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Liu, H., Ye, Q., Simpson, K.J. orcid.org/0000-0001-6673-227X et al. (2 more authors) (2022) Can evolutionary history predict plant plastic responses to climate change? New Phytologist, 235 (3). pp. 1260-1271. ISSN 0028-646X

https://doi.org/10.1111/nph.18194

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Article type : Full paper

Can evolutionary history predict plant plastic responses to climate change?

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Received: *3 December 2021*
Accepted: *24 April 2022*

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Summary

- 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) of 434 species and their variation under four environmental change scenarios (warming, drought, elevated CO_2, or nitrogen addition) 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 but did not change directions of conventional pairwise trait relationships, suggesting the co-evolved leaf trait pairs have consistent responses across 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.

Key words: climate change, leaf nitrogen content, photosynthetic rate, phylogenetic signal, phenotypic variation, specific leaf area.

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 ~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 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 with phylogenetic signal seen for a trait but only 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 imperatively 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 (Am, carbon assimilation per unit leaf mass) and resource acquisition, but at the cost of longevity (Reich, 2014). At the other end of the spectrum is longer-lived species that invest highly in leaf material, and are thus associated with low SLA, Nm and Am. LES 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 way that plants optimize their performance in a rapidly-changing environment (*e.g.*, 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 the production of coordinated evolution (Garland *et al.*, 1992). Thus testing how the pair-wise trait relationships shift within a phylogenetic context could reveal information of 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, allow 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, to different aspects of climate change, such as elevated temperatures (Shao *et al.*, 2019) and CO_2 concentrations (Temme *et al.*, 2017). Recent work has summarized 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 CO_2, and nitrogen addition. By combining this dataset with a phylogeny of the study species, we aim to test for: (1) Phylogenetic signal in leaf traits and their variation under altered environmental conditions, and (2) phylogenetic influences on pair-wise trait relationships (*e.g.*, the relationship between SLA and Am across species). We propose three hypotheses that could describe and explain various phylogenetic patterns in traits and their variation (Fig. 1).

For question (1), we predict 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 expect hypothesis 3 has small probability to be supported, because many plant traits in natural conditions show significant phylogenetic signal (*e.g.*, 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 question (2), we predict that pair-wise trait relationships will weaken based on other coordinated leaf traits, 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, cm^2 g^{-1}), and mass-based leaf nitrogen content (Nm, %), from experiments that mimic four aspects of climate change (warming, drought, elevated CO_2, 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. In Cui *et al.* (2020), studies were classified into field experiments (garden or natural habitat experiments; 102 studies) and environmentally controlled experiments (greenhouse, 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 C_3 vs. C_4 species) were all tested separately and showed consistent patterns with very few exceptions (Fig. S4-S7 in Cui *et al.*, 2020). Therefore, to focus on phylogenetic questions and guarantee enough species numbers to validate 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 CO_2 experiments with lower CO_2 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 Table S1.

We transformed all data by natural logarithm prior to analysis to homogenize variance. We then characterized phenotypic variation as the ln-transformed response ratio (RR). RR is calculated as $\ln(RR)=\ln(T_t)-\ln(T_c)$, where T_t and T_c are the experimental treatment mean and control mean, respectively (Hedges *et al.*, 1999). All the response ratios of leaf traits to the four treatments were normally distributed (Fig. 2 in Cui *et al.*, 2020). For all the 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 involve "study" as a factor into the following models. Next we added standard errors (SE) 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 did phylogenetic analyses on both datasets here to account for trait variation in case it may affect phylogenetic signal and phylogenetic pair-wised trait relationships.

Although the original studies were not designed to test phylogenetic hypotheses, our dataset incorporates large plant species diversity, which has much stronger 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 of the 434 species using the package *V.PhyloMaker* (Jin & Qian, 2019) in the R language and environment (R Core Team, 2018). The mega-tree implemented 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 half-way point of the genus branch, which originated from the software 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 in Smith & Brown (2018), using *multi2di* in the R package *ape* (Paradis *et al.*, 2004). Finally, with the 300 imputation trees, we repeated all the following phylogenetic analyses 300 times, in order to account for phylogenetic uncertainties. Furthermore, the ideal phylogeny is certainly a tree based on molecular information of each species, thus for future comparative analyses, care should be taken in constructing phylogeny with missing or unsolved species and interpreting results. All 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 λ=1 implies trait variation completely depends on phylogeny, while λ=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.*, 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 such as *picante* because *phylosig* allows standard error (SE) in its formula, and can calculate both Pagel's λ and Blomberg's K by setting different methods (Table S2). Values of 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). K values are 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 is 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 are obtained for comparing K values with those 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). PHL equals 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)/standard deviation], in order to avoid the influences from 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 the traits under all 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 elevations and slopes (Table S4a), thus figures of trait relationships were plotted based on one tree results. Furthermore, we added standard error (SE) of each trait pairs into 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 their SLA (+6.9%; hereinafter ‘significantly affect’ means $P<0.05$, whereas ‘not affect’ means $P>0.05$), but not in Am and Nm (+1.2% and -1.3%, respectively). Drought significantly decreased Am (-38.3%) and SLA (-8.7%), and increased Nm (+6.5%). Elevated CO_2 significantly increased Am (+12.6%), while 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 (55%, 53% and 66% positive responses for Am, Nm and SLA, respectively), and responses of Am and SLA under nitrogen addition similarly varied (65%, 92% and 53% positive responses for Am, Nm and SLA, respectively). In contrast, response directions to drought were more certain (9%, 62% and 28% positive responses for Am, Nm and SLA, respectively), as were responses to eCO_2 (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 present in almost all leaf traits under control conditions for the different environmental factors (10 out of 12 trait-treatment combinations; λ=0.40-0.91; Table 1a). The only exceptions were Am and Nm in control plants 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 (λ=0.62-0.83), elevated CO_2 (λ=0.37-0.52) and nitrogen addition (λ=0.37-0.85), but only in SLA under warming conditions (λ=0.53; Table 1a). In 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 CO_2 (λ=0.69; Fig. S1). Results stayed the same based on either 300 imputation trees (Table 1a; Fig. S3) or adding standard error (SE) of each trait in the models (Table S2a).

Phylogenetic patterns of Blomberg's *K* were very similar as those of Pagel's λ, except the lower absolute magnitude of *K* values (Table 1b). The three traits showed *K* values ranging from 0.24-0.71, and 0.22-0.80 for control and treatment, respectively (not including Am and Nm under warming with $P>0.05$), whereas *K* values of phenotypic variation of three traits were smaller (K=0.10-0.25) than those under control and treatment conditions. The only differences from λ results were significant *P* values 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). *K* values of most traits under control or treatment conditions were significantly higher than 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. Whereas *K* values of traits and trait variation did not differ from K_{WN} and were significantly lower than K_{BM}, indicating convergent or highly labile phenotypic variation. Again, results were nearly the same based on either 300 imputation trees (Table 1b) or adding standard error (SE) of each trait in the models (Table S2b).

Values of α, based on OU models, were generally low for all the three leaf traits under control conditions (mean=0.02; range=0.01-0.05) and under the 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 0.02 of SLA under elevated CO_2, the same pattern as for λ; Table 1c). This indicates higher adaptive evolutionary rates for trait phenotypic variation. Similarly, phylogenetic half-life (PHL) of all the three leaf traits was long under control conditions (31.12 Myr; 12.65-48.15) and the four treatments (28.82; 3.83-58.84, one extreme value of 3.83 for Nm under warming), whereas PHL of plastic responses of the three traits was quite short (4.01; 0.25-19.14; except the only extreme value 41.98 of 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 well predict leaf traits under control and treatment conditions, whereas short PHL of 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 agree on the strong phylogenetic dependences of the three leaf traits, but not their variation (Fig 1 Hypothesis 2). Because of the good agreement of these different metrics, and as λ generally outperforms *K* in detecting phylogenetic signal and PGLS allows adding factors into models, we focus here on using λ to analyze traits and their variation considering treatment strength. Similar results were found that all the phylogenetic signal of the three traits under four environmental factors became significantly strong, whereas phylogenetic signal of trait variation was near 0 or not significantly different from λ=0, except that of SLA under doubled CO_2 concentration (Table S3). This suggests that evolutionary history has little influence on 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 using the mean result. 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 evolutionary history explains a proportion of 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 none were under warming with λ values near zero (P>0.05 for λ=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 versus treatment being nearly equal (not different from the 1:1 line; Fig. S5ab), but intercepts varied under different environmental factors (Fig. 3; Table S4a). Adding the standard error (SE) of each trait into PGLS models changed intercepts and slopes, with most slopes becoming flatter (Table S4b). PGLS with SE also showed equal slopes between control versus treatment (Table S4b; Fig. S5c), indicating a symmetrical trait variation of the three leaf traits.

Discussion

Using a large dataset of plant traits, spanning a substantial number of species, and different growth forms, we explore the influence of evolutionary history on plant trait responses to multiple aspects of environmental change. We do this at an unprecedented scale (434 species) and on 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 that, whilst leaf traits were phylogenetically conserved under ambient conditions (as has been found elsewhere: Cornwell *et al.* (2014) and Liu *et al.* (2015)) and under manipulated environmental conditions, the ability to change leaf traits was not dependent on phylogeny, adding new evidence to the previous few and contrasting results (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 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 been frequently reported across species mainly within one or a few families (Cavender-Bares *et al.*, 2006; Liu *et al.*, 2015), but here we show they exist in the three leaf traits widely across 71 families, both under present and future predicted 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 the results, such as the intrinsic phylogenetic differences (Cornwell *et al.*, 2014) and restricted genetic variation (Prinzing *et al.*, 2001), stabilizing selection and environmental constraint (Donoghue, 2008) on these traits across species. Warming was the only treatment under which plant traits did not consistently exhibit significant phylogenetic signal: SLA was associated with phylogenetic relatedness across species (λ=0.53), but neither Am or 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), suggests 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; Table 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 had previously investigated phylogenetic signal in trait variation (Kembel & Cahill, 2005; Godoy *et al.*, 2011; Burns & Strauss, 2012; Stotz *et al.*, 2021). However, our results tend to support three out of four previous studies (Godoy *et al.*, 2011; Burns & Strauss, 2012; Stotz *et al.*, 2021), increasing the credibility of hypothesis 2. We also expanded 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 possibility that different traits under different treatment strengths may exhibit phylogenetic signal, we advance this topic by analyzing traits in a substantial sample of species, which allows us overcome problems of limited statistical power associated with small sample sizes in previous studies.

It is intriguing that leaf traits showed high phylogenetic signal under both control and treatment conditions, but that 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 in Stotz *et al.* (2021). For our data, Am was very flexible in both directions even through Am significantly increasing under eCO_2 overall (mean=+12.6%, range from -24.4% to +43.5%), with 64% species response positively and 36% negatively (randomly distributed black and red bars in Fig. 2a; Fig. S1). Secondly, even if the direction of phenotypic variation is predictable under treatments, its strength varies dramatically, possibly resulting from different treatment strengths (Burns & Strauss, 2012). For example, although Nm of 92% species decreased significantly under eCO_2 (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 both direction and strength of phenotypic variation, but such within-species variation did not affect across-species patterns, because adding the standard error (SE) of each trait into 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 CO_2 (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 eCO_2 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 cm^2 g^{-1}) limit their ability to decrease SLA further under eCO_2. Eudicots have higher SLA values (177.8 cm^2 g^{-1}) and thus can respond to a much greater extent in SLA under eCO_2, and have been found responding stronger than monocots (Bloor *et al.*, 2008). Monocots, however, have high SLA values (190.5 cm^2 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 eCO_2. 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 non-adaptive 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 continuously 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 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 lower slopes of PGLS than SMA models emphasized that phylogeny should be considered in interpreting leaf trait relationships (Fig. 3). The eco-physiological meanings of trait relationships and their responses to climate change have been discussed in 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 high phylogenetic signal in traits and their relationships (Table 1; Table S4). The flatter slopes of phylogenetic models have been observed before, either based on 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 on trait relationships with or without phylogeny exhibited similar changing patterns 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 reflects intrinsic evolutionary differences, whereas trait variation within species represents short-term adaptions 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 in detecting lineages rich in species showing similar traits, and tracing general patterns of trait relationships across species in future environments.

Acknowledgements

We thank the editor and reviewers for their constructive suggestions and comments on earlier versions of this manuscript. This work was supported by the National Natural Science Foundation of China (31825005 and 31670411), Key Special Project for Introduced Talents Team of Southern Marine Science and Engineering Guangdong Laboratory (Guangzhou) (GML2019ZD0408), Institution of South China Sea Ecology and Environmental Engineering, Chinese Academy of Sciences (ISEE2020YB01), and the Youth Innovation Promotion Association of the Chinese Academy of Sciences (2019339). KJS was supported by Natural Environment Research Council grant NE/T000759/1. 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.

Data availability

Full dataset of this study is available in supplementary Table S1. All the R codes used in this study are available in Notes S2.

References

Ackerly DD. **2009**. Conservatism and diversification of plant functional traits: Evolutionary rates versus phylogenetic signal. *Proceedings of the National Academy of Sciences* **106**: 19699–19706.

Ackerly DD, Donoghue MJ. **1998**. Leaf size, sapling allometry, and Corner's rules: phylogeny and correlated evolution in maples (*Acer*). *The American Naturalist* **152**: 767–791.

Asao S, Hayes L, Aspinwall MJ, Rymer PD, Blackman C, Bryant CJ, Cullerne D, Egerton JJG, Fan Y, Innes P, *et al.* 2020. Leaf trait variation is similar among genotypes of *Eucalyptus camaldulensis* from differing climates and arises in plastic responses to the seasons rather than water availability. *New Phytologist* **227**: 780–793.

Ashton KG. **2004**. Comparing phylogenetic signal in intraspecific and interspecific body size datasets. *Journal of Evolutionary Biology* **17**: 1157–1161.

Ayrinhac A, Debat V, Gibert P, Kister AG, Legout H, Moreteau B, Vergilino R, David JR. **2004**. Cold adaptation in geographical populations of *Drosophila melanogaster*: Phenotypic plasticity is more important than genetic variability. *Functional Ecology* **18**: 700–706.

Blomberg SP, Garland T, Ives AR. **2003**. Testing for phylogenetic signal in comparative data: behavioral traits are more labile. *Evolution* **57**: 717–745.

Bloor JMG, Barthes L, Leadley PW. **2008**. Effects of elevated CO_2 and N on tree-grass interactions: an experimental test using *Fraxinus excelsior* and *Dactylis glomerata*. *Functional Ecology* **22**: 537–546.

Borges RM. **2009**. Phenotypic plasticity and longevity in plants and animals: cause and effect? *Journal of Biosciences* **34**: 605–611.

Burns JH, Strauss SY. **2012**. Effects of competition on phylogenetic signal and phenotypic plasticity in plant functional traits. *Ecology* **93**: S126–S137.

Cadotte MW, Davies TJ, Peres-Neto PR. **2017**. Why phylogenies do not always predict ecological differences. *Ecological Monographs* **87**: 535–551.

Cavender-Bares J, Keen A, Miles B. **2006**. Phylogenetic structure of Floridian plant communities depends on taxonomic and spatial scale. *Ecology* **87**: S109–S122.

Chevin L-M, Lande R, Mace GM. **2010**. Adaptation, Plasticity, and Extinction in a Changing Environment: Towards a Predictive Theory. *PLoS Biology* **8**: e1000357.

Cornelissen JHC, Lavorel S, Garnier E, Díaz S, Buchmann N, Gurvich DE, Reich PB, Steege H ter, Morgan HD, Heijden MGA van der, *et al.* 2003. A handbook of protocols for standardised and easy measurement of plant functional traits worldwide. *Australian Journal of Botany* **51**: 335.

Cornwell WK, Westoby M, Falster DS, FitzJohn RG, O'Meara BC, Pennell MW, McGlinn DJ, Eastman JM, Moles AT, Reich PB, *et al.* 2014. Functional distinctiveness of major plant lineages. *Journal of Ecology* **102**: 345–356.

Crisp MD, Arroyo MTK, Cook LG, Gandolfo MA, Jordan GJ, McGlone MS, Weston PH, Westoby M, Wilf P, Linder HP, *et al.* 2009. Phylogenetic biome conservatism on a global scale. *Nature* **458**: 754–756.

Cui E, Weng E, Yan E, Xia J. **2020**. Robust leaf trait relationships across species under global environmental changes. *Nature Communications* **11**: 2999.

Diaz S, Hodgson JG, Thompson K, Cabido M, Cornelissen JHC, Jalili A, Montserrat ‐ Martí G, Grime JP, Zarrinkamar F, Asri Y, *et al.* 2004. The plant traits that drive ecosystems: Evidence from three continents. *Journal of Vegetation Science* **15**: 295–304.

Díaz S, Kattge J, Cornelissen JHC, Wright IJ, Lavorel S, Dray S, Reu B, Kleyer M, Wirth C, Colin Prentice I, *et al.* 2016. The global spectrum of plant form and function. *Nature* **529**: 167–171.

Donoghue MJ. **2008**. A phylogenetic perspective on the distribution of plant diversity. *Proceedings of the National Academy of Sciences of the United States of America* **105**: 11549–11555.

Felsenstein J. **1985**. Phylogenies and the Comparative Method. *The American Naturalist* **125**: 1–15.

Flores O, Garnier E, Wright IJ, Reich PB, Pierce S, Dìaz S, Pakeman RJ, Rusch GM, Bernard-Verdier M, Testi B, *et al.* 2014. An evolutionary perspective on leaf economics: Phylogenetics of leaf mass per area in vascular plants. *Ecology and Evolution* **4**: 2799–2811.

Freckleton RP, Harvey PH, Pagel M, Freckleton, Harvey, Pagel. **2002**. Phylogenetic analysis and comparative data: a test and review of evidence. *The American Naturalist* **160**: 712–726.

De Frenne P, Rodríguez-Sánchez F, De Schrijver A, Coomes DA, Hermy M, Vangansbeke P, Verheyen K. **2015**. Light accelerates plant responses to warming. *Nature Plants* **1**: 15110.

Garland T, Harvey PH, Ives AR. **1992**. Procedures for the Analysis of Comparative Data Using Phylogenetically Independent Contrasts. *Systematic Biology* **41**: 18–32.

Godoy O, Valladares F, Castro-Díez P. **2011**. Multispecies comparison reveals that invasive and native plants differ in their traits but not in their plasticity. *Functional Ecology* **25**: 1248–1259.

Hansen TF. **1997**. Stabilizing selection and the comparative analysis of adaptation. *Evolution* **51**: 1341–1351.

Hedges L V., Gurevitch J, Curtis PS. **1999**. The Meta-Analysis of Response Ratios in Experimental Ecology. *Ecology* **80**: 1150–1156.

Huang M, Piao S, Ciais P, Peñuelas J, Wang X, Keenan TF, Peng S, Berry JA, Wang K, Mao J, *et al.* **2019**. Air temperature optima of vegetation productivity across global biomes. *Nature Ecology and Evolution* **3**: 772–779.

Jin Y, Qian H. **2019**. V.PhyloMaker: an R package that can generate very large phylogenies for vascular plants. *Ecography* **42**: 1353–1359.

Kattge J, Bönisch G, Díaz S, Lavorel S, Prentice IC, Leadley P, Tautenhahn S, Werner GDA, Aakala T, Abedi M, *et al.* **2020**. TRY plant trait database – enhanced coverage and open access. *Global Change Biology* **26**: 119–188.

Kembel SW, Cahill JF. **2005**. Plant phenotypic plasticity belowground: A phylogenetic perspective on root foraging trade-offs. *American Naturalist* **166**: 216–230.

Leigh A, Sevanto S, Close JD, Nicotra AB. **2017**. The influence of leaf size and shape on leaf thermal dynamics: does theory hold up under natural conditions? *Plant Cell and Environment* **40**: 237–248.

Liu H, Xu Q, He P, Santiago LS, Yang K, Ye Q. **2015**. Strong phylogenetic signals and phylogenetic niche conservatism in ecophysiological traits across divergent lineages of Magnoliaceae. *Scientific Reports* **5**: 12246.

Losos JB. **2008**. Phylogenetic niche conservatism, phylogenetic signal and the relationship between phylogenetic relatedness and ecological similarity among species. *Ecology Letters* **11**: 995–1003.

Ma Z, Guo D, Xu X, Lu M, Bardgett RD, Eissenstat DM, McCormack ML, Hedin LO. **2018**. Evolutionary history resolves global organization of root functional traits. *Nature* **555**: 94–97.

Des Marais DL, Hernandez KM, Juenger TE. **2013**. Genotype-by-Environment Interaction and Plasticity: Exploring Genomic Responses of Plants to the Abiotic Environment. *Annual Review of Ecology, Evolution, and Systematics* **44**: 5–29.

Moran E V., Hartig F, Bell DM. **2016**. Intraspecific trait variation across scales: implications for understanding global change responses. *Global Change Biology* **22**: 137–150.

Neto‐Bradley BM, Whitton J, Lipsen LPJ, Pennell MW. **2021**. Macroevolutionary history predicts flowering time but not phenological sensitivity to temperature in grasses. *American Journal of Botany* **108**: 893–902.

Nicotra AB, Atkin OK, Bonser SP, Davidson AM, Finnegan EJ, Mathesius U, Poot P, Purugganan MD, Richards CL, Valladares F, *et al.* 2010. Plant phenotypic plasticity in a changing climate. *Trends in Plant Science* **15**: 684–692.

Orme D, Freckleton R, Thomas G, Petzoldt T, Fritz S, Isaac N, Pearse W. **2018**. The caper package: comparative analysis of phylogenetics and evolution in R. Verion 1.0.1 https://CRAN.R-project.org/package=caper.

Pagel M. **1999**. Inferring the historical patterns of biological evolution. *Nature* **401**: 877–884.

Paradis E, Claude J, Strimmer K. **2004**. APE: analyses of phylogenetics and evolution in R language. *Bioinformatics* **20**: 289–290.

Pennell MW, Eastman JM, Slater GJ, Brown JW, Uyeda JC, FitzJohn RG, Alfaro ME, Harmon LJ. **2014**. geiger v2.0: an expanded suite of methods for fitting macroevolutionary models to phylogenetic trees. *Bioinformatics* **30**: 2216–2218.

Perez TM, Feeley KJ. **2021**. Weak phylogenetic and climatic signals in plant heat tolerance. *Journal of Biogeography* **48**: 91–100.

Poorter H, Niinemets Ü, Poorter L, Wright IJ, Villar R. **2009**. Causes and consequences of variation in leaf mass per area (LMA): a meta‐analysis. *New Phytologist* **182**: 565–588.

Poorter H, Niklas KJ, Reich PB, Oleksyn J, Poot P, Mommer L. **2012**. Biomass allocation to leaves, stems and roots: meta-analyses of interspecific variation and environmental control. *New Phytologist* **193**: 30–50.

Prinzing A, Durka W, Klotz S, Brandl R. **2001**. The niche of higher plants: evidence for phylogenetic conservatism. *Proceedings of the Royal Society B: Biological Sciences* **268**: 2383–2389.

Prinzing A, Pavoine S, Jactel H, Hortal J, Hennekens SM, Ozinga WA, Bartish I V., Helmus MR, Kühn I, Moen DS, *et al.* 2021. Disturbed habitats locally reduce the signal of deep evolutionary history in functional traits of plants. *New Phytologist* **232**: 1849–1862.

R Core Team. **2018**. R: A language and environment for statistical computing (version 3.5.1). R Foundation for Statistical Computing, Vienna, Austria.

Reich PB. **2014**. The world-wide 'fast–slow' plant economics spectrum: a traits manifesto. *Journal of Ecology* **102**: 275–301.

Reich PB, Walters MB, Ellsworth DS. **1997**. From tropics to tundra: Global convergence in plant functioning. *Proceedings of the National Academy of Sciences of the United States of America* **94**: 13730–13734.

Reich PB, Wright IJ, Cavender-Bares J, Craine JM, Oleksyn J, Westoby M, Walters MB. **2003**. The evolution of plant functional variation: traits, spectra, and strategies. *International Journal of Plant Sciences* **164**: S143–S164.

Relyea RA, Stephens PR, Barrow LN, Blaustein AR, Bradley PW, Buck JC, Chang A, Collins JP, Crother B, Earl J, *et al.* 2018. Phylogenetic patterns of trait and trait plasticity evolution: Insights from amphibian embryos. *Evolution* **72**: 663–678.

Revell LJ. **2012**. phytools: an R package for phylogenetic comparative biology (and other things). *Methods in Ecology and Evolution* **3**: 217–223.

Shao J, Yuan T, Li Z, Li N, Liu H, Bai SH, Xia J, Lu M, Zhou X. **2019**. Plant evolutionary history mainly explains the variance in biomass responses to climate warming at a global scale. *New Phytologist* **222**: 1338–1351.

Smith SA, Brown JW. **2018**. Constructing a broadly inclusive seed plant phylogeny. *American Journal of Botany* **105**: 302–314.

Snell-Rood EC, Kobiela, ME, Sikkink, KL, Shephard AM. **2018**. Mechanisms of Plastic Rescue in Novel Environments. *Annual Review of Ecology, Evolution, and Systematics* **49**: 331–354.

Song J, Wan S, Piao S, Knapp AK, Classen AT, Vicca S, Ciais P, Hovenden MJ, Leuzinger S, Beier C, *et al.* 2019. A meta-analysis of 1,119 manipulative experiments on terrestrial carbon-cycling responses to global change. *Nature Ecology and Evolution* **3**: 1309–1320.

Stotz GC, Salgado‐Luarte C, Escobedo VM, Valladares F, Gianoli E. **2021**. Global trends in phenotypic plasticity of plants. *Ecology Letters* **24**: 2267–2281.

Swenson NG, Enquist BJ. **2009**. Opposing assembly mechanisms in a Neotropical dry forest: implications for phylogenetic and functional community ecology. *Ecology* **90**: 2161–2170.

Temme AA, Liu JC, van Hal J, Cornwell WK, Cornelissen JHC, Aerts R. **2017**. Increases in CO_2 from past low to future high levels result in "slower" strategies on the leaf economic spectrum. *Perspectives in Plant Ecology, Evolution and Systematics* **29**: 41–50.

Warton DI, Duursma RA, Falster DS, Taskinen S. **2012**. smatr 3-an R package for estimation and inference about allometric lines. *Methods in Ecology and Evolution* **2**: 257–259.

Webb CO, Ackerly DD, Kembel SW. **2008**. Phylocom: software for the analysis of phylogenetic community structure and trait evolution. *Bioinformatics* **24**: 2098–2100.

Westerband AC, Funk JL, Barton KE. **2021**. Intraspecific trait variation in plants: a renewed focus on its role in ecological processes. *Annals of Botany* **127**: 397–410.

Westoby M, Falster DS, Moles AT, Vesk PA, Wright IJ, Falster DS, Westoby M, Wright IJ, Vesk PA. **2002**. Plant ecological strategies: some leading dimensions of variation between species. *Annual Review of Ecology and Systematics* **33**: 125–159.

Wiens JJ. **2004**. Speciation and ecology revisited: phylogenetic niche conservatism and the origin of species. *Evolution* **58**: 193–197.

Wright IJ, Reich PB, Westoby M, Ackerly DD, Baruch Z, Bongers F, Cavender-Bares J, Chapin T, Cornelissen JHC, Diemer M, *et al.* 2004. The worldwide leaf economics spectrum. *Nature* **428**: 821–827.

Zanne AE, Tank DC, Cornwell WK, Eastman JM, Smith SA, FitzJohn RG, McGlinn DJ, O'Meara BC, Moles AT, Reich PB, *et al.* 2014. Three keys to the radiation of angiosperms into freezing environments. *Nature* **506**: 89–92.

Supplemental Information

The following Supporting Information is available for this article:

Notes S1 300 imputation trees used in this study.

Notes S2 R codes in this study.

Fig. S1 Original and scaled values of three leaf traits across phylogenetic trees for the elevated CO_2 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 SMA and PGLS models.

Table S1 Full dataset in this study (provided in a separated excel file).

Table S2. Phylogenetic signal in leaf traits and their variation considering standard errors of each trait.

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

Table S4 Coefficients of PGLS and SMA models on pair-wise trait relationships.

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

Figure 1. A schematic diagram and hypotheses demonstrating 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 the trait of 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 those more distantly-related (*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.

Figure 2. Phylogenetic trees with leaf traits and their variation under elevated CO_2 treatment. Phylogenetic groups are highlighted (gymnosperms, green; eudicots, yellow; monocots, orange) with corresponding ln-transformed trait values of (a) Am, (b) Nm and (c) SLA arranged in the order of control (purple 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 is found (SLA variation; Table 1), while figures for other three treatments are very similar. Scaled values of the same dataset are shown in Supporting Information Fig. S1. Am, mass-based net photosynthetic rate; SLA, specific leaf area; Nm, mass-based leaf nitrogen content.

Figure 3. Leaf trait relationships under different manipulated environmental factors. Data from control (black dots and lines) and treatment (colored dots and lines; a-c, warming, red; d-f, drought, yellow; g-i, elevated CO_2 (eCO_2), blue; and j-l, nitrogen addition, green) 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 in Supporting

Information Table S4, with only lines of significant models plotted. Am, mass-based net photosynthetic rate; SLA, specific leaf area; Nm, mass-based leaf nitrogen content.

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 ($K>K_{\mathrm{WN}}$) and Brownian motion evolution ($K<K_{\mathrm{BM}}$) along the phylogeny; (c) α and phylogenetic half-life (PHL) based on the Ornstein–Uhlenbeck (OU) models.

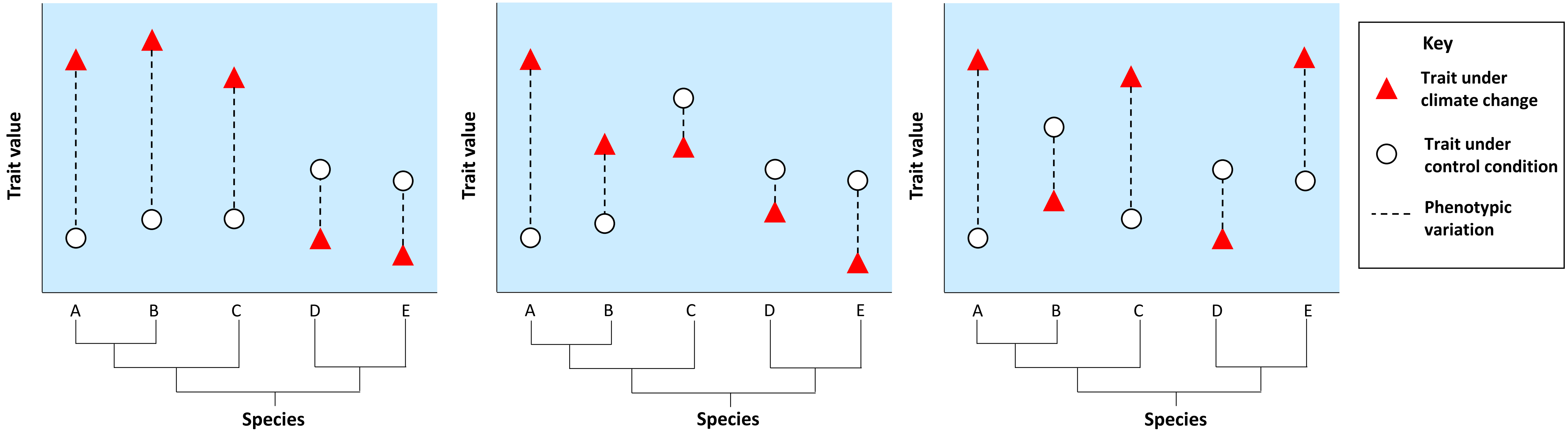
(a) Pagel's λ (300 trees)		Warming (n=39, 30 and 53 for Am, Nm and SLA)		Drought (48, 52, 75)		eCO$_2$ (116, 135, 158)		Nitrogen (116, 141, 140)	
	Trait	λ	$P_{(\lambda=0)}$	λ	$P_{(\lambda=0)}$	λ	$P_{(\lambda=0)}$	λ	$P_{(\lambda=0)}$
Control	Am	0.18±0.000	ns	0.89±0.001	***	0.41±0.000	***	0.91±0.000	***
	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* ($K>K_{\mathrm{WN}}$)	*P* ($K<K_{\mathrm{BM}}$)	*K*	*P* ($K>K_{\mathrm{WN}}$)	*P* ($K<K_{\mathrm{BM}}$)	*K*	*P* ($K>K_{\mathrm{WN}}$)	*P* ($K<K_{\mathrm{BM}}$)	*K*	*P* ($K>K_{\mathrm{WN}}$)	*P* ($K<K_{\mathrm{BM}}$)
Control	Am	0.24±0.000	ns	*	0.69±0.002	**	ns	0.28±0.000	**	ns	0.71±0.000	**	ns
	Nm	0.26±0.000	ns	*	0.41±0.000	*	ns	0.24±0.000	**	ns	0.26±0.000	**	ns
	SLA	0.30±0.000	**	ns	0.31±0.000	*	ns	0.27±0.000	**	ns	0.34±0.001	**	ns
Treatment	Am	0.23±0.000	ns	*	0.80±0.001	**	ns	0.28±0.001	**	ns	0.54±0.000	**	ns
	Nm	0.27±0.000	ns	*	0.50±0.000	**	ns	0.23±0.000	**	ns	0.22±0.000	*	ns
	SLA	0.29±0.000	**	ns	0.34±0.000	**	ns	0.25±0.000	**	ns	0.30±0.000	**	ns
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	**	ns	0.14±0.000	ns	*

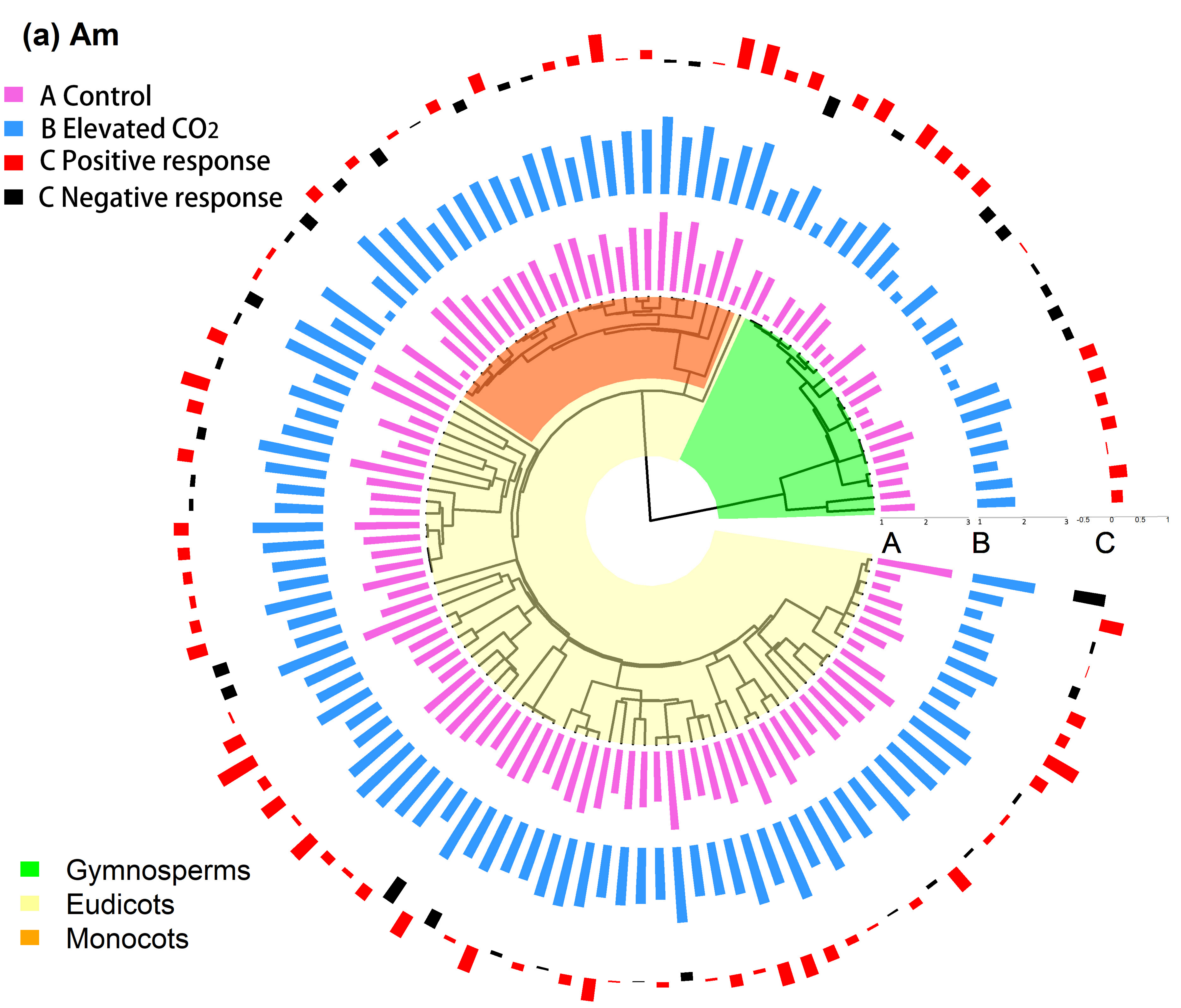
(c) OU models (300 trees)	Warming (39, 30, 53)	Drought (48, 52, 75)	eCO$_2$ (116, 135, 158)	Nitrogen (116, 141, 140)

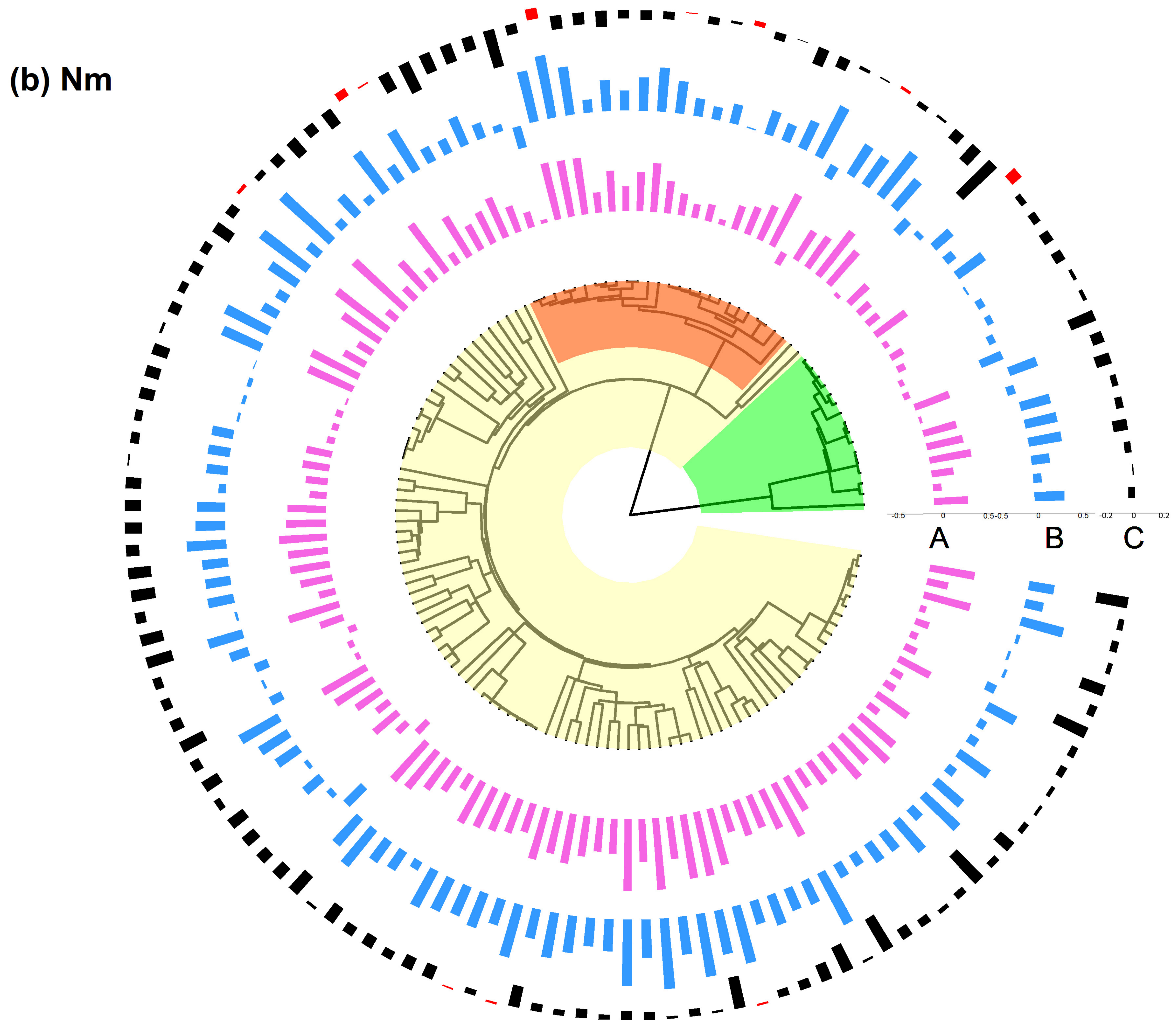
	Trait	*α*	PHL	*α*	PHL	*α*	PHL	*α*	PHL
Control	Am	0.04 ±0.000	16.05 ±0.000	0.02 ±0.000	42.65 ±0.135	0.02 ±0.000	32.47 ±0.000	0.02 ±0.000	36.89 ±0.010
	Nm	0.03 ±0.000	20.31 ±0.000	0.02 ±0.000	28.11 ±0.013	0.02 ±0.002	33.11 ±0.048	0.02 ±0.000	39.45 ±0.006
	SLA	0.02 ±0.000	31.24 ±0.003	0.05 ±0.001	32.65 ±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 ±0.001	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

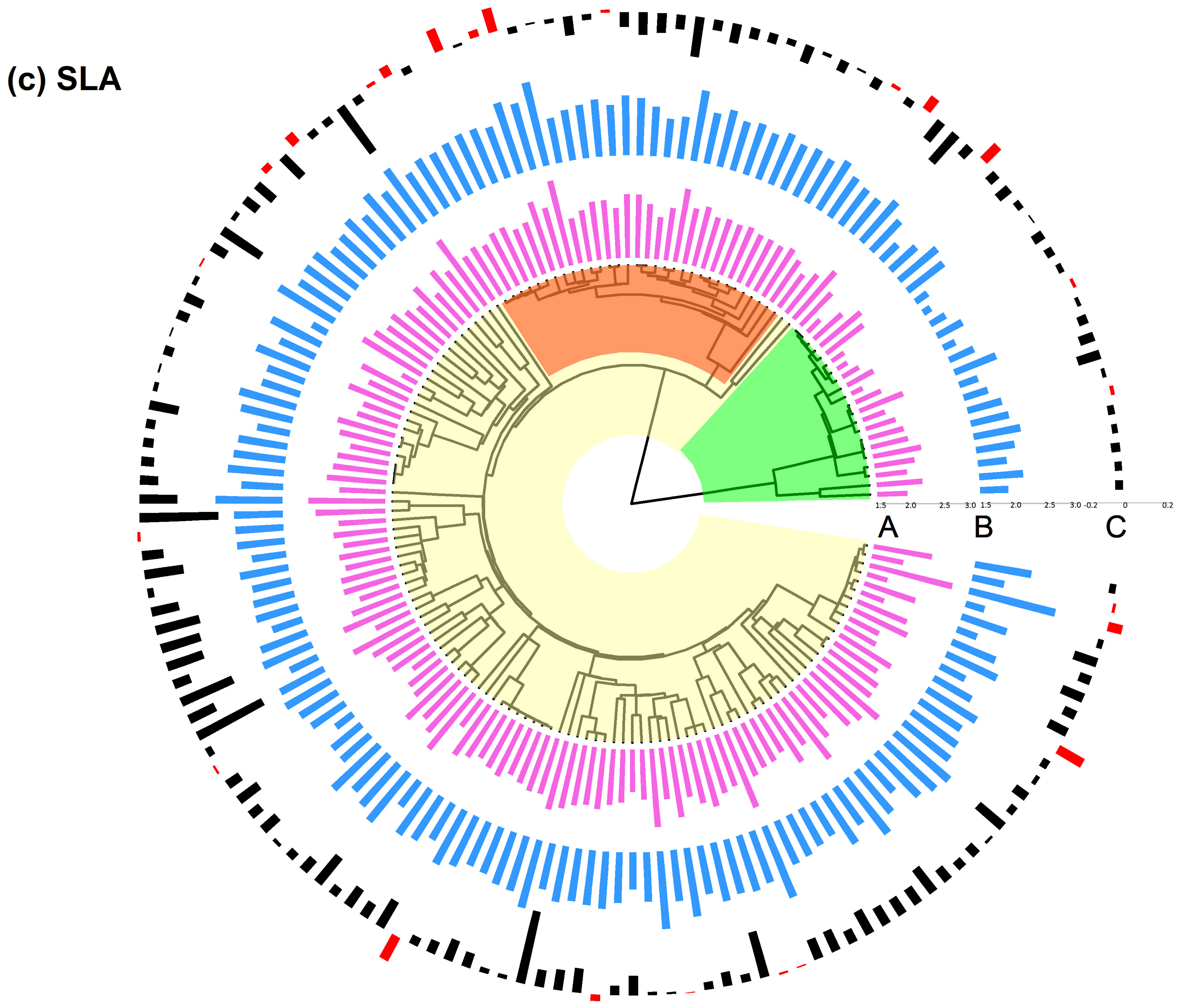
All the results are mean ± standard errors based on 300 imputation trees. Sample sizes (*n*) of three traits under four manipulated environmental factors are reported in corresponding brackets. Am, mass-based net photosynthetic rate; SLA, specific leaf area; Nm, mass-based leaf nitrogen content; eCO_2, elevated CO_2; Nitrogen, nitrogen addition. Phenotypic variation is the response ratio between experimental treatment mean and control mean. Level of significance: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; ns, not significant.

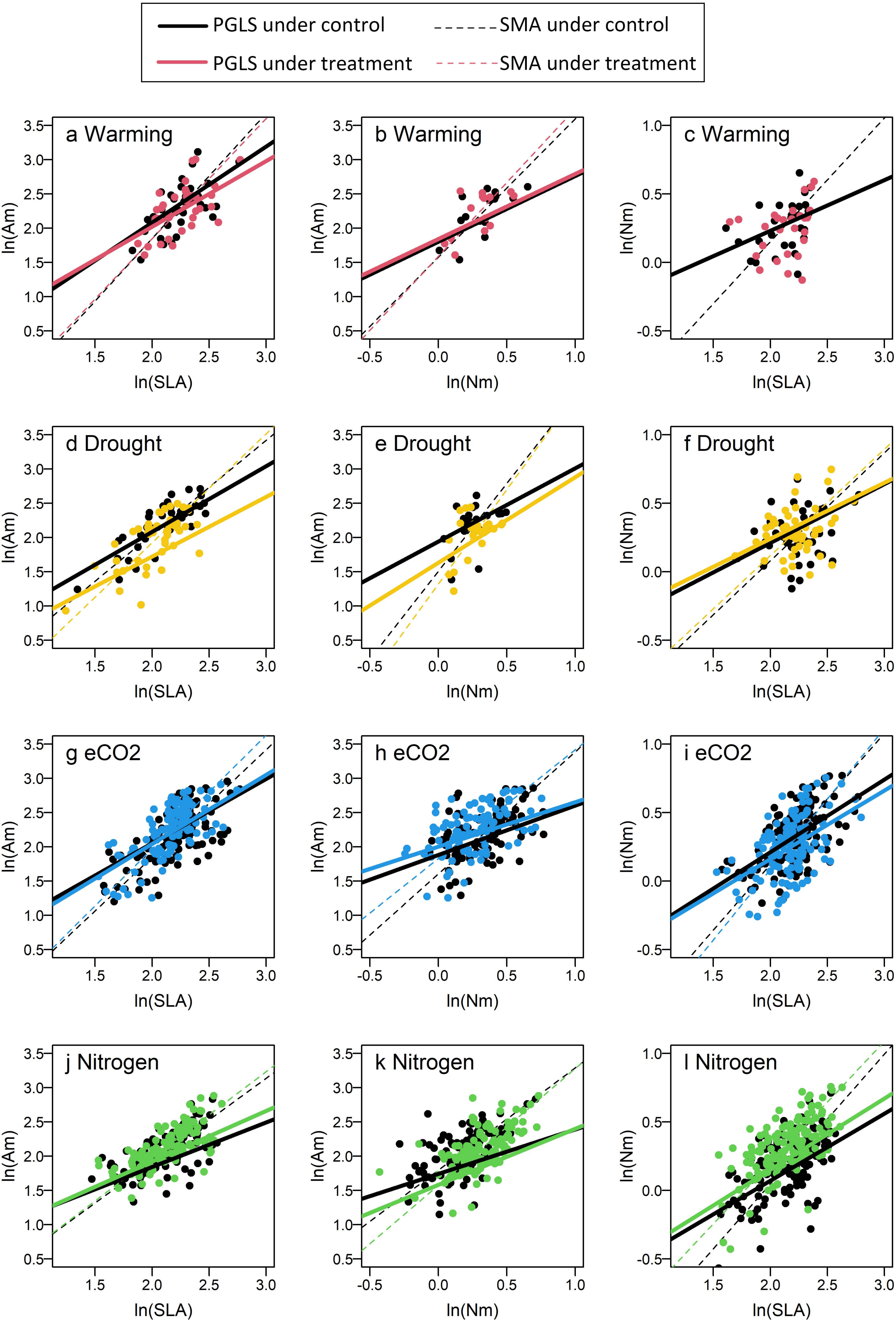


	H1. Evolution-driven trait response	**H2. Environment-driven trait response**	**H3. Random trait response**
Hypothesis	Phylogenetic signal in trait and phenotypic variation. Traits under both control and climate change, and phenotypic variation, are conserved within lineages.	Phylogenetic signal in trait but not phenotypic variation. Trait is conserved within lineages under control and/or treatment, but phenotypic variation is not.	No phylogenetic signal in trait or phenotypic variation. Trait is not conserved within lineages under control or treatment, and neither is phenotypic variation.
Possible mechanism	Intrinsic phylogenetic differences dominate how species adjust their traits when environmental conditions change. Reasons include phylogenetic niche conservatism, stabilizing selection and environmental constraints on traits and their responses.	Intrinsic phylogenetic differences exist for absolute trait values, however, the direction and strength of relative trait changes are mainly driven by environment rather than phylogeny.	Traits respond flexibly to environmental changes, or traits are not under selection from current environmental pressure. Therefore no evolutionary or ecological patterns could be detected.


(a) Am
A Control
B Elevated CO2
C Positive response
C Negative response
Gymnosperms
Eudicots
Monocots
A
B
C


(b) Nm
A
B
C


(c) SLA
A
B
C


PGLS under control
SMA under control
PGLS under treatment
SMA under treatment
a Warming
b Warming
c Warming
d Drought
e Drought
f Drought
g eCO2
h eCO2
i eCO2
j Nitrogen
k Nitrogen
l Nitrogen
ln(Am)
ln(Nm)
ln(SLA)
ln(Nm)
0.5
1.0
1.5
2.0
2.5
3.0
3.5
-0.5
0.0
0.5
1.0
1.5
2.0
2.5
3.0