

Can evolutionary history predict plant plastic responses to climate change?

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Summary

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- Plant plastic responses are critical to the adaptation and survival of species under climate change, but whether they are constrained by evolutionary history (phylogeny) is largely unclear. Plant leaf traits are key in determining plants' performance in different environments, and if these traits and their variation are phylogenetically dependent, predictions could be made to identify species vulnerable to climate change.
- We compiled data on three leaf traits (photosynthetic rate, specific leaf area, and leaf nitrogen content) and their variation under four environmental change scenarios (warming, drought, elevated CO₂, or nitrogen addition) for 434 species, from 210 manipulation experiments.
- We found phylogenetic signal in the three traits but not in their variation under the four scenarios. This indicates that closely related species show similar traits but that their plastic responses could not be predicted from species relatedness under environmental change. Meanwhile, phylogeny weakened the slopes but did not change the directions of conventional pairwise trait relationships, suggesting that co-evolved leaf trait pairs have consistent responses under contrasting environmental conditions.
- Phylogeny can identify lineages rich in species showing similar traits and predict their relationships under climate change, but the degree of plant phenotypic variation does not vary consistently across evolutionary clades.

Introduction

Environmental variation in nature is ubiquitous, and in response, many species have functional traits that can be altered under changing conditions in order to maximize fitness. Unlike evolution, this trait flexibility or 'phenotypic plasticity' does not involve genetic changes and can occur over very short time scales (Nicotra *et al.*, 2010). These quick, plastic responses are possible because of the capacity of an individual's genetic makeup (genotype) to give rise to variable observable traits (multiple phenotypes), within their lifetime (Westerband *et al.*, 2021). Anthropogenic climate change is drastically altering environmental conditions over short time frames, and therefore the ability of organisms to quickly adapt via phenotypic variation is critical to the survival of species (Chevin *et al.*, 2010; Snell-Rood *et al.*, 2018). However, the ability to plastically alter traits when exposed to changing environmental conditions differs across species (Cui *et al.*, 2020), and if conserved across evolutionary

clades, trait plasticity under environmental change may be predictable from evolutionary history.

Given that closely related species tend to share more similar traits than distantly related ones (indicated by strong trait phylogenetic signal; Wiens, 2004; Losos, 2008; Crisp *et al.*, 2009), trait variation may be also constrained by evolutionary history, with closely related species reacting more similarly to environmental change than distantly related species (Fig. 1, Hypothesis 1, evolution-driven trait response). Evidence for the phylogenetic conservation of phenotypic variation comes from a study of root growth traits under soil heterogeneity across *c.* 100 plant species (Kembel & Cahill, 2005). However, other studies have found no phylogenetic effect on variation across multiple plant traits (Fig. 1, Hypothesis 2 or 3, environment-driven or random trait responses, depending upon whether trait values show no phylogenetic signal in addition to trait variation). For example, in 20 invasive–native species pairs from the Mediterranean region, plastic responses of leaf- and plant-level traits were not explained by

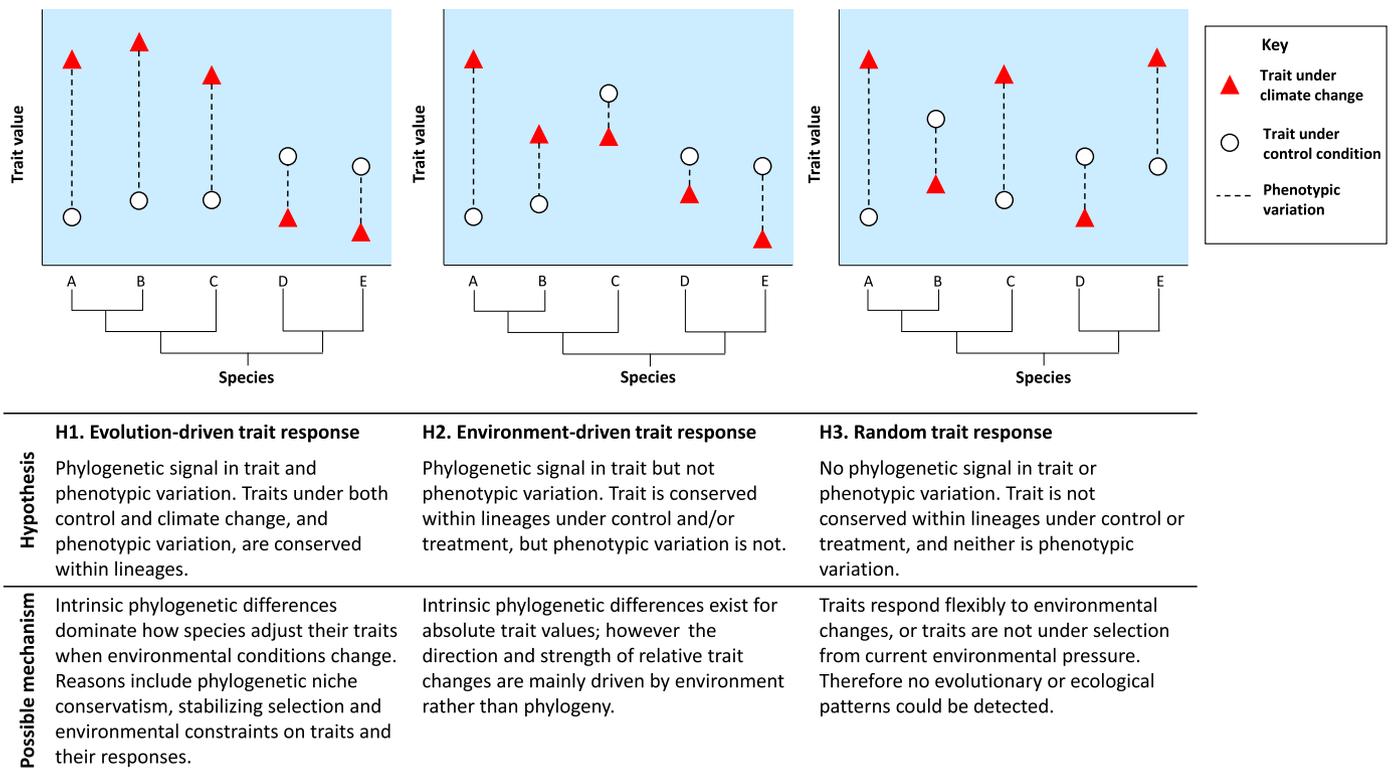


Fig. 1 A schematic diagram illustrating three hypotheses regarding the presence or absence of phylogenetic signal in a hypothetical plant trait under climate change and its phenotypic variation. In Hypothesis 1 (evolution-driven trait response), evolutionary history influences a trait in species A–E under control conditions (white circles), and under climate change (red triangles), as well as the ability to change the trait under different conditions (phenotypic variation; the dashed line). Therefore, closely related species show more similar trait values and phenotypic variation than more distantly related species (i.e. there is phylogenetic signal in the trait and its variation). In Hypothesis 2 (environment-driven trait response), phylogenetic signal exists in trait values but not in phenotypic variation. In Hypothesis 3 (random trait response), there is no phylogenetic signal in either the trait or phenotypic variation. The phylogeny below each panel shows the evolutionary relationships between the five hypothetical species, with species A most closely related to B, then C, and most distantly related to D and E.

the phylogenetic structure of the species (Godoy *et al.*, 2011). Similarly, a recent study reported that phenotypic variation of four trait types (leaf morphology, plant allocation, size and performance) is phylogenetically independent (Stotz *et al.*, 2021). In addition, the presence of environmental stress may be important, that is, phylogenetic signal may only be observed for a trait under stressful conditions (Burns & Strauss, 2012). These mixed findings are similarly reflected in the few similar studies on animal trait variation (Ashton, 2004; Relyea *et al.*, 2018). A further examination of broad patterns in the plastic responses of species within a phylogenetic context is very much needed, and would provide useful information about the vulnerability of species to environmental change (Moran *et al.*, 2016; Shao *et al.*, 2019).

Being immobile organisms, the ability to alter traits in response to a changing environment is particularly important for plants (Borges, 2009). Traits relating to the ‘leaf economic spectrum’ (LES) are key in determining plant performance in a given environment (Westoby *et al.*, 2002; Cornelissen *et al.*, 2003; Diaz *et al.*, 2004; Kattge *et al.*, 2020), and therefore plasticity in these traits is crucial for survival when conditions change. This spectrum is a well-established axis of adaptive variation, where a set of coordinated leaf traits that relate to resource investment is associated with different evolutionary strategies across plant species (Reich *et al.*, 1997, 2003; Wright *et al.*, 2004; Díaz *et al.*, 2016).

Fast-living species that produce ‘cheap’ leaves, characterized by low investment in tissue density (i.e. high values of specific leaf area (SLA); or a large leaf area for a given leaf dry mass) and nutrients (i.e. low leaf nitrogen content for a given leaf mass (Nm)), fall at one end of this spectrum. Such species show high rates of photosynthesis (A_m , carbon assimilation per unit leaf mass) and resource acquisition, but at the cost of longevity (Reich, 2014). At the other end of the spectrum are longer-lived species that invest highly in leaf material, and are thus associated with low SLA, Nm and A_m . Leaf economic spectrum traits are highly plastic, and they change flexibly in response to environmental changes through a diverse array of physiological, morphological and ecological mechanisms (Des Marais *et al.*, 2013), providing a key means by which plants optimize their performance in a rapidly changing environment (Huang *et al.*, 2019). However, whether this flexibility is constrained by evolutionary history is still unclear.

Furthermore, although the coordination between LES traits has been widely recognized (Reich *et al.*, 1997, 2003; Wright *et al.*, 2004; Díaz *et al.*, 2016), as evolutionary history explains a significant proportion of trait variation (Shao *et al.*, 2019), such relationships could remain or could disappear once phylogeny is accounted for (Liu *et al.*, 2015). The latter outcome indicates that the apparent correlation observed on the raw data is an artifact of

their evolutionary history (Felsenstein, 1985), while the former means that the trait correlation is a product of coordinated evolution (Garland *et al.*, 1992). Thus, testing how the pair-wise trait relationships shift within a phylogenetic context could reveal information regarding the evolutionary trajectory of co-adapted traits, which is also valuable for predicting plant responses under climate change.

Manipulation experiments, in which plants are subjected to ambient and contrasting environmental conditions, enable the quantification of leaf trait variation (Poorter *et al.*, 2009; De Frenne *et al.*, 2015). A plethora of studies have measured plastic changes in leaf economic traits in response to different aspects of climate change, such as elevated temperatures (Shao *et al.*, 2019) and CO₂ concentrations (Temme *et al.*, 2017). Recent work has summarized the findings of many such studies, and has uncovered general patterns in species' plastic responses to environmental change (Song *et al.*, 2019; Cui *et al.*, 2020). By examining these patterns within a phylogenetic context, the influence of evolutionary history on plastic plant responses can be determined across hundreds of species.

Here we explore phylogenetic influences on a trait dataset spanning 434 plant species grown under ambient and manipulated environmental conditions, from a meta-analysis of 210 manipulation experiments (Cui *et al.*, 2020). This dataset quantified the response of three key leaf economic traits (SLA, Nm and Am) to four types of environmental change: increased temperatures, reductions in water availability, elevated atmospheric CO₂, and nitrogen addition. By combining this dataset with a phylogeny of the study species, we aim to test for the following: (i) phylogenetic signal in leaf traits and their variation under altered environmental conditions, and (ii) phylogenetic influences on pair-wise trait relationships (e.g. the relationship between SLA and Am across species). We propose three hypotheses (given in Fig. 1) that could describe and explain various phylogenetic patterns in traits and their variation.

In our investigation of (i), we predict that either Hypothesis 1 or 2 will be supported by our data (phylogenetic signal in both traits and their variation, or just in traits) based on the mixed results on phenotypic variation in the existing literature. We believe that support for Hypothesis 3 is unlikely, because many plant traits in natural conditions show significant phylogenetic signal (Ackerly, 2009; Flores *et al.*, 2014; Liu *et al.*, 2015; Ma *et al.*, 2018), including SLA (Cornwell *et al.*, 2014; Flores *et al.*, 2014), Nm and Am (Liu *et al.*, 2015). For (ii), we predict that pair-wise trait relationships will weaken, rather than disappear under phylogenetic influence, because the coordination between LES traits is more likely to be co-adapted under evolutionary pressure (Reich *et al.*, 1997, 2003; Wright *et al.*, 2004; Díaz *et al.*, 2016).

Materials and Methods

Data compilation

To examine the influence of evolutionary history on plant phenotypic variation and trait relationships, we developed models based

on a recent dataset from Cui *et al.* (2020). Data on three key leaf economic traits, mass-based net photosynthetic rate (Am, nmol g⁻¹ s⁻¹), specific leaf area (SLA, cm² g⁻¹), and mass-based leaf nitrogen content (Nm, %), from experiments that mimic four aspects of climate change (warming, drought, elevated CO₂, or nitrogen addition) were collated from a literature search. Data from articles that measured at least two of the three traits made it into the final dataset, which included data for 434 plant species in 210 manipulative experiments. Those studies were classified into field experiments (garden or natural habitat experiments; 102 studies) and environmentally controlled experiments (glasshouse, growth chamber, or pot experiments; 108 studies). Trait relationships for field or environmentally controlled experiments, and for different functional groups (angiosperms vs gymnosperms, monocots vs eudicots, and C₃ vs C₄ species) were all tested separately and showed consistent patterns with very few exceptions (figs S4–S7 in Cui *et al.*, 2020). Therefore, to focus on phylogenetic questions and guarantee a high enough number of species for the validation of phylogenetic models, we combined data for all the species within each of the four environmental factors for analysis. Furthermore, we calculated and considered the strength of the treatment as an important factor in the following models. Treatment strength was calculated as the ratio of treatment level to the control level for elevated CO₂ experiments with lower CO₂ concentration as control. Otherwise treatment strength was the difference between control and treatment levels for warming, drought and nitrogen addition experiments, with lower temperature, higher water availability and lower nitrogen levels as control, respectively. We reported all the raw data with detailed treatment conditions in Supporting Information Table S1.

All data were natural-logarithm transformed before analysis to homogenize variance. We then characterized phenotypic variation as the log_e-transformed response ratio (RR). Response ratio is calculated as log_e(RR) = log_e(T_t) – log_e(T_c), where T_t and T_c are the experimental treatment mean and control mean, respectively (Hedges *et al.*, 1999). All of the response ratios of leaf traits to the four treatments were normally distributed (fig. 2 in Cui *et al.*, 2020). For all nine variables (Am, SLA, and Nm under control, treatment, and their phenotypic variation), we first calculated mean values of each species from the same or multiple studies; thus, we could no longer include 'study' as a factor in subsequent models. Next we incorporated the SE values of each variable into phylogenetic models to consider intraspecific variation. Although the two datasets (with and without SE) showed the same response patterns of conventional trait relationships under different treatments (fig. S9 in Cui *et al.*, 2020), we performed phylogenetic analyses on both datasets here to account for trait variation in case it had an effect on phylogenetic signal and phylogenetic pair-wise trait relationships.

Although the original studies were not designed to test phylogenetic hypotheses, our dataset incorporates a large diversity of plant species, which results in an increased statistical power to detect phylogenetic signal in both trait values and trait variation compared with previous tests based on few species (Godoy *et al.*, 2011; Burns & Strauss, 2012; Stotz *et al.*, 2021). Specifically, the

434 species belong to 189 genera and 71 families, covering gymnosperms (58 species) and angiosperms (376 species), and a variety of life forms (83 grasses, 95 herbs, 26 shrubs and 230 trees).

Phylogenetic tree

We generated phylogenetic trees for the 434 species using the package `V.PHYLOMAKER` (Jin & Qian, 2019) in the R language and environment (R Core Team, 2018). The mega-tree used in `V.PHYLOMAKER` (GBOTB.extended.tre) includes 74 531 species of 479 families, the largest dated phylogeny for seed plants, which is derived from two recently published mega-trees, based on fossil records, molecular data from GenBank and phylogenetic data from the OPEN TREE OF LIFE (Zanne *et al.*, 2014; Smith & Brown, 2018). For the 65 missing species, we used the branch length adjuster (BLADJ) method to attach them to their close relatives, which was done automatically by `V.PHYLOMAKER`. BLADJ binds the tip for a new species to the halfway point of the genus branch, which originated from the software package `PHYLOCOM` (Webb *et al.*, 2008). For unsolved species, including 12 three-species polytomies, two five-species polytomies and one seven-species polytomy, we randomly resolved these polytomies 300 times, as done by Smith & Brown (2018), using `multi2di` in the R package `APE` (Paradis *et al.*, 2004). Finally, with the 300 imputation trees, we repeated all subsequent phylogenetic analyses 300 times, in order to account for phylogenetic uncertainties. Furthermore, since the ideal phylogeny should be a tree based on molecular information for each species, for future comparative analyses, care should be taken in constructing phylogeny with missing or unsolved species, and in interpreting results. The 300 trees are provided in Notes S1.

Data analyses

To determine phylogenetic signal in the three leaf traits (both under control and treatment conditions) and in their variation, we first estimated Pagel's λ using phylogenetic generalized least square (PGLS) models in the R package `CAPER` (Orme *et al.*, 2018). According to the prediction of a Brownian model (BM) of trait evolution (Pagel, 1999), Pagel's λ is a value between 0 and 1, where $\lambda = 1$ implies that trait variation completely depends on phylogeny, while $\lambda = 0$ indicates no phylogenetic dependence (Freckleton *et al.*, 2002). We obtained P -values to evaluate the validity of whether λ was significantly different to 0 (i.e. that a phylogenetic signal exists). Significantly high λ values indicate that closely related species are more similar than would be expected by chance. Next, we estimated Blomberg's K as a complementary phylogenetic signal, using `phylosig` in the R package `PHYTOOLS` (Revell, 2012). We did not use other, similar functions/packages (e.g. `PICANTE`) because `phylosig` allows SE in its formula, and it is possible to calculate both Pagel's λ and Blomberg's K by using different settings within the same function (Table S2). $K = 0$ indicates no phylogenetic signal, and $K > 1$ suggests stronger similarities among closely related species than expected under Brownian motion (Blomberg *et al.*, 2003). The K

values were compared to a null distribution based on a white noise (WN) model, in which trait values are randomly swapped across tips 1000 times. Then a BM null model was simulated based on BM trait evolution over the phylogeny 1000 times; K values less than the 95% distribution of the simulations indicate they are less divergent than expected by Brownian motion (Blomberg *et al.*, 2003). P -values were obtained to enable a comparison between the K values and simulated K values based on both white noise (K_{WN}) and BM (K_{BM}) null models. Finally, we also fitted Ornstein–Uhlenbeck (OU) models for each trait, in order to estimate phylogenetic half-life (PHL), which is another complementary phylogenetic signal, using `fitContinuous` in the R package `GEIGER` (Pennell *et al.*, 2014). Phylogenetic half-life is equal to \log_2/α , where α is the attraction strength of the evolutionary optimum. If α is near 0, then the OU model resembles a BM model, indicating strong phylogenetic signal. Meanwhile, PHL quantifies the extent to which the trait displays evolutionary inertia (Hansen, 1997). If PHL is long relative to the depth of the phylogeny, then the macro-evolutionary history of a trait is a good predictor of its current value; if it is short, it is not (Relyea *et al.*, 2018; Neto-Bradley *et al.*, 2021).

We also tested phylogenetic signal based on scaled data ((species specific value – mean across species)/SD), in order to avoid influences from the data structure (i.e. only positive values for traits, but both positive and negative values for phenotypic variation; Fig. S1). However, phylogenetic signal in the original and scaled datasets was exactly the same for all of the traits under all of the treatments. Furthermore, to eliminate the influence of treatment strength (the magnitude of the environmental difference between control and treatment conditions) on plant plastic responses (Fig. S2), we tested phylogenetic signal in traits and trait variation by adding treatment strength as a factor in the PGLS models, and by using data subsets under the same treatment strength to confirm (Table S3).

To analyze phylogenetic influences on pair-wise trait relationships (e.g. SLA vs Am), we compared relationships when evolutionary history is accounted for (using PGLS models) and when it is not. The conventional relationships not controlling for phylogeny were modeled using standardized major axis (SMA) regressions in the R package `SMATR` (Warton *et al.*, 2012). We also ran PGLS models for the 300 imputation trees, to account for phylogenetic uncertainties. Results based on 300 trees converged very well, with negligible variation in intercepts and slopes (Table S4a); thus, trait relationships were plotted based on one-tree results. Furthermore, we added the SE for each trait pair to the PGLS models, using `pgls.Ives` in the R package `PHYTOOLS` (Revell, 2012).

Results

General patterns

On average, plants responded to experimental warming significantly positively in terms of their SLA (+6.9%; hereafter, 'significantly affected' means $P < 0.05$, whereas 'not affected' means $P > 0.05$), but not in terms of Am or Nm (+1.2% and –1.3%,

respectively). Drought significantly decreased Am (−38.3%) and SLA (−8.7%), and increased Nm (+6.5%). Elevated CO₂ significantly increased Am (+12.6%), and decreased Nm (−16.1%) and SLA (−12.6%; Fig. 2). Furthermore, nitrogen addition significantly increased Am (+12.8%) and Nm (+34.0%), but did not affect SLA (−0.6%; $P > 0.05$).

Plant responses across the phylogeny were highly variable in direction. For example, positive and negative responses to warming were nearly equal in number (55%, 53% and 66% positive responses for Am, Nm and SLA, respectively), and responses of Am and SLA to nitrogen addition were similarly varied (65%, 92% and 53% positive responses for Am, Nm and SLA, respectively). By contrast, the directions of the responses to drought were less varied (9%, 62% and 28% positive responses for Am, Nm and SLA, respectively), as were responses to elevated CO₂ (eCO₂; 64%, 8% and 13% positive responses for Am, Nm and SLA, respectively; Fig. 2).

Phylogenetic signal in leaf traits and phenotypic variation

Based on Pagel's λ (λ hereafter), we found significant phylogenetic signal for almost all leaf traits under control conditions (10 out of 12 trait–treatment combinations; $\lambda = 0.40$ – 0.91 ; Table 1a). The only exceptions were Am and Nm in control plants subjected to warming treatment ($P < 0.05$ for $\lambda = 0$). The traits of plants under 'climate change' treatments showed similar patterns in their phylogenetic signal to their controls: there was significant phylogenetic signal in the three traits when grown under drought ($\lambda = 0.62$ – 0.83), elevated CO₂ ($\lambda = 0.37$ – 0.52) and nitrogen addition ($\lambda = 0.37$ – 0.85), but only in SLA under warming conditions ($\lambda = 0.53$; Table 1a). By contrast, phylogenetic signal in phenotypic variation was rare (Fig. 2; Table 1a). A significant phylogenetic signal was found only in one instance, in SLA variation in response to CO₂ ($\lambda = 0.69$; Fig. S1). The results were almost identical when using 300 imputation trees (Table 1a; Fig. S3) or adding SE for each trait to the models (Table S2a).

The patterns for Blomberg's K were very similar to those for Pagel's λ , except the K values exhibited a lower absolute magnitude (Table 1b). The three traits showed K values ranging from 0.24 to 0.71, and 0.22 to 0.80 for control and treatment, respectively (not including Am and Nm under warming, with $P > 0.05$), whereas the K values for the phenotypic variation of three traits were smaller ($K = 0.10$ – 0.25) than those under control and treatment conditions. The only differences from the λ results were the significant P -values found for variation in SLA under warming, and in Am and SLA under drought, but their K values were still very small ($K = 0.10$ – 0.13). The K values of most traits under control or treatment conditions were significantly higher than those of null distributions based on the white noise model (K_{WN}), and did not differ from the Brownian motion model (K_{BM}), suggesting that these traits were phylogenetically conserved. For SLA variation under warming and drought and Am variation under drought, K values differed significantly from both K_{WN} and K_{BM} , implying an intermediate conservatism. K values of traits and trait variation, however, did not differ from

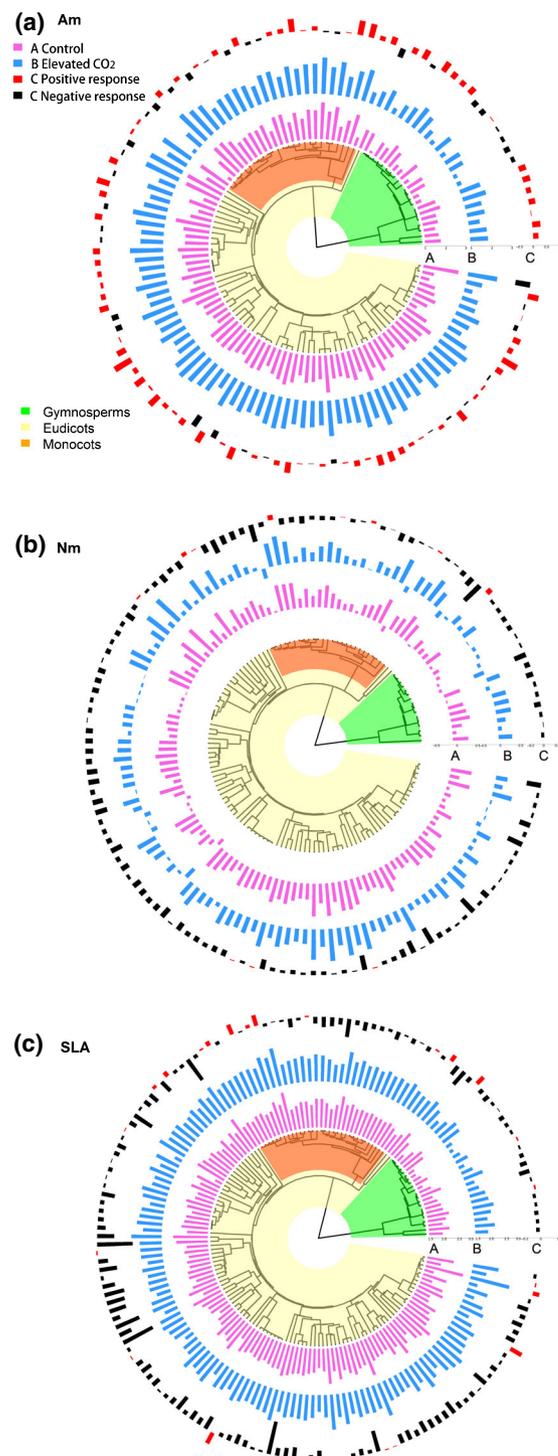


Fig. 2 Phylogenetic trees with leaf traits and their variation under elevated CO₂ treatment. Phylogenetic groups are highlighted (gymnosperms, green; eudicots, yellow; monocots, orange) with corresponding log_e-transformed trait values of (a) Am, (b) Nm and (c) SLA arranged in the following order, from inner to outer circle: control (pink bars), treatment (blue bars) and phenotypic variation (positive response, red bars; negative response, black bars). This treatment is plotted because it is the only one in which significant phylogenetic signal in phenotypic variation was found (SLA variation; Table 1), while the figures for the other three treatments are very similar. Scaled values for the same dataset are shown in Supporting Information Fig. S1. Am, mass-based net photosynthetic rate; Nm, mass-based leaf nitrogen content; SLA, specific leaf area.

Table 1 Phylogenetic signal in leaf traits and phenotypic variation. (a) Pagel's λ , P -values for $\lambda = 0$; (b) Blomberg's K , P -values for comparing K values with null distributions simulated by random tip swaps ($K > K_{\text{WN}}$) and Brownian motion evolution ($K < K_{\text{BM}}$) along the phylogeny; (c) α and phylogenetic half-life (PHL) based on the Ornstein–Uhlenbeck (OU) models.

(a) Pagel's λ (300 trees)	Warming ($n = 39, 30$ and 53 for Am, Nm and SLA)			Drought (48, 52, 75)			eCO ₂ (116, 135, 158)			Nitrogen (116, 141, 140)		
	Trait	λ	$P_{(\lambda=0)}$	λ	$P_{(\lambda=0)}$	λ	$P_{(\lambda=0)}$	λ	$P_{(\lambda=0)}$	λ	$P_{(\lambda=0)}$	
Control	Am	0.18 ± 0.000	ns	0.89 ± 0.001	***	0.41 ± 0.000	***	0.91 ± 0.000	***			
	Nm	0.04 ± 0.000	ns	0.40 ± 0.000	*	0.54 ± 0.000	***	0.58 ± 0.000	***			
	SLA	0.53 ± 0.001	**	0.54 ± 0.000	*	0.40 ± 0.000	***	0.62 ± 0.000	***			
Treatment	Am	0.18 ± 0.000	ns	0.81 ± 0.000	***	0.42 ± 0.000	***	0.85 ± 0.000	***			
	Nm	0.00 ± 0.000	ns	0.83 ± 0.000	**	0.52 ± 0.000	**	0.37 ± 0.000	**			
	SLA	0.53 ± 0.000	**	0.62 ± 0.000	*	0.37 ± 0.000	***	0.45 ± 0.000	***			
Phenotypic variation	Am	0.00 ± 0.000	ns	0.33 ± 0.000	ns	0.00 ± 0.000	ns	0.00 ± 0.000	ns			
	Nm	0.00 ± 0.000	ns	0.26 ± 0.000	ns	0.00 ± 0.000	ns	0.00 ± 0.000	ns			
	SLA	0.23 ± 0.000	ns	0.34 ± 0.000	ns	0.69 ± 0.000	***	0.00 ± 0.000	ns			

(b) Blomberg's K (300 trees; rep = 1000)	Warming (39, 30, 53)			Drought (48, 52, 75)			eCO ₂ (116, 135, 158)			Nitrogen (116, 141, 140)		
	Trait	K	P ($K > K_{\text{WN}}$)	K	P ($K > K_{\text{WN}}$)	K	P ($K > K_{\text{WN}}$)	K	P ($K > K_{\text{WN}}$)	K	P ($K > K_{\text{WN}}$)	
Control	Am	0.24 ± 0.000	ns	0.69 ± 0.002	**	0.28 ± 0.000	**	0.71 ± 0.000	**			
	Nm	0.26 ± 0.000	ns	0.41 ± 0.000	*	0.24 ± 0.000	**	0.26 ± 0.000	**			
	SLA	0.30 ± 0.000	**	0.31 ± 0.000	**	0.27 ± 0.000	**	0.34 ± 0.001	**			
Treatment	Am	0.23 ± 0.000	ns	0.80 ± 0.001	**	0.28 ± 0.001	**	0.54 ± 0.000	**			
	Nm	0.27 ± 0.000	ns	0.50 ± 0.000	**	0.23 ± 0.000	**	0.22 ± 0.000	*			
	SLA	0.29 ± 0.000	**	0.34 ± 0.000	**	0.25 ± 0.000	**	0.30 ± 0.000	**			
Phenotypic variation	Am	0.20 ± 0.000	ns	0.12 ± 0.000	**	0.16 ± 0.000	ns	0.19 ± 0.000	ns			
	Nm	0.13 ± 0.000	ns	0.10 ± 0.000	ns	0.18 ± 0.000	ns	0.19 ± 0.000	ns			
	SLA	0.10 ± 0.000	*	0.13 ± 0.000	*	0.25 ± 0.000	**	0.14 ± 0.000	ns			

(c) OU models (300 trees)	Warming (39, 30, 53)			Drought (48, 52, 75)			eCO ₂ (116, 135, 158)			Nitrogen (116, 141, 140)		
	Trait	α	PHL	α	PHL	α	PHL	α	PHL			
Control	Am	0.04 ± 0.000	16.05 ± 0.000	0.02 ± 0.000	42.65 ± 0.135	0.02 ± 0.000	32.47 ± 0.000	0.02 ± 0.000	36.89 ± 0.010			
	Nm	0.03 ± 0.000	20.31 ± 0.000	0.02 ± 0.000	28.11 ± 0.013	0.02 ± 0.002	33.11 ± 0.048	0.02 ± 0.000	39.45 ± 0.006			
	SLA	0.02 ± 0.000	31.24 ± 0.003	0.05 ± 0.001	32.65 ± 0.014	0.02 ± 0.001	32.41 ± 0.044	0.01 ± 0.000	48.15 ± 0.043			
Treatment	Am	0.05 ± 0.000	13.76 ± 0.000	0.03 ± 0.000	23.88 ± 0.077	0.02 ± 0.000	33.71 ± 0.000	0.02 ± 0.000	25.50 ± 0.008			
	Nm	0.18 ± 0.000	3.83 ± 0.000	0.01 ± 0.000	58.84 ± 0.125	0.02 ± 0.003	32.27 ± 0.088	0.03 ± 0.001	24.01 ± 0.001			
	SLA	0.02 ± 0.000	32.07 ± 0.005	0.02 ± 0.000	32.94 ± 0.019	0.02 ± 0.001	28.77 ± 0.049	0.02 ± 0.000	36.30 ± 0.016			
Phenotypic variation	Am	0.70 ± 0.000	0.99 ± 0.000	0.12 ± 0.001	5.78 ± 0.037	2.71 ± 0.000	0.26 ± 0.001	2.72 ± 0.003	0.25 ± 0.000			
	Nm	2.72 ± 0.001	0.25 ± 0.000	0.24 ± 0.002	2.97 ± 0.022	0.18 ± 0.000	3.80 ± 0.004	1.43 ± 0.000	0.48 ± 0.000			
	SLA	0.07 ± 0.000	9.91 ± 0.018	0.04 ± 0.000	19.14 ± 0.009	0.02 ± 0.000	41.98 ± 0.013	2.72 ± 0.002	0.25 ± 0.000			

Am, mass-based net photosynthetic rate; eCO₂, elevated CO₂; Nitrogen, nitrogen addition; Nm, mass-based leaf nitrogen content; SLA, specific leaf area. All the results are mean values ± SE based on 300 imputation trees. Sample sizes (n) of three traits under four manipulated environmental conditions are given in parentheses. Phenotypic variation is the response ratio between experimental treatment mean and control mean. Asterisks indicate statistical significance (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ns, not significant).

K_{WN} and were significantly lower than K_{BM} , indicating convergent or highly labile phenotypic variation. Again, the results were almost identical when using either 300 imputation trees (Table 1b) or adding the SE for each trait to the models (Table S2b).

Values of α were generally low for all three leaf traits under control conditions (mean = 0.02; range = 0.01–0.05) and under all four treatments (0.04; 0.01–0.18), while α values were higher for trait phenotypic variation (1.24; 0.04–2.72; except the only extreme value of 0.02 for SLA under elevated CO_2 , the same pattern as for λ ; Table 1c). This indicates higher adaptive evolutionary rates for trait phenotypic variation. Similarly, the phylogenetic half-life (PHL) of the three leaf traits was long under control conditions (31.12 million years (Myr); 12.65–48.15) and all four treatments (28.82; 3.83–58.84, one extreme value of 3.83 for Nm under warming), whereas the PHL of plastic responses of the three traits was quite short (4.01; 0.25–19.14; except the only extreme value of 41.98 for SLA under elevated CO_2 , the same pattern as for λ). Results across 300 imputation trees converged well, confirming higher evolutionary rates of trait variation than those of the traits themselves based on both PHL and α values (Table 1c). Thus, long PHL suggested that evolutionary history can reliably predict leaf traits under control and treatment conditions, whereas the short PHL for phenotypic variation indicated weak phylogenetic influences on current values.

We tested the agreement between the three metrics of phylogenetic signal, and found significant positive relationships between them (Fig. S4). Therefore, all of the metrics agree on the strong phylogenetic dependence of the three leaf traits, but not their variation (Fig. 1, Hypothesis 2). Because of the good agreement between these different metrics, and as λ generally outperforms K in detecting phylogenetic signal and PGLS models allow extra factors to be added, we focus here on using λ to analyze traits and their variation considering treatment strength. Results were similar after adding treatment strength into PGLS models: phylogenetic signal was found to be significantly strong for all three traits under the influence of four environmental factors, whereas phylogenetic signal was close to zero or not significantly different from $\lambda = 0$ for trait variation, except that of SLA under doubled CO_2 concentration (Table S3). This suggests that evolutionary history has little influence on the variation of these traits.

The phylogenetic influence on pair-wise trait relationships under contrasting environmental conditions

Results based on the 300 imputation trees showed very little variation (Table S4a); thus, we plotted patterns from PGLS and SMA models using the mean results. There were positive relationships between each pair of traits across all treatments, regardless of whether the relationship accounted for phylogeny or not (Fig. 3). However, the slopes of all the relationships were much flatter when controlling for phylogeny (i.e. lower gradients in PGLS models in comparison to SMA models, but still significant; Fig. 3), suggesting that evolutionary history explains some proportion of the variation in these trait relationships.

Based on the mean values across results from 300 trees, phylogenetic signal was strong in all six trait–trait relationships under drought, eCO_2 and nitrogen addition, but not under warming, for which λ values were close to zero ($P > 0.05$ for $\lambda = 0$; Table S4a). Meanwhile, R^2 values of PGLS were slightly lower than those of SMA, except Am~Nm and Nm~SLA relationships under drought (Table S4a). The changing patterns of trait relationships for both PGLS and SMA were similar, with slopes of control vs treatment being nearly equal (not different from the 1 : 1 line; Fig. S5a,b), but intercepts varied under different environmental factors (Fig. 3; Table S4a). Adding the SE of each trait into the PGLS models changed intercepts and slopes, with most slopes becoming flatter (Table S4b). PGLS with SE also showed equal slopes between control and treatment (Table S4b; Fig. S5c), indicating symmetrical trait variation for the three leaf traits.

Discussion

Using a large dataset of plant traits, spanning a substantial number of species and different growth forms, we have explored the influence of evolutionary history on plant trait responses to multiple aspects of environmental change. We have done this at an unprecedented scale (434 species) and for key adaptive plant traits that define the diversity of functional traits onto a single axis of variation. In doing so, we make a substantial contribution to the study of drivers of phenotypic variation. Our data supports Hypothesis 2: whilst leaf traits were phylogenetically conserved under ambient conditions (as has been found elsewhere: Cornwell *et al.*, 2014; Liu *et al.*, 2015) and under manipulated environmental conditions, the ability to change leaf traits was not dependent on phylogeny; this finding provides new evidence on a subject which has not been studied in depth, and for which previously published results have been contrasting (Kembel & Cahill, 2005; Godoy *et al.*, 2011; Burns & Strauss, 2012; Stotz *et al.*, 2021). We also found that the significant positive relationships between leaf trait pairs under all conditions are weakened when phylogeny is accounted for, but are still significant. Overall, this study expands the knowledge of adaptation and coordination of leaf economic traits under climate change from an evolutionary perspective.

Phylogenetic signal in leaf traits

Phylogenetic signal in Am, SLA and Nm has frequently been reported across species, mainly within one or a few families (Cavender-Bares *et al.*, 2006; Liu *et al.*, 2015), but here it was observed for three leaf traits across 71 families, under both present and predicted future climatic conditions, and using three different estimates of phylogenetic signal (Pagel's λ , Blomberg's K , and phylogenetic half-life). Many processes could lead to these results, such as intrinsic phylogenetic differences (Cornwell *et al.*, 2014) and restricted genetic variation (Prinzing *et al.*, 2001), stabilizing selection and environmental constraints (Donoghue, 2008) on these traits across species. Warming was the only treatment under which plant traits did not consistently exhibit

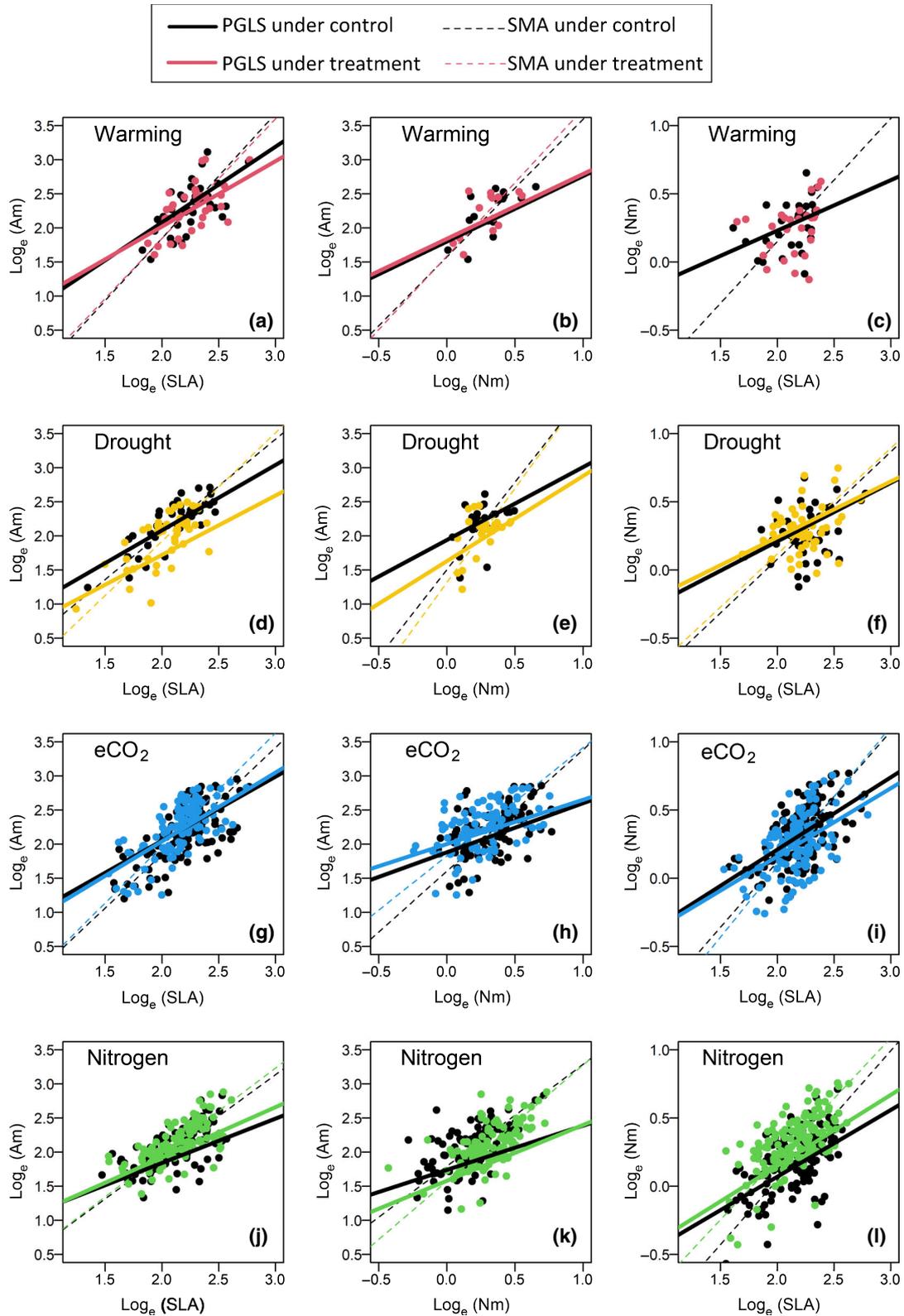


Fig. 3 Leaf trait relationships under different manipulated environmental conditions. Data from control (black dots and lines) and treatment (colored dots and lines; (a–c) warming, red; (d–f) drought, yellow; (g–i) elevated CO₂ (eCO₂), blue; and (j–l) nitrogen addition, green) conditions are modeled separately. Phylogenetic generalized least square (PGLS) models account for relatedness across species, whilst standardized major axis (SMA) models do not. Coefficients for all models are given in Supporting Information Table S4, with only the lines of significant models plotted. Am, mass-based net photosynthetic rate; Nm, mass-based leaf nitrogen content; SLA, specific leaf area.

significant phylogenetic signal: SLA was associated with phylogenetic relatedness across species ($\lambda = 0.53$), but neither Am nor Nm were. A recent study similarly found that the photosynthetic heat tolerance of a wide range of species was not phylogenetically conserved (Perez & Feeley, 2021). The ability to tolerate elevated temperatures may be better explained by other thermoregulatory traits, such as leaf size or differences in microclimate (Leigh *et al.*, 2017; Perez & Feeley, 2021). However, the findings from the other treatments (and for SLA under warming), suggest that phylogenetic relatedness can be useful in predicting variation in leaf traits in species under future climatic conditions.

The lack of phylogenetic signal in leaf phenotypic variation

Whilst leaf traits showed significant phylogenetic signal in almost all treatment conditions, the same was not true for the variation of these traits (following Hypothesis 2 in Fig. 1). The lack of phylogenetic signal in leaf phenotypic variation across a wide range of species (11 out of 12 trait–treatment combinations; Tables 1, S2), indicates that it is not phylogenetically conserved, and the main determining factor affecting the direction and strength of plant responses was environmental variation rather than genetic differences. It is still difficult to thoroughly assess whether our findings are pervasive as very few studies have investigated phylogenetic signal in trait variation (Kembel & Cahill, 2005; Godoy *et al.*, 2011; Burns & Strauss, 2012; Stotz *et al.*, 2021). However, our results were consistent with those of three out of four previous studies on the subject (Godoy *et al.*, 2011; Burns & Strauss, 2012; Stotz *et al.*, 2021), increasing the credibility of Hypothesis 2. We also expanded the number of trait types under LES, and considered more environmental factors, compared to previous studies focused on morphological traits (Godoy *et al.*, 2011), or for many traits considered together (Stotz *et al.*, 2021). Although there is still a possibility that different traits under different treatment strengths may exhibit phylogenetic signal, in this study we have advanced this topic through the analysis of a variety of traits in a substantial sample of species, which allows us overcome the problem of the limited statistical power associated with small sample sizes seen in previous studies.

It is intriguing that leaf traits showed high phylogenetic signal under both control and treatment conditions, but their variation did not (Table 1). This result could be explained in two ways. Firstly, the direction of plant trait response may be unpredictable, as found by Stotz *et al.* (2021). For our data, Am was very flexible in both directions, even though Am significantly increasing under eCO₂ overall (mean = +12.6%, range from –24.4% to +43.5%), with 64% of species responding positively and 36% negatively (randomly distributed black and red bars in Figs 2a, S1). Secondly, even if the direction of phenotypic variation is predictable under the various treatments, its strength varies dramatically, possibly as a result of different treatment strengths (Burns & Strauss, 2012). For example, although Nm decreased significantly under eCO₂ for 92% of species (mean = –16.1%), it ranged from –27.4% to +8.3% (Fig. 2b). The very small phylogenetic half-life of leaf trait variation (< 5 Myr) also indicated that the retention of information on this variation throughout

the phylogeny is relatively short-lived (Hansen, 1997; Neto-Bradley *et al.*, 2021). Furthermore, field studies without genetic control on individuals may further amplify trait variation in terms of both the direction and strength of phenotypic variation, but such within-species variation did not affect across-species patterns, because adding the SE of each trait to the models produced the same results (Table S2). Together, these mechanisms could contribute to the paucity of phylogenetic signal seen in our study, despite the traits themselves being phylogenetically structured.

The only phylogenetic signal we found in trait variation was that for SLA under elevated CO₂ (its Blomberg's *K* was not different from the BM null models, indicating high phylogenetic conservatism). This can largely be explained by the deep divergence in SLA variation between eudicots and other lineages: eudicots reduced their SLA in response to eCO₂ to a much greater extent (mean = –6.9%) than monocots (–2.1%) and gymnosperms (–2.5%, the only significant difference among the three groups found for the 12 analyses; Table S5). Differences in leaf anatomy among these groups may further explain these findings. The very low SLA of gymnosperms (mean SLA = 81.2 cm² g^{–1}) limits their ability to decrease SLA further under eCO₂. Eudicots have higher SLA values (177.8 cm² g^{–1}) and thus can respond to a much greater extent in SLA under eCO₂, and they have been found to respond more strongly than monocots (Bloor *et al.*, 2008). Monocots, however, have high SLA values (190.5 cm² g^{–1}), but their relative 'fast-living' and 'resource acquisitive' strategies keep them building 'cheap' high-SLA leaves even when more carbon is fixed (higher Am) under eCO₂. Such different allocation patterns across the three groups have also been observed in a previous meta-analysis (Poorter *et al.*, 2012).

Overall, there are no significant phylogenetic constraints on trait variation, meaning higher levels of phenotypic plasticity are not related to particular groups of closely related taxa, but instead are randomly distributed across the phylogeny. This suggests that, in the study species here, phenotypic variation is a convergent evolutionary strategy, and may be more strongly influenced by other factors, such as environmental stresses. Alternatively, although trait variation is often assumed to be adaptive, it could be neutral or nonadaptive or even maladaptive (Westerband *et al.*, 2021); thus, the genetic basis of trait variation is hard to detect based on measured phenotypes. Indeed, studies on molecular ecology have repeatedly found that phenotypic plasticity played a more important role than population genetic differences in explaining the variation of different leaf traits in populations (Ayrinhac *et al.*, 2004; Asao *et al.*, 2020), indicating that intraspecific phenotypic variation is released from population genetics. Consequently, phenotypic variation across species may also have a small probability of being phylogenetically dependent. In addition, both biotic and abiotic environmental changes affect the direction and strength of trait variation, making short-term ecological responses hard to predict from phylogeny (Cadotte *et al.*, 2017). For example, a study on 12 plant species found a significant phylogenetic signal in a trait (root : shoot ratio) only under competition, but not under control conditions (Burns & Strauss, 2012), implying that more stressful or disturbed environments could reveal a greater expression of phylogenetic

differences in traits and facilitate species co-existence (Swenson & Enquist, 2009; Prinzing *et al.*, 2021).

Phylogeny weakened but did not affect patterns of conventional relationships under climate change

The obviously flatter slopes of PGLS than SMA models emphasized the fact that phylogeny should be considered when interpreting leaf trait relationships (Fig. 3). The ecophysiological meanings of trait relationships and their responses to climate change have been discussed in an earlier study (Cui *et al.*, 2020), so here we focused on their phylogenetic implications. In theory, divergent evolution was stronger among large lineages than among descendants within them, which would weaken the conventional relationships (Felsenstein, 1985). In agreement with this, we did find that gymnosperms, monocots and eudicots were three main lineages with contrasting trait values (Table S5), leading to strong phylogenetic signal in traits and their relationships (Tables 1, S4). The flatter slopes of phylogenetic models have been observed before, based on either phylogenetic independent contrast (PIC) values (Ackerly & Donoghue, 1998; Ma *et al.*, 2018) or PGLS (Liu *et al.*, 2015). These findings support the generality of coordination and trade-offs among LES traits (Wright *et al.*, 2004), even within a phylogenetic structure.

Models of trait relationships with or without phylogeny exhibited similar patterns of change under different environmental factors (Fig. 3). This consistency was important in predicting plant responses to climate change, because trait coordination affects plant growth and adaptive strategies (Westoby *et al.*, 2002; Cornelissen *et al.*, 2003; Diaz *et al.*, 2004; Kattge *et al.*, 2020). For example, at a given SLA, Am decreased under drought (Fig. 3d) and increased with nitrogen addition (Fig. 3j), indicating that future productivity might decrease under drought but increase with nitrogen deposition at the same cost of leaf carbon investment, and such responses are evolutionary co-related and predictable.

Conclusion

Phylogenetic signal exists in key plant traits but not in their variation, demonstrating that different trait values across species reflect intrinsic evolutionary differences, whereas trait variation within species represents short-term adaptations that are largely shaped by environmental constraints. Phylogeny was also important in relationships between leaf traits, with the slopes of trait relationships being much flatter when phylogeny was accounted for, emphasizing the co-evolved trait pairs and their potential in predicting plant responses under changing climate. Therefore, although phylogeny cannot be used to predict phenotypic variation, it is still important for the detection of lineages rich in species showing similar traits, and for the tracing of general patterns of trait relationships across species in future environments.

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Competing interests

The authors declare no conflict of interest.

Author contributions

HL and QY designed the study. EC, JX collected data. HL and KJS performed the analyses. HL drafted the first manuscript and all authors contributed substantially to revisions.

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Data availability

The full dataset for this study is given in Table S1. All R code used in this study is available in Notes S2.

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Supporting Information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

Fig. S1 Original and scaled values of three leaf traits across phylogenetic trees for the elevated CO₂ experiments.

Fig. S2 Responses of leaf traits to different treatment strengths and duration.

Fig. S3 Histograms of Pagel’s λ values from 300 phylogenetic trees.

Fig. S4 Relationships between three metrics of phylogenetic signal.

Fig. S5 Comparisons of slope values of trait relationships between control and treatment groups based on standardized major axis and phylogenetic generalized least square models.

Notes S1 The 300 imputation trees used in this study.

Notes S2 R code used in this study.

Table S1 Full dataset for this study.

Table S2 Phylogenetic signal in leaf traits and their variation considering the SE of each trait.

Table S3 Phylogenetic signal in leaf traits and their variation considering treatment strength.

Table S4 Coefficients of phylogenetic generalized least square and standardized major axis models for pair-wise trait relationships.

Table S5 Comparisons of leaf traits and their variation across early divergent lineages.

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