbury, 1928). We hypothesised that these trends would be true regionally, i.e. that  $s$  and  $d$  would shift such that  $f$  would be higher for communities with greater climatic aridity and soil fertility, and that  $f$  would be higher in trees than shrubs than herbs.

At the leaf level, intrinsic WUE may be measured with a gas exchange system, as the ratio of photosynthetic rate to transpiration rate, or may be estimated using the carbon isotope ratio ( $\delta^{13}\text{C}$ ) (Hultine & Marshall, 2000; Luo, Zang, & Li, 2006). In general, at the leaf level, lower stomatal conductance reduces water loss more than  $\text{CO}_2$  gain, and, hence, corresponds to higher WUE (Franks & Beerling, 2009; Franks, Doheny-Adams, Britton-Harper, & Gray, 2015; Taylor et al., 2012). On the one hand, one might expect a negative relationship of  $f$  to WUE, if a higher  $f$  reflects higher operating stomatal conductance, which would result in lower WUE at the ecosystem scale, as is generally found at the leaf scale. Alternatively, we hypothesised that there might be a positive relationship of  $f$  with WUE at the ecosystem scale. Because  $f$  can reflect the maximum stomatal conductance to a large extent, plants in drier areas would have a higher  $f$  to achieve higher rates of gas exchange (Hetherington & Woodward, 2003), but would reduce their stomatal conductance during dry periods, such that overall these plants would have higher  $f$

and higher WUE, driving a positive relationship between  $f$  and WUE at the regional scale.

We measured the stomatal traits of 737 species from nine forest ecosystems along the north-south transect of eastern China (NSTEC). The first aim of the study was to test hypotheses at a regional scale for the association of stomatal traits with climate, soil type and growth form. Our second aim was to test the regional scale hypotheses for the association of stomatal traits with WUE.

## 2 | MATERIALS AND METHODS

### 2.1 | Site description

The regional transect passed through Huzhong, Liangshui, Changbai Mountain, Dongling, Taiyue, Shennongjia, Jiulian, Dinghu and Jianfengling, which contain most of the forest types in the Northern Hemisphere (Figure S1). These forests range in latitude from 18.7°N to 51.8°N, in mean annual temperature (MAT) from -3.67 to 23.2°C, and in mean annual precipitation (MAP) from 472 to 2,266 mm (Wang et al., 2015). The soil types vary from cold temperature brown soils with high organic matter to tropical red soils with low organic matter (Table S1). The vegetation types include cold temperate coniferous forest, temperate deciduous forest, subtropical evergreen forest and tropical rainforest.

### 2.2 | Sampling and measurements

The field survey was conducted between July and August 2013. To minimise the effect of anthropogenic disturbance, sampling plots were set up within well-protected national nature reserves with relatively continuous vegetation representative for the given forest ecosystems. Four experimental plots (30 m × 40 m) were established in each forest. Geographical information (latitude, longitude and altitude), plant species composition, and community structure were determined for each plot.

### 2.3 | Analysis of plant samples and the community-weighted means of stomatal traits

Leaves were collected from trees, shrubs and herbs within the plots, using long-handle shears or by climbing. For each species within the plot, 20 mature leaves were collected from four healthy individuals from the top of the canopy and were then pooled, placed in sealed plastic bags, and immediately stored in a cool box with ice.

Eight to 10 leaves from the pooled sample were cut into small pieces (1.0 cm × 0.5 cm) along the main vein, and were fixed in 75% alcohol: formalin: glacial acetic acid: glycerin (90:5:5:5). Stomatal traits were observed by scanning electron microscope (S-3400N, Hitachi, Japan). Three small pieces were selected from the pooled sample, and each replicate was photographed twice on the lower surface. In each image, the number of stomata ( $N_{\text{photo}}$ ) was recorded, and five stomata were randomly selected for measurement of SL and stomatal width (SW,  $\mu\text{m}$ ) and measured, using MIPS (Optical

Instrument Co., Ltd., Chongqing, China).  $d$ ,  $s$  and  $f$  were calculated as follows:

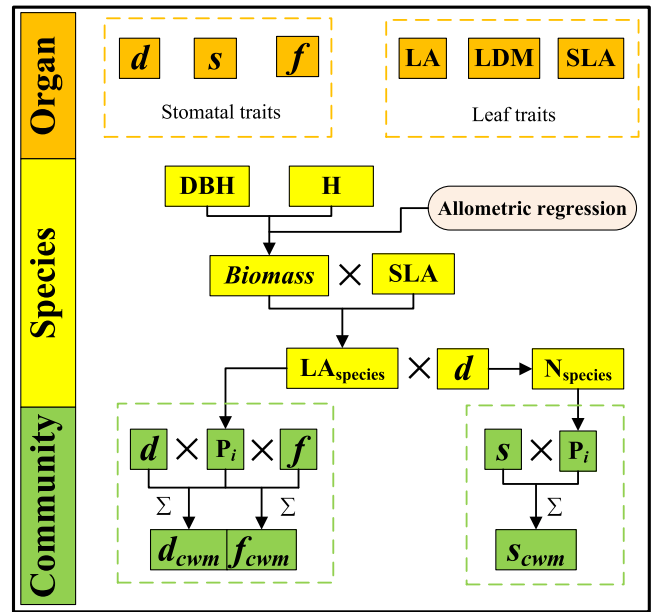
$$d = \frac{N_{\text{Photo}}}{1.12 \times 10^{-2}} \quad (1)$$

$$s = \frac{\pi}{4} \times \text{SL} \times \text{SW} \quad (2)$$

$$f = s \times d \times 10^{-4}, \quad (3)$$

where  $1.12 \times 10^{-2}$  was the measured image area ( $\text{mm}^2$ ).

Overall our selection was guided by the measurement approaches most typical in the literature. (1) Given that most forest plant species are hypostomatous (i.e. with stomata distributed on the lower epidermis), we therefore focused on this surface for standard sampling (Wang et al., 2015). Given 737 plant species, and collection of data for stomatal traits using a scanning electron microscope and manual measurements of stomata, labour and expense precluded additional measurements of the upper leaf epidermis. (2) We measured SL and stomatal width as reliable metrics of stomatal size. Stomatal pore length can also be measured reliably, but pore width is less clear in the images, and varies with the external environment. Thus, we calculated and presented  $f$  rather than stomatal pore area fraction. This approach was also informed by the typical assumption in the literature that  $f$  would impose a major constraint on stomatal pore area fraction, resulting in a close relationship between these two variables, and in turn that both would act as proxies for the anatomical constraint on the maximum stomatal conductance (Franks & Beerling, 2009). In the previous literatures, there was the assumption that stomatal conductance would be related to the circumference rather than the area of the stomatal pore, based on early experiments and theory developed for leaves in still air, but later work showed that in moving air, diffusion across stomata would relate to the area of the pore (Boyer & Kramer, 1995). Community-weighted means were used to scale up the stomatal traits from the species level to the community level. The mass ratio hypothesis (Grime, 1998) considers that the extent to which the trait of a given species affects ecosystem properties depends on the relative contribution of that species to the total community biomass. However, the mass weighted method is not necessarily to scaling stomatal traits to the community level, and we developed an approach based on weighted averages according to leaf area and stomata number (Figure 1). We calculated  $d$  and  $f$  at the community level ( $d_{\text{CWM}}$ ,  $f_{\text{CWM}}$ ) by weighting the value for each species by its contribution to the total leaf area of plant community, where the leaf area of each species ( $\text{LA}_{\text{Species}}$ ) was calculated as the product of the specific leaf area (SLA,  $\text{mm}^2/\text{mg}$ ) and leaf biomass (*Biomass*). We calculated  $s$  at the community level ( $s_{\text{CWM}}$ ) by weighting the value for each species by its contribution to the total number of stomata in the community, where the total number of stomata of each species ( $N_{\text{Species}}$ ) was calculated as the product of  $\text{LA}_{\text{Species}}$  and  $d$ . The leaf biomass of trees and shrubs was calculated, using species-specific allometric regressions based on measured values of diameter at breast height (DBH) and tree height (H), which has been



**FIGURE 1** Scaling-up stomatal traits from the levels of organ, species, to plant community. Also  $s$ ,  $d$  and  $f$  are stomatal size, density and area fraction, respectively; LA, leaf area; LDM, leaf dry mass; SLA, specific leaf area; DBH, diameter at breast height; H, height; *Biomass*, leaf biomass;  $\text{LA}_{\text{Species}}$ , total leaf area of a given species;  $N_{\text{Species}}$ , total number of stomata of a given species;  $P_i$ , the percent of  $\text{LA}_{\text{Species}}$  or  $N_{\text{Species}}$  in the community;  $s_{\text{CWM}}$ ,  $d_{\text{CWM}}$  and  $f_{\text{CWM}}$  are the corresponding parameters of  $s$ ,  $d$  and  $f$  at the community level

reported previously (Wang et al., 2015). For herbs, the aboveground biomass was measured directly after oven-drying. To obtain the SLA, we measured leaf area (LA,  $\text{mm}^2$ ) for 16 leaves (4 groups), using a scanner (Cano Scan LIDE 100, Japan) and Photoshop CS (Adobe, USA). Then, after oven-drying to constant mass ( $60^\circ\text{C}$ ), we measured leaf dry mass (LDM, mg), and determined SLA as  $\text{LA}/\text{LDM}$ . The data of SLA for all plant species have been reported previously (Wang et al., 2016).

The  $d_{\text{CWM}}$ ,  $s_{\text{CWM}}$ , and  $f_{\text{CWM}}$  were calculated as:

$$d_{\text{CWM}} = \sum_{i=1}^n \frac{\text{LA}_{\text{Species } i}}{\sum_{i=1}^n \text{LA}_{\text{Species } i}} \times d_i \quad (4)$$

$$s_{\text{CWM}} = \sum_{i=1}^n \frac{N_{\text{Species } i}}{\sum_{i=1}^n N_{\text{Species } i}} \times s_i \quad (5)$$

$$f_{\text{CWM}} = \sum_{i=1}^n \frac{\text{LA}_{\text{Species } i}}{\sum_{i=1}^n \text{LA}_{\text{Species } i}} \times f_i \quad (6)$$

where  $n$  was the number of species sampled in the plant community.

## 2.4 | Collection and analysis of soil samples

More than 30 soil samples in each plot were collected from the 0–10 cm layer, using a soil-drilling method. All soils were mixed and then sieved through a 2 mm mesh to remove roots and visible organic debris. We measured soil moisture, pH, texture and concentrations

of organic carbon, nitrogen, and phosphorus. Soil moisture was measured by the oven drying method, while soil pH was measured by the potentiometric method. The soil texture index is defined as the ratio of the mass of clay (<2  $\mu\text{m}$ ) to that of soil. Soil particle size was measured, using MALVERN2000. The concentrations of carbon and nitrogen in these soils were measured, using an elemental analyzer (Elementar, Vario Max CN, Germany). The concentration of soil total phosphorus was measured, using molybdenum antimony spectrophotometry and a continuous flow analyzer (AutoAnalyzer3 Continuous- Flow Analyzer; Bran Luebbe, Germany).

## 2.5 | Climate data and ecosystem-scale WUE

Mean annual temperature and precipitation (MAT and MAP, respectively) of the 9 forests were derived from the Wang et al. (2015). The de Martonne aridity index (DI) (de Martonne, 1926) was calculated to describe water availability:

$$DI = \frac{MAP}{MAT + 10} \quad (7)$$

Notably, a lower DI value corresponds to greater climatic aridity.

Yu et al. (2008) showed that the spatial patterns of WUE (annual mean growing season ecosystem water-use efficiency, measured from 2003 to 2005 with eddy covariance systems) along the NSTEC are strongly associated with MAT and MAP. Thus, the WUE of the natural forest community in the growing season can be estimated as follows:

$$\begin{aligned} WUE &= 11.742 + 0.00003 MAP - 0.266 MAT, \\ R^2 &= 0.85, p = .008 \end{aligned} \quad (8)$$

## 2.6 | Data analysis

Stomatal traits were  $\log_{10}$  transformed to plot histograms showing frequency distributions.

To compare differences in stomatal traits among different growth forms, and to partition the variances of the stomatal traits within-site from among site, we applied one-way ANOVA with least significant difference. Two-way analysis of variance was used to partition the contribution of family, site and their interaction to variances of stomatal traits. Data analyses and graphical presentation were conducted, using SPSS 13.0 (Chicago, IBM Corp., USA) and SIGMAPLOT 10 software (Systat Software, Point Richmond, CA).

For relating the variation of stomatal traits to species, and to climate and soil variables, we applied a series of partial redundancy analyses (RDA) using the “NNET” and “VEGAN” packages in R. We conducted analyses considering (1) all factors as explanatory, or (2) single-grouped factors (species, climate variables or soil variables) as explanatory with the others as covariates, or (3) two grouped factors as explanatory and the other as covariates, and in each analysis calculated the total effect and pure effect of soil factors, climate factors, and species on stomatal traits and their joint effects.

## 3 | RESULTS

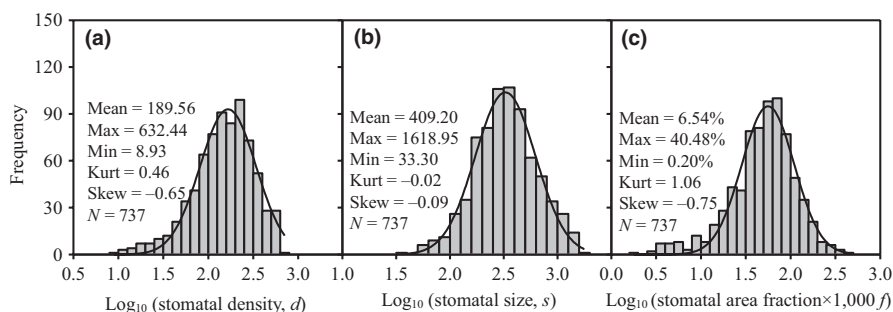
### 3.1 | Stomatal traits and influencing factors

Across all plant species, the  $d$ ,  $s$  and  $f$  were distributed log-normally, and showed major variation across species and biomes (Figure 2a–c). The  $d$ ,  $s$  and  $f$  ranged 8.93–632 pores per  $\text{mm}^2$ , 33.3–1,619  $\mu\text{m}^2$ , and 0.20%–40.5%, respectively, with average values of 190 pores per  $\text{mm}^2$ , 409  $\mu\text{m}^2$ , and 6.54%. The  $d$  and  $f$  were positively correlated with MAT and MAP, whereas  $s$  was negatively related to MAT and MAP (Table 1). The  $d$  and  $f$  were also positively correlated with soil nutrients, i.e. the concentrations of carbon, nitrogen and phosphorus in soils, and  $s$  showed the opposite trends (Table 1). The contribution of interspecific variation to the spatial patterns of leaf stomatal traits was more than 90% for all three stomatal traits, whereas climate and soil had comparatively small influences (Figure S2a–c).

The variance of stomatal traits was mainly explained by within-site variation, with only a small portion arising from among-site variation (Table 2). Plant family had a large effect on stomatal traits, and the interaction of site and plant family also had a significant influence (Figure 3).

### 3.2 | Variation in leaf stomatal traits among plant functional groups

The  $d$ ,  $s$  and  $f$  all differed significantly among plant growth forms (trees, shrubs, and herbs) ( $p < .001$ ; Figure 4). Trees tended to have higher  $d$ , lower  $s$  and higher  $f$  than shrubs and/or herbs. The  $d$  was higher in broad-leaved than coniferous trees, but  $s$  was lower, and the two groups did not differ on average in  $f$ . We found no statistical differences in any of the stomatal traits between evergreen and deciduous species.



**FIGURE 2** Frequency distributions of stomatal density (a), stomatal size (b) and stomatal area fraction (c)

**TABLE 1** Pearson's coefficients between leaf stomatal traits and environmental variables

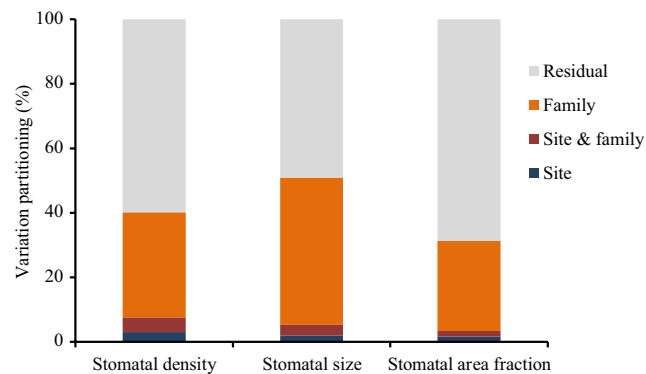
|              | Stomatal density ( <i>d</i> ) | Stomatal size ( <i>s</i> ) | Stomatal area fraction ( <i>f</i> ) |
|--------------|-------------------------------|----------------------------|-------------------------------------|
| MAT          | <b>0.20***</b>                | <b>-0.10**</b>             | <b>0.11***</b>                      |
| MAP          | <b>0.22***</b>                | <b>-0.11***</b>            | <b>0.11***</b>                      |
| Soil C       | <b>-0.21***</b>               | <b>0.14***</b>             | <b>-0.10**</b>                      |
| Soil N       | <b>-0.19***</b>               | <b>0.11**</b>              | <b>-0.10**</b>                      |
| Soil P       | <b>-0.12***</b>               | 0.04                       | <b>-0.10**</b>                      |
| pH           | <b>-0.14***</b>               | 0.06                       | -0.06                               |
| Soil texture | -0.02                         | <b>0.13***</b>             | <b>0.08*</b>                        |

MAT, mean annual temperature; MAP, mean annual precipitation; Soil C, soil carbon concentration; Soil N, soil nitrogen concentration; Soil P, soil total phosphorus concentration; Soil texture, the ratio of clay weight (<2  $\mu\text{m}$ ) to soil weight. Bold values indicated that the correlations between variables were significant.

\* $p < .05$ , \*\* $p < .01$ , \*\*\* $p < .001$ .

**TABLE 2** Partitioning of the variance of stomatal traits within- and among-site

| Stomatal traits        | Within-site | Among-site | <i>F</i> | <i>p</i> |
|------------------------|-------------|------------|----------|----------|
| Stomatal density       | 92.4%       | 7.58%      | 9.18     | <.001    |
| Stomatal size          | 94.7%       | 5.28%      | 6.24     | <.001    |
| Stomatal area fraction | 96.6%       | 3.39%      | 3.93     | <.001    |

**FIGURE 3** The contributions of family, site and their interaction to variation in stomatal traits

### 3.3 | Latitudinal patterns in stomatal traits at the community level

Both  $d_{\text{CWM}}$  and  $f_{\text{CWM}}$  showed humpbacked-shaped relationships with latitude, whereas  $s_{\text{CWM}}$  showed a positive linear trend (Figure 5). There were significant correlations between the community-weighted means of stomatal traits and climate (Figure 6). For  $d_{\text{CWM}}$  plotted against MAT,  $s_{\text{CWM}}$  against MAP and  $f_{\text{CWM}}$  against MAT, the relationships showed increases followed by decreases, with significant quadratic relationships ( $R^2 = 0.24$ ,  $p < .02$ ;  $R^2 = 0.48$ ,  $p < .001$ ;  $R^2 = 0.41$ ,

$p < .001$ ; respectively). For  $d_{\text{CWM}}$  versus DI,  $s_{\text{CWM}}$  versus MAT, and  $f_{\text{CWM}}$  versus DI, negative relationships were observed ( $R^2 = 0.46$ ,  $p < .001$ ;  $R^2 = 0.18$ ,  $p = .01$ ;  $R^2 = 0.63$ ,  $p < .001$ ; respectively). No significant correlations between stomatal traits and soil characteristics at the community level were observed (Table S2).

### 3.4 | Relationships between the community-weighted means of stomatal traits and WUE

The community-weighted means of stomatal traits were related to WUE across the natural forest communities (Figure 7a–c).  $d_{\text{CWM}}$  and  $s_{\text{CWM}}$  showed significant quadratic relationships with WUE ( $R^2 = 0.27$ – $0.29$ ,  $p = .007$ – $.01$ ; respectively), while  $f_{\text{CWM}}$  was positively related to WUE ( $R^2 = 0.25$ ,  $p = .003$ ).

## 4 | DISCUSSION

### 4.1 | Variation in leaf stomatal traits

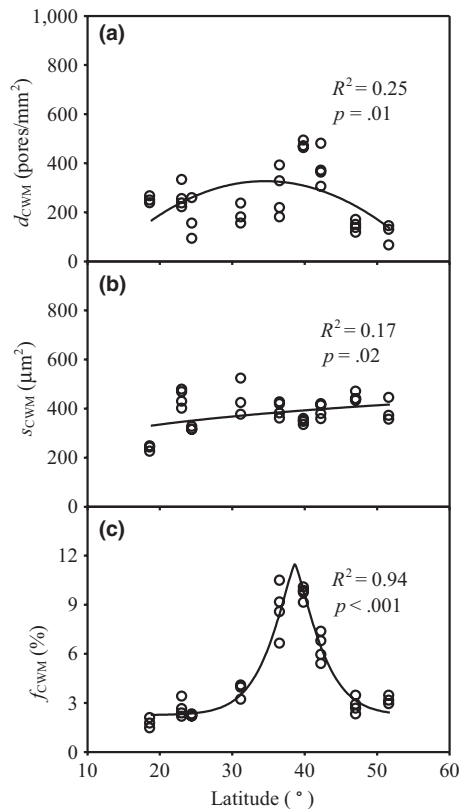
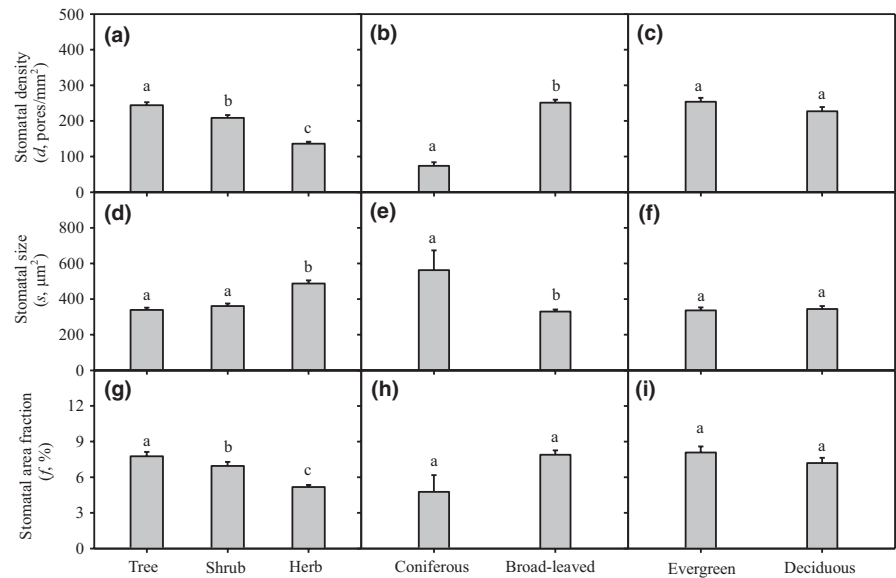
We quantified the ranges of *d*, *s* and *f* for 737 plant species from cold temperate to tropical forests. This study confirmed and expanded the dramatic variation of *d* and *s* shown by Wang et al. (2015), and provided the first dataset on *f* for a large number of diverse species across a large region. In this study, stomatal traits were only measured on the lower surface, given that hypostomatous species were dominant in these forests, the results are expected to be robust and representative of differences within and among communities. A previous study of 90 species of woody and semi-woody plants showed that *f* ranged from 2.8% to 33% (Cornelissen et al., 2003), and our study extended the range of *f* from 0.20% to 40.5%. This 200-fold variation in *f* across species would have strong functional consequences, as *f* reflects variation in stomatal pore index (across our species, SPI was tightly correlated with *f*;  $R^2 = 0.94$ ,  $p < .001$ ; Figure S3a), and in the anatomical maximum stomatal conductance (Sack & Buckley, 2016;  $R^2 = 0.43$ ,  $p < .001$ ; Figure S3b).

Significant differences in stomatal traits were observed among the plant functional groups, consistent with patterns previously reported within forests: *d* was highest in trees, followed by shrubs and herbs, and *s* showed the opposite trend. These trends across the regional species pool extend the findings for British woodlands (Salisbury, 1928) and the study of Wang et al. (2015). This variation resulted in *f* values being higher for trees than shrubs than herbs. Such a pattern is consistent with the leaves of trees being more “dry-adapted,” consistent with their greater hydraulic limitation and transpiration demand, and with an ability to achieve higher rates of gas exchange in the more limited times when water is available. Indeed, higher *f* for trees than shrubs and for shrubs than herbs is consistent with anatomical maximum stomatal conductance calculated from our data (Figure S4).

### 4.2 | Functional implications of variation in *f* across environments

The strong variation in *f* arises from variation in both *d* and *s*, despite the frequently observed negative correlations between them (Franks

**FIGURE 4** Changes in leaf stomatal traits among different plant functional groups. In each panel, data represent the  $M \pm 1$  SE, and data with same lowercase letters represent no significant differences at  $p = .05$  level



**FIGURE 5** Latitudinal patterns of stomatal density ( $d_{CWM}$ , a), stomatal size ( $s_{CWM}$ , b) and stomatal area fraction ( $f_{CWM}$ , c) at the community level. The parameters were calculated on community-weighted means across plant species

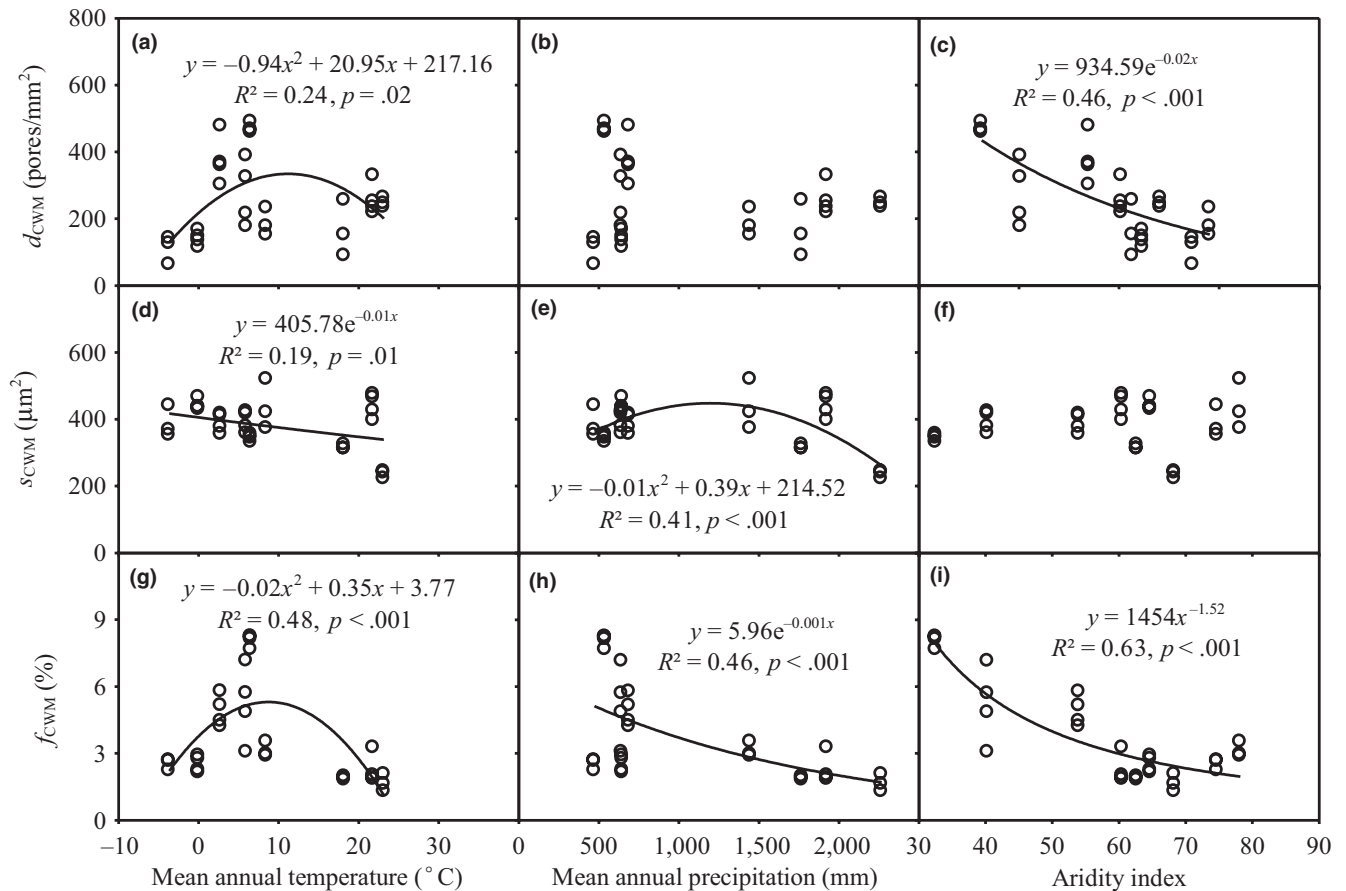
et al., 2009; Hetherington & Woodward, 2003). This negative correlation might arise from physical and/or energetic constraints. Thus, a trade-off between  $s$  and  $d$  that would constrain  $f$  has been hypothesised to arise from simple limits on embedding stomata in the epidermis to minimise the effect of stomatal interference on stomatal

conductance (Franks et al., 2009), or from energetic constraints if leaves maximise their stomatal conductance and, thereby, photosynthetic rate, while minimising investment in stomatal area or volume (Franks & Beerling, 2009). However, our finding of extensive variation in  $f$  indicates that the  $s$ - $d$  trade-off does not necessarily arise from, or imply, a tightly constrained  $f$ , and underlines the fact that  $f$  differs significantly among species, plant functional groups, and communities (Figure 4g).

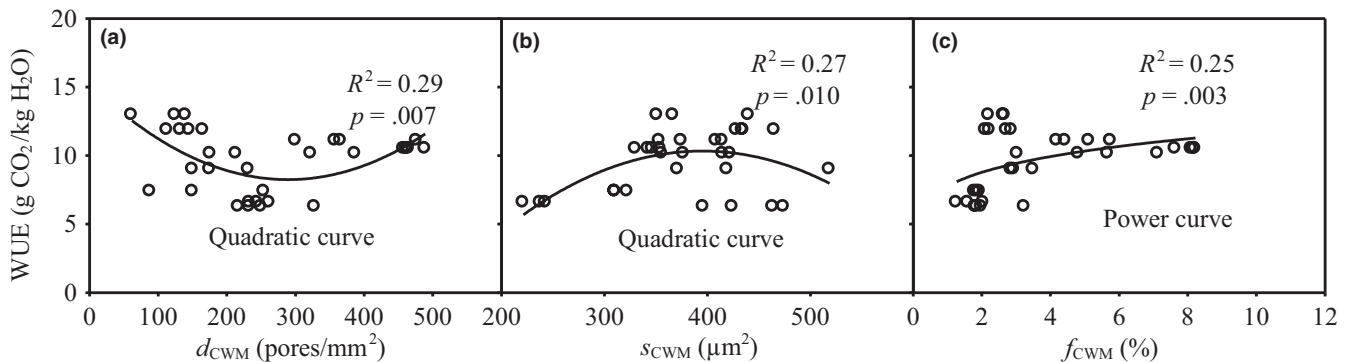
The community-weighted variable  $f_{CWM}$  was negatively related to the aridity index ( $R^2 = 0.63$ ,  $p < .001$ ). This finding is consistent with the concept that adaptation to drier environments often includes a counter-intuitive increase in the capacity for maximum gas exchange (Grubb, 1998; Hetherington & Woodward, 2003; Maximov, 1931). Plant communities living in the drier area often show an opportunistic mechanism with respect to moisture, i.e. being adapted to achieving high rates of stomatal conductance, photosynthesis and growth during periods when water is available (Grubb, 1998; Hetherington & Woodward, 2003; Sack et al., 2003). Such higher  $f$ , and  $g_{max}$ , and WUE would not preclude drought tolerance via dormancy or dieback when water availability is low. Our study is the first to our knowledge, to show regional and continental variation of  $f$  linked to climate, highlighting the importance of opportunistic mechanisms for adaptation to aridity.

Our study also highlighted and extended that relationships between plant traits and climate at the community level were tighter than that at the species level. Individual species diversify in niches within their ecosystem, adapting in their phenology and in response to contrasting local microclimates, resource availabilities, neighbourhoods and life history strategies. Consequently, even when traits shift strongly with climate across communities, within-site variation tends to contribute most to species-level variation, as previously shown, e.g. for leaf mass per area, foliar nutrient concentration, seed mass and stem hydraulic conductance (Choat, Sack, & Holbrook, 2007; Wright, Reich, & Westoby, 2001; Zhao et al., 2016).





**FIGURE 6** Relationships between climate and the community-weighted means of stomatal density ( $d_{CWM}$ ), stomatal size ( $s_{CWM}$ ) and stomatal area fraction ( $f_{CWM}$ )



**FIGURE 7** Relationships between water-use efficiency (WUE) and stomatal density ( $d_{CWM}$ , a), stomatal size ( $s_{CWM}$ , b), and stomatal area fraction ( $f_{CWM}$ , c) at the community level. Based on Yu et al. (2008), the WUE of the natural forest community along the NSTEC in a vegetative season was estimated as follows:  $WUE = 11.742 + 0.00003 \text{ MAP} - 0.266 \text{ MAT}$ ,  $R^2 = 0.85$ ,  $p = .008$

### 4.3 | Relationship of community stomatal traits to WUE at the regional scale

Plant traits are recognised as important determinants of ecosystem functioning (Garnier & Navas, 2012; Lavorel & Grigulis, 2012; Reichstein, Bahn, Mahecha, Kattge, & Baldocchi, 2014). We found a strong positive association of  $f_{CWM}$  with WUE across natural forest communities ( $R^2 = 0.25$ ,  $p = .003$ ). Given that lower stomatal

conductance would be a driver of high WUE at leaf scale, this result does not support the hypothesis that the anatomical constraint on maximum stomatal conductance, represented by  $f$ , is a control on WUE at regional or continental scale. Rather, our findings are consistent with the operating stomatal conductance over the course of the growing season, being independent of the anatomical maximum stomatal conductance across communities regionally. Thus, plant species in arid environments tend to have higher  $f$ , and, thus, higher

anatomically determined maximum stomatal conductance, yet this would be coupled with the ability to operate with lower stomatal opening for most of the productive season, conferring a higher WUE. Thus, our findings suggest that at regional scale, the anatomical adaptation of maximum stomatal conductance is independent from, and often in opposite direction to, the adaptation for stomatal opening.

## 5 | CONCLUSIONS

This study is the first to quantify variation in stomatal traits  $d$ ,  $s$  and  $f$  from cold temperate to tropical forests. These traits varied significantly across species, functional groups, and communities, and the variation was mainly controlled by interspecific variation within forests. The  $f$  has strong functional implications, relating to the anatomical maximum stomatal conductance, and increased from herbs to shrubs to trees and varied strongly across communities with relation to climate. The close positive linkage between  $f_{CWM}$  and WUE in natural forest communities indicated that WUE was not related directly to maximum stomatal conductance. Rather, this linkage supported classical hypotheses that species in environments of greater aridity have a greater capacity not only for higher potential gas exchange when water is available, but also tend to have lower operational stomatal conductance on average across the photosynthetic period of the year. These findings importantly broaden current knowledge on the adaptation of stomatal traits and provide direct evidence of how plant traits are linked to ecosystem functioning across communities and regions.

## AUTHORS' CONTRIBUTIONS

N.H. and G.Y. conceived the ideas and designed methodology; C.L., J.Z., Y.L. and Q.W. collected the data; N.H. and C.L. analysed the data; C.L., N.H. and L.S. led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

## DATA ACCESSIBILITY

Data used in this manuscript are available from Dryad Digital Repository <https://doi.org/10.5061/dryad.qp727> (Liu et al., 2017). Requests for data or other materials should contact to N.H. ([henp@igsnr.ac.cn](mailto:henp@igsnr.ac.cn)).

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#### SUPPORTING INFORMATION

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