STUDIES

Phenotypic plasticity of stomatal and photosynthetic features of four Picea species in two contrasting common gardens

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Abstract.

Global climate change is expected to affect mountain ecosystems significantly. Phenotypic plasticity, the ability of any genotype to produce a variety of phenotypes under different environmental conditions, is critical in determining the ability of species to acclimate to current climatic changes. Here, to simulate the impact of climate change, we compared the physiology of species of the genus Picea from different provenances and climatic conditions and quantified their phenotypic plasticity index (PPI) in two contrasting common gardens (dry vs. wet), and then considered phenotypic plastic effects on their future adaptation. The mean PPI of the photosynthetic features studied was higher than that of the stomatal features. Species grown in the arid and humid common gardens were differentiated: the stomatal length (SL) and width (SW) on the adaxial surface, the transpiration rate (Tr) and leaf mass per area (LMA) were more highly correlated with rainfall than other traits. There were no significant relationships between the observed plasticity and the species’ original habitat, except in P. crassifolia (from an arid habitat) and P. asperata (from a humid habitat). Picea crassifolia exhibited enhanced instantaneous efficiency of water use (PPI = 0.52) and the ratio of photosynthesis to respiration (PPI = 0.10) remained constant; this species was, therefore, considered to the one best able to acclimate when faced with the effects of climate change. The other three species exhibited reduced physiological activity when exposed to water limitation. These findings indicate how climate change affects the potential roles of plasticity in determining plant physiology, and provide a basis for future reforestation efforts in China.

Keywords: Climate change; phenotypic plasticity; photosynthetic features; Picea; stomatal features.

Introduction

The global climate will change significantly over the coming century, with overall increases in temperature and a greater frequency of drought events in many regions (Gitay et al. 2002). This will affect the exchange of water, carbon, nutrients, and energy between plants and the environment, particularly in mountain ecosystems (IPCC 2007; Grossiord et al. 2017a). Previous research indicates that plant species can adjust to climate change through phenotypic plasticity—the range of phenotypes that a genotype can express as a function of the range of environmental conditions. However, the range of variation within genotypes varies...
depending on the scale of the particular study (species, population, etc.) (Miner et al. 2005; Nicotra et al. 2010; Pigliucci 2010). In the context considered here, we can hypothesize that species may have the greatest capacity for acclimation as a result of increased phenotypic plasticity. Generally, species may be constrained by the selective pressures acting in their particular native climate. So the species exposed to more climatic variation in their distribution range are reported to have more phenotypic plasticity than species exposed to restricted climatic variation in their ranges (Valladares et al. 2014). Therefore, it is valuable to quantify a species’ ability to rapidly adjust functional traits, both morphological and physiological (Gruulke 2010; Nicotra et al. 2010; Frank et al. 2017).

The growth and survival of plants during periods of drought depends on those traits affecting the absorption, transport and conservation of water; these include stomatal regulation of water loss, osmotic adjustment of the leaf turgor loss point, and the structure of the xylem and the roots (Brodribb and Holbrook 2003; McDowell et al. 2008; Bartlett et al. 2016; Pfautsch et al. 2016). Moreover, stomata regulate gas exchange, a key process enabling plants to cope with rapid climate change (Nicotra et al. 2010; Grossiord et al. 2017a). Stomatal structure and behaviour have particularly important effects on water loss, hydraulic characteristics and carbon acquisition, and have therefore been studied in several plant species (Brodribb and Holbrook 2003; Cheoat et al. 2011; Bourne et al. 2017). For instance, Brodribb et al. (2003) demonstrated that the point of stomatal closure occurs prior to cavitation and relates to stem vulnerability and the water potential at the turgor loss point in eight tropical dry forest trees. In addition, Wang et al. (2007) concluded that the density, size and distribution of the stomata all had important effects on water exchange between plants and their environment. There is also evidence that stomatal density increases and/or stomatal size decreases in more arid climates and at higher temperatures (Franks and Farquhar 2001; Zhao et al. 2015; Carlson et al. 2016). Leaf mass per unit area and water use efficiency are key adaptive plastic traits for assessing plants’ responses to climate change (Hetherington and Woodward 2003; Wright et al. 2004; Seibt et al. 2008). However, it is not clear how the plasticity of stomatal density and size relate to the plasticity of gas exchange capacity in arid climates (Locosselli and Ceccantini 2012), especially when focusing on plants belonging to a single genus.

Picea species, which are important components of the alpine and subalpine coniferous forests of the Northern Hemisphere, may be exposed to frequent drought events as a result of climate change. Picea meyeri, P. crassifolia, P. asperata and P. wilsonii are species endemic to China (Fu et al. 1999), and climate change is expected to make the regions in which they are found drier than they are at present (Fu and Ma 2008; Huang et al. 2016). Coincidentally, the mean annual precipitation in the native ranges of P. meyeri and P. crassifolia is lower (491 and 417 mm, respectively) than that in the native ranges of P. asperata and P. wilsonii (714 and 690 mm, respectively). Therefore, the existence of four Picea species from contrasting climates provides us with an opportunity to evaluate the phenotypic plasticity of stomatal and photosynthetic features in spruce saplings under simulated climate change scenarios. Generally, species that originate from arid regions may benefit from maintaining a low stomatal conductance and increasing water use efficiency by minimizing transpiration (Bourne et al. 2017). Conversely, plants from humid areas may benefit from high rates of assimilation and productivity (Fichot et al. 2009). Therefore, we gathered seeds from a site at the centre of the distribution range of each of the four species and then reared in a greenhouse, then planted the saplings into two different common gardens (wet and dry), where confounding environmental and phylogenetic factors were minimized, to simulate the impact of climate change. Stomatal anatomic traits were measured separately on the adaxial and abaxial surface due to their differing morphology, according to classical taxonomy (Fu et al. 1999). To assess the functional status of the stomata, photosynthetic features were also measured, including net photosynthetic rate (Pn), the instantaneous efficiency of water use (iWUE) and photosynthesis/respiration ratio (P/R), which can be used to evaluate the balance of water loss and carbon acquisition. Finally, we aimed to investigate the degree and relationships between stomatal and photosynthetic plasticity of the four Picea species and the relationship between their original habitats. More specifically, we expect the species from dry habitats to achieve higher plasticity with respect to regulating water loss, whilst species from humid habitats could be expected to exhibit greater variability in physiological traits related to productivity. Furthermore, a better understanding of the phenotypic plasticity of Picea species will assist in compiling guidelines for future reforestation efforts in China and elsewhere.

Materials and Methods

Study site and experimental design

One of the common gardens is in the southeast of Gansu Province, at Zhangjia forest station in Liangdang County (106.5575°E, 34.1186°N; WCG denoting the wet common garden). This region has a medium latitude monsoon climate, with a mean annual precipitation and temperature of 754 mm and 7.8 °C, respectively. The other common garden is in the middle of Gansu Province, at the Plant Germplasm Repository of Lanzhou University in Yuzhong County (104.1562°E, 55.9391°N; DCG denoting the dry common garden). This region has a temperate semi-arid climate, with a mean annual precipitation and temperature of 385 mm and 7.1 °C, respectively. The two sites thus have similar annual temperatures but different levels of precipitation, making them suitable for simulating the effect of a transition from a wet climate to a dry one.

Seeds of each species (Picea meyeri, P. crassifolia, P. asperata and P. wilsonii) were collected from their respective central distribution ranges during 2004 and 2005. As noted above, the habitats of P. crassifolia and P. meyeri have lower mean annual precipitation levels than those of P. asperata and P. wilsonii. All seeds were germinated at a tree nursery and grown on until they were 5 years old, after which saplings were successively transplanted to the two study sites in the spring of 2010. Planting consisted of four blocks (replicates); seedlings from each species were planted 1 m apart in 3 × 3 square matrices (9 individuals per block), and randomly assigned within each block (Oleksyn et al. 1998).

Anatomy of stomatal features

Ten individuals per species were randomly chosen from four blocks for anatomical measurements. Current-year needles were collected in mid-September 2013 to ensure that the collected leaves were fully expanded and mature. On a sunny morning, current-year twigs all with the same orientation were cut down and immediately placed in 10-mL centrifuge tubes containing modified FAA solution (50 % alcohol, formalin, glacial acetic acid and glycerinum in a volume ratio of 90:5:5:5) to prevent leaf deformation and stomatal closure. All samples were then taken back to the laboratory for the measurement of anatomical characteristics. Twenty needles from each twig were randomly
selected and dissociated in sodium hypochlorite (10% available chlorine). Because needles of spruce are quadrangular in cross section with four ridges, we sliced the mid-ridge of one side to distinguish the adaxial and abaxial surface and also to allow easy dissociation of the mesophyll tissue in sodium hypochlorite. After immersion for 8 h, a banister brush was used carefully to clean the residual mesophyll cells without destroying the epidermis. The transparent epidermis pieces were then dyed with safranine (1%) to make temporary sections, which were imaged using an Olympus photomicroscope (BX51, Olympus (China) Co., Ltd) at ×40 and ×400 magnification (Fig. 1, an example of P. crassifolia).

Stomatal density (SD, mm⁻²) values were calculated as the total number of stomata per 1 mm² area on the adaxial or abaxial surface. The number of stomatal rows (N) was manually counted using the ×40 images (incomplete rows were assigned a value of 0.5), while stomatal counts on the epidermis were conducted near the middle of the needle on the same surface to avoid potential variation at the base or tip (Fig. 1A). The ×400 images were used to determine the stomatal pore length (SL, μm) and width (SW, μm) by measuring the longest axis and the widest point perpendicular to this (Fig. 1B). The mean stomatal pore area (S, μm²) was defined as an ellipse with major axis equal to SL and minor axis equal to SW, as described by Dow et al. (2013). Stomatal pore index (SI) was the pore area per leaf area and was calculated as SD multiplied by S. All image analysis was performed using the Image-Pro Plus 5.0.1 (Media Cybernetics, Inc., USA) software package.

**Measurement of photosynthetic features**

We measured photosynthetic features on single current-year sun-exposed twigs of the selected individuals in mid-September. The features examined were the leaf mass per unit area (LMA, kg m⁻²), net photosynthesis rate (PN, μmol cm⁻² s⁻¹), stomatal conductance (Gs, mol cm⁻² s⁻¹), transpiration rate (Tr, mmol cm⁻² s⁻¹), respiration rate during daytime darkness (Rday, mol cm⁻² s⁻¹), instantaneous efficiency of water use (iWUE, μmol mmol⁻¹) and P/R (photosynthesis/respiration ratio). At least three randomly chosen twigs per selected individual in the four blocks were measured with a Li-6400XT infrared gas exchange system (LI-COR Biosciences, Lincoln, NE, USA). Measurements were taken in the morning (between 08:30 and 11:30 a.m.) when the highest stomatal conductance could be expected.

Measurements were made using a 6400-22L conifer chamber (RGB sources) with a reference CO₂ concentration of 380 μmol mol⁻¹, a reference chamber temperature of 25 °C, a flow rate of 300 μmol s⁻¹, and two light levels of 1000 (saturated light) and 0 μmol m⁻² s⁻¹ to obtain the maximum gas exchange and daytime respiration, respectively (see Drake et al. 2013). After that, all needles were scanned to measure their projected areas and then dried to constant mass (>48 h) in an oven at 72 °C to allow us to calculate LMA.

**Phenotypic plasticity index**

For all studied traits, a phenotypic plasticity index (PPI) was calculated as described by Larcher et al. (2015) and Santiso and Retuerto (2015), using the following expression: PPI = (species mean 1 – species mean 2)/(the larger species mean). The index varies from 0 (no plasticity) to 1 (the maximum plasticity), and allows comparisons between traits with different units (Valladares et al. 2006; Larcher et al. 2015; Santiso and Retuerto 2015).

**Data analysis**

A one-way ANOVA was used to test whether trait means differed significantly among species and between the two gardens; analysis was conducted using SPSS 16.0 (SPSS Inc., Chicago, IL, USA). Differences were considered statistically significant if P < 0.05. The PPI values were calculated in Excel after the analysis above. To understand how the stomatal and photosynthetic traits differed between species and study sites, and to describe the relationships between the studied traits, we performed a principal component analysis (PCA) using the Past 2.17c software (Hammer et al. 2001) after removing non-independent and highly correlated traits. Intraspecific comparisons between the two common gardens were performed via independent samples t-tests with SPSS 16.0 and variation trends for all traits were plotted using SigmaPlot 10.0 (Systat Software, Inc., Chicago, IL, USA).

**Results**

**Differences in stomatal and photosynthetic features for species and sites**

For stomatal features, significant differences were observed between adaxial and abaxial surfaces among the four Picea

![Figure 1.](http://example.com/figure1.png)
species from the two sites. That is, on the adaxial surface, *P. asperata* had the highest stomatal pore length (SL), stomatal pore width (SW), stomatal area (S) and stomatal pore index (SI); *P. crassifolia* exhibited the highest adaxial stomatal density (SD) and the lowest SL and S; and *P. wilsonii* had the lowest number of stomatal rows (N) among the four species when grown in WCG (Table 1). On the abaxial surface, *P. meyeri* exhibited the highest values of all stomatal traits except for N and SL, *P. asperata* had the highest SD and the lowest SL and S; and *P. wilsonii* had the lowest number of stomatal rows (N) among the four species when grown in WCG (Table 1). However, in DCG, these interspecific differences on the adaxial surface were different: *P. meyeri* exhibited the highest SD and SI, and *P. crassifolia* tended to have the highest S on the adaxial surface among the four species (Table 2). However, the interspecific differences on the abaxial surface in DCG were similar to those found in WCG: *P. meyeri* had the highest S and SI and *P. wilsonii* tended to have the lowest SD and N among the four species (Tables 1 and 2).

Significant differences were also observed in photosynthetic features among species. In WCG, *P. meyeri* showed the highest net photosynthetic rate (Pn) and stomatal conductance (Gs), while *P. asperata* tended to have the lowest transpiration rate; *P. crassifolia* had the lowest respiration rate during daytime darkness (Rday) and the lowest ratio of photosynthesis to respiration (P/R) (Table 1). In DCG, *P. asperata* had the highest Pn and Gs, *P. crassifolia* had the highest instantaneous photosynthesis/respiration ratio (P/R) (Table 1). However, the interspecific differences on the abaxial surface in DCG were similar to those found in WCG: *P. meyeri* had the highest S and SI and *P. wilsonii* tended to have the lowest SD and N among the four species (Tables 1 and 2).

**Table 1.** Stomatal and photosynthetic features of four *Picea* species grown in WCG. Row legends: SD (stomatal density); N (number of stomatal rows); SL (stomatal pore length); SW (stomatal pore width) and S (stomatal pore area); SI (stomatal pore index); Pn (net photosynthetic rate); Gs (stomatal conductance); Tr (transpiration rate); Rday (respiration rate during daytime darkness); iWUE (instantaneous efficiency of water use); P/R (photosynthesis/respiration ratio); LMA (leaf mass per area). Data are presented as mean ± SE. Significance of interspecies differences is based on one-way ANOVA and marked with A, B and C.

| Features | *P. meyeri* | *P. crassifolia* | *P. asperata* | *P. wilsonii* |
|----------|-------------|-----------------|---------------|--------------|
| SD (mm⁻²) |             |                 |               |              |
| Adaxial  | 42 ± 3B     | 53 ± 3A         | 38 ± 0B       | 40 ± 2B      |
| Abaxial  | 32 ± 1A     | 30 ± 0B         | 30 ± 1B       | 23 ± 1C      |
| N        | 3.6 ± 0.2A  | 3.8 ± 0.1A      | 3.4 ± 0.2A    | 2.9 ± 0.1B   |
| SL (μm)  | 34 ± 1B     | 27 ± 1C         | 37 ± 1A       | 34 ± 1B      |
| SW (μm)  | 15 ± 0.4BC  | 14 ± 0.5C       | 18 ± 0.7A     | 16 ± 0.1B    |
| S (μm²)  | 410 ± 13B   | 299 ± 16C       | 520 ± 26A     | 422 ± 9B     |
| SI       | 0.017 ± 0.001AB | 0.016 ± 0.002B  | 0.012 ± 0.001B | 0.009 ± 0.000C |
| Pn (µmol cm⁻² s⁻¹) | 9.4 ± 0.5A | 7.8 ± 0.2B | 6.7 ± 0.7B | 6.8 ± 0.3B |
| Gs (mol cm⁻² s⁻¹) | 0.11 ± 0.01A | 0.09 ± 0.00AB | 0.08 ± 0.01B | 0.07 ± 0.00B |
| Tr (mmol cm⁻² s⁻¹) | 2.1 ± 0.25A | 2.0 ± 0.19A | 1.4 ± 0.15B | 2.1 ± 0.13A |
| Rday (µmol cm⁻² s⁻¹) | −1.1 ± 0.1A | −1.8 ± 0.3B | −1.1 ± 0.1A | −0.8 ± 0.1A |
| iWUE (µmol mmol⁻¹) | 4.7 ± 0.8A | 3.9 ± 0.5AB | 4.7 ± 0.2AB | 3.2 ± 0.1B |
| P/R      |              |                 |               |              |
| LMA (kg m⁻²) | 0.19 ± 0.001AB | 0.20 ± 0.02A | 0.22 ± 0.01A | 0.16 ± 0.01B |

| Features | *P. meyeri* | *P. crassifolia* | *P. asperata* | *P. wilsonii* |
|----------|-------------|-----------------|---------------|--------------|
| SD (mm⁻²) |             |                 |               |              |
| Adaxial  | 47 ± 2A     | 39 ± 2B         | 37 ± 1B       | 35 ± 1B      |
| Abaxial  | 32 ± 1A     | 32 ± 2A         | 30 ± 1A       | 22 ± 1B      |
| N        | 4.3 ± 0.1A  | 4.1 ± 0.3A      | 3.4 ± 0.1B    | 3.0 ± 0.1B   |
| SL (μm)  | 3.3 ± 0.1B  | 3.8 ± 0.1A      | 3.1 ± 0.0B    | 2.0 ± 0.1C   |
| SW (μm)  | 36 ± 1B     | 38 ± 1A         | 35 ± 1B       | 40 ± 1A      |
| S (μm²)  | 548 ± 10B   | 579 ± 8A        | 507 ± 6C      | 521 ± 9BC    |
| SI       | 0.026 ± 0.001A | 0.023 ± 0.001B  | 0.019 ± 0.000C | 0.018 ± 0.001C |
| Pn (µmol cm⁻² s⁻¹) | 7.1 ± 0.1AB | 7.5 ± 0.6A | 6.9 ± 0.3AB | 6.2 ± 0.2B |
| Gs (mol cm⁻² s⁻¹) | 0.07 ± 0.004B | 0.10 ± 0.003A | 0.07 ± 0.003B | 0.07 ± 0.002B |
| Tr (mmol cm⁻² s⁻¹) | 1.3 ± 0.0B | 1.6 ± 0.1A | 0.9 ± 0.1C | 1.7 ± 0.1A |
| Rday (µmol cm⁻² s⁻¹) | −1.9 ± 0.3A | −2.0 ± 0.2A | −1.4 ± 0.1A | −2.0 ± 0.4A |
| iWUE (µmol mmol⁻¹) | 5.6 ± 0.2B | 4.9 ± 0.3B | 8.2 ± 0.6A | 3.6 ± 0.3C |
| P/R      |              |                 |               |              |
| LMA (kg m⁻²) | 0.27 ± 0.02B | 0.34 ± 0.01A | 0.36 ± 0.01A | 0.28 ± 0.01B |
efficiency of water use (iWUE) and lowest transpiration rate (Tr), while *P. wilsonii* exhibited the lowest iWUE among the four species (Table 2).

**Plasticity of stomatal and photosynthetic features**

These four species exhibited different plastic responses between the two sites with their different amounts of rainfall. That is, *P. meyeri* tended to have higher N, SW and S on both the adaxial and abaxial surfaces in DCG than in WCG, but its photosynthetic features (Pn, Gs, Tr and P/R) in DCG had lower values than in WCG (Figs 2 and 3; *P* < 0.05). Similarly, *P. crassifolia* exhibited higher SL, S and SI on both the adaxial and abaxial surfaces, as well as higher abaxial N, higher adaxial SW and higher iWUE in DCG than in WCG; while its adaxial SD, Gs and Tr in DCG were lower than in WCG (Figs 2 and 3; *P* < 0.05). *P. asperata* also had higher SL on both the adaxial and abaxial surfaces, higher abaxial N and adaxial SW and S in DCG than in WCG, but its Tr, Rday and P/R values were lower in DCG than in WCG (Figs 2 and 3; *P* < 0.05). *P. asperata* had higher abaxial N in DCG than in WCG, but its abaxial S and Rday in DCG were lower than in WCG (Figs 2 and 3; *P* < 0.05). Additionally, the leaf mass per area (LMA) of all species was higher in DCG than in WCG (Fig. 4). The correlation analysis indicated that SL and SW on the adaxial surface, Tr and LMA were correlated with many traits [see Supporting Information—Table S1], which would imply that changes in these characteristics were important to species acclimation.

Overall, the morphological features of stomata exhibited limited plasticity: the mean PPI values of independent traits for all species were below 0.18 and the highest PPI value was observed in *P. crassifolia* (0.31); the non-independent traits, stomatal pore area (S) and stomatal pore index (SI) showed more

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**Figure 2.** Differences in stomatal traits between WCG and DCG for each species. Yellow symbols indicate the species from the dry habitat and blue symbols indicate the species from the wet habitat. The lines show mean values and standard error of means across three replicates for each measurement, plotted against drought treatments. (A) SD is stomatal density; (B) N is number of stomatal rows; (C) SL is stomatal pore length; (D) SW is stomatal pore width; (E) S is stomatal pore area; (F) SI is stomatal pore index. *P* < 0.05 and **P** < 0.01.
plasticity in *P. meyeri* and *P. crassifolia* than in the other species (Table 3). The mean PPI values of photosynthetic features were higher than those of stomatal features (Table 3). In addition, the species that originated in arid regions (*P. meyeri* and *P. crassifolia*) exhibited higher gas exchange plasticity in terms of Gs and Tr than those which originated in humid habitats (*P. asperata* and *P. wilsonii*). In contrast, the plasticity of Rday in species from humid habitats was higher than in those from arid areas.

**PCA of stomatal and photosynthetic features from two sites**

A PCA was performed to reveal the relationships between traits in each species when grown at the arid and humid sites. Three axes were identified (each with an eigenvalue > 1) that together explained 84.9 % of the total variation at the two sites (Fig. 5). Due to similarity between the proportion of variation explained by axis 2 (23.3 %) and axis 3 (20.2 %), two
graphs were produced to show the relationships (Fig. 5A and B). Apparently, species in the arid and humid gardens were separated along principle axis 1 (PC1) (Fig. 5). For axis 1, SL and SW on the adaxial surface, Tr and LMA contributed greatly to the distribution, while SL and SW on the abaxial surface accounted more for the distributions on axis 2 and axis 3, respectively [see Supporting Information—Table S2].

**Discussion**

Transplanting saplings into humid and arid common gardens can simulate a climate change scenario (Gavazov et al. 2013; Cácer et al. 2017). A previous study suggested that patterns of stomatal distribution on the two leaf surfaces generally change with the environment (Fraser et al. 2009), and stomata can operate independently across and between surfaces of dorsi-ventral leaves (Fanourakis et al. 2015; Richardson et al. 2017). We found that there were significant differences on both the adaxial and abaxial surface between every species in the two common gardens (Figs 2 and 3). Research examining the adaxial and abaxial surface of conifers has shown that a reduction in stomatal density on both surfaces and reduced stomatal conductance may have important consequences for the response of pine trees to water stress during long-term growth at elevated CO₂ concentrations (Pearson et al. 1995; Lin et al. 2001).
result, however, was different, suggesting that, during long-term growth under drought conditions, stomatal density remained constant while stomatal conductance decreased (Figs 2 and 3). This is consistent with data on Pinus edulis in a 7-year experiment (Guérin et al. 2018) and with previous reports suggesting that physiological responses tend to be stronger and faster than morphological ones (Valladares et al. 2000; Hodge 2004; Wyka et al. 2007, 2012; Grossiord et al. 2017a, b). Wyka et al. (2012) attributed this to the structural demands on conifers’ leaves resulting from their perennial life cycle, and also to the ghosts of this lineage’s evolutionary past. Thus, physiological processes such as the rate of photosynthesis and stomatal conductance were generally highly plastic (Fig. 3A and B). This plasticity may function as an adaptive mechanism: unlike morphological traits, photosynthetic features can be quickly restored to their original state when extreme conditions no longer prevail, without requiring tissue reconstruction or substantial maintenance costs (Metlen et al. 2009).

Leaf gas exchange capacity depends on both stomatal traits (notably the number and/or size of the stomata) and their behaviour (Hetherington and Woodward 2003; Zwie niecki et al. 2016). Although there was no further analysis of non-independent traits, their physiological significance could not be ignored. In our research, we noticed that iWUE did not differ significantly between WCG and DCG, whilst the P/R for all species except P. crassifolia was different between the common gardens (Fig. 3E and F). For P. crassifolia, the reason may be the greater decline of Tr at the arid site to prevent water loss, representing a positive response to drought stress. For the other three species, P/R decreased no matter whether Pn, Gs and Tr increased or not from WCG to DCG (Fig. 3). Similar examples can be found in P. edulis and Juniperus monosperma which, when grown with reduced levels of precipitation, exhibit lower rates of photosynthesis and stomatal conductance together with increased water use efficiency (Grossiord et al. 2017b). Stomatal pore index (SI) and its relationship with total leaf area is also important with respect to gas exchange (Zwie niecki et al. 2016). Our results revealed that changes in SL, SW and S were not associated with much change in SI, which could help us to understand why photosynthetic traits changed in the ways described above.

Most previous research about the plasticity in traits related to species’ original habitats has been based on experiments comparing plants grown under controlled conditions, such as paired shaded and full-sunlight plots (Moreira et al. 2013). In the wild, we expect plasticity to be influenced by both precipitation (the main factor in this study) and other environmental factors. Valladares et al. (2007), indeed, argued that ecological limits resulting from abiotic and biotic factors would influence phenotypic plasticity in unpredictable ways. However, in our study, species that originated from arid habitats exhibited non-significant plasticity compared to those from humid habitats (Table 3), although species grown in the two common gardens did differ (Fig. 5). The main trait variations in each species are revealed by the direction of change in the graph. It appears that each species exhibits a different strategy when modifying traits to respond to growing in a dry environment. This result suggests that the phenotypic plasticity of these four species was site-specific rather than, as we first expected, associated with their original habitats. In fact, P. crassifolia (arid habitat) and P. asperata (humid habitat) did respond in the way we expected: P. crassifolia reduced water loss and enhanced iWUE while P. asperata maintained high photosynthetic capacity but with high respiration (Fig. 3). Thus, our results indicate that P. crassifolia may survive well when suffering long-term water limitation by enhancing iWUE without reducing P/R (Fig. 3E and F). It was, however, also limited by traits other than these, which may be why Valladares et al. (2007) proposed ecological limits to plant phenotypic plasticity and Nicotra et al. (2010) aimed to synthesize multiple factors to examine the potential roles of plasticity in determining plant response to and effects of climate change. Therefore, long-term observation and molecular evidence (e.g. gene differential expression) of phenotypes could provide more substantive evidence explaining this hypothesis (Matesanz et al. 2010).

Conclusions

High resolution sampling of adaxial and abaxial leaf surfaces combined with direct measurement of carbon acquisition, water use and gas exchange in four Picea species provided a new insight into the plasticity of stomatal density, size and photosynthetic performance in the leaves of individuals growing in two regions with different levels of precipitation. The four species in the same genus analysed in this study exhibited comparatively high photosynthetic plasticity and low stomatal plasticity in the two common gardens. The differences in the plasticity of the independent traits resulted in divergence of the iWUE and P/R between species when comparing the results obtained in the arid and humid gardens. This could be expected, in turn, to affect the species’ relative capacities for rates of water loss and carbon accumulation. Our results also indicated that site-specific conditions can mask habitat variations, and thus should always be considered in studies of habitat trends in the future. Finally, we can make a preliminary suggestion that P. crassifolia should survive best because of its ability to enhance iWUE and keep P/R unchanged during long-term drought stress, while the other species slow down physiological activities to acclimate to drought stress. This result may be useful when planning plant reforestation efforts. In the future, synthesizing multiple factors to examine how climate change affects the potential roles of plasticity in determining plant responses would be valuable.

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Contributions by the Authors

C.M.Z., M.H.W. and J.R.W. conceived and designed the experiment. M.H.W. and A.P.Z. performed the experiment and analysed data with the help of X.W.Z. M.H.W. wrote the manuscript. S.S. and C.M.Z. discussed and revised the manuscript.

Conflict of Interest

None declared.

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Supporting Information

The following additional information is available in the online version of this article—

Table S1. Analysis of Pearson’s correlation between stomatal and photosynthetic traits.

Table S2. Values of loadings and their cumulative proportions for the four species in the principal component analysis.

Literature Cited

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