

## RESEARCH ARTICLE

# Nitrogen use strategy drives interspecific differences in plant photosynthetic CO<sub>2</sub> acclimation

Erqian Cui<sup>1</sup>  | Jianyang Xia<sup>1</sup>  | Yiqi Luo<sup>2</sup> 

<sup>1</sup>Zhejiang Tiantong Forest Ecosystem National Observation and Research Station, Center for Global Change and Coastal Ecosystems, Institute of Eco-Chongming, School of Ecological and Environmental Sciences, East China Normal University, Shanghai, China

<sup>2</sup>School of Integrative Plant Science, Cornell University, Ithaca, New York, USA

## Correspondence

Jianyang Xia, Zhejiang Tiantong Forest Ecosystem National Observation and Research Station, Center for Global Change and Coastal Ecosystems, Institute of Eco-Chongming, School of Ecological and Environmental Sciences, East China Normal University, Shanghai, China. Email: [jyxia@des.ecnu.edu.cn](mailto:jyxia@des.ecnu.edu.cn)

## Funding information

Shanghai Key Laboratory for Urban Ecological Processes and Eco-Restoration, Grant/Award Number: SHUES2021B03; The Fellowship of China Postdoctoral Science Foundation, Grant/Award Number: 2022T150216; Shanghai Pilot Program for Basic Research, Grant/Award Number: TQ20220102

## Abstract

Rising atmospheric CO<sub>2</sub> concentration triggers an emergent phenomenon called plant photosynthetic acclimation to elevated CO<sub>2</sub> (PAC). PAC is often characterized by a reduction in leaf photosynthetic capacity ( $A_{\text{sat}}$ ), which varies dramatically along the continuum of plant phylogeny. However, it remains unclear whether the mechanisms responsible for PAC are also different across plant phylogeny, especially between gymnosperms and angiosperms. Here, by compiling a dataset of 73 species, we found that although leaf  $A_{\text{sat}}$  increased significantly from gymnosperms to angiosperms, there was no phylogenetic signal in the PAC magnitude along the phylogenetic continuum. Physio-morphologically, leaf nitrogen concentration ( $N_m$ ), photosynthetic nitrogen-use efficiency (PNUE), and leaf mass per area (LMA) dominated PAC for 36, 29, and 8 species, respectively. However, there was no apparent difference in PAC mechanisms across major evolutionary clades, with 75% of gymnosperms and 92% of angiosperms regulated by the combination of  $N_m$  and PNUE. There was a trade-off between  $N_m$  and PNUE in driving PAC across species, and PNUE dominated the long-term changes and inter-specific differences in  $A_{\text{sat}}$  under elevated CO<sub>2</sub>. These findings indicate that nitrogen-use strategy drives the acclimation of leaf photosynthetic capacity to elevated CO<sub>2</sub> across terrestrial plant species.

## KEYWORDS

elevated CO<sub>2</sub>, nitrogen acquisition, nitrogen utilization, photosynthetic acclimation, plant phylogeny

## 1 | INTRODUCTION

Terrestrial vegetation absorbs a considerable proportion of anthropogenic CO<sub>2</sub> emissions through photosynthesis and plays a vital role in stabilizing the climate system (Chen et al., 2022; Friedlingstein et al., 2022). There is compelling evidence that terrestrial photosynthesis largely depends on the coexistence of diverse plant species in the ecosystems (Chen et al., 2018; Liang et al., 2016). Recently, satellite- and ground-based evidence point to an atmospheric CO<sub>2</sub>-induced increase in terrestrial photosynthesis (Haverd et al., 2020; Wang et al., 2020). In the long term, however, plants growing at

elevated CO<sub>2</sub> often fail to sustain the initial stimulation of photosynthetic capacity ( $A_{\text{sat}}$ ), a process known as photosynthetic acclimation to elevated CO<sub>2</sub> (PAC; Gunderson & Wullschleger, 1994; Halpern et al., 2019; Luo et al., 1994). In recent decades, numerous studies have investigated photosynthetic acclimation in elevated CO<sub>2</sub> experiments and provided extensive data sets to understand PAC for various species. The magnitude of photosynthetic acclimation varies widely, ranging from positive to negative among functional groups (Lee et al., 2001, 2011; Medlyn et al., 1999). However, the mechanisms responsible for such sizeable interspecific divergence in the extent and pattern of PAC are not well understood, effectively

limiting projections of future terrestrial photosynthesis (Ainsworth & Long, 2005; Dusenage et al., 2020).

Photosynthetic acclimation to elevated CO<sub>2</sub> reflects a combination of adjustments in leaf biochemical and morphological traits (Ainsworth & Rogers, 2007; Luo et al., 1994). The classical photosynthetic biochemical model of Farquhar et al. (1980) has discussed the mechanistic basis of leaf nitrogen limitation on photosynthetic characteristics. Then leaf photosynthetic characteristics are commonly fitted as a function of leaf nitrogen per unit leaf area (N<sub>a</sub>; Field, 1983; Harley et al., 1992). Therefore, photosynthetic capacity can be jointly determined by leaf nitrogen concentration (N<sub>m</sub>) and leaf dry mass per area (LMA), where N<sub>a</sub> = N<sub>m</sub> × LMA (Luo et al., 1994). For plants grown in nitrogen-limiting soils, the magnitude of PAC is often tightly coupled to the reduction in N<sub>m</sub> under elevated CO<sub>2</sub> concentration (Crous et al., 2010; Warren et al., 2015). The decreased N<sub>m</sub> has frequently been attributed to dilution by carbohydrate accumulation, progressive nitrogen limitation, and excessive demands for nitrogen to support plant growth (Feng et al., 2015; Kitaoka et al., 2016; Luo et al., 2004). In addition, PAC is also accompanied by increased leaf mass per area (LMA) under elevated CO<sub>2</sub> concentration. This morphological change involves increased nonstructural carbohydrates storage, leaf thickness, and mesophyll tissue, partially compensating for the decreased photosynthetic capacity limited by N<sub>m</sub> (Gardiner et al., 2009; Wang et al., 2022; Yin, 2002).

Meanwhile, many empirical studies have shown that the relationship between A<sub>sat</sub> and N<sub>a</sub> varies with the atmospheric CO<sub>2</sub> concentration (Crous et al., 2008; Peterson, Ball, Luo, Field, Reich, et al., 1999). Therefore, photosynthetic capacity per unit leaf nitrogen, termed photosynthetic nitrogen-use efficiency (PNUE), has been considered another crucial biochemical trait to characterize PAC (Onoda et al., 2017). The optimal PNUE is constrained by available nitrogen and responds dynamically to environmental changes (Quebbeman & Ramirez, 2016). Free Air CO<sub>2</sub> Enrichment (FACE) experiments have shown various CO<sub>2</sub>-induced shifts in PNUE among species (Leakey et al., 2009; Lee et al., 2011). Variation in PNUE indicates a flexible nitrogen allocation strategy of plants. To maximize photosynthesis under elevated CO<sub>2</sub>, plants could reinvest leaf nitrogen from Rubisco to other photosynthetic processes limiting potential leaf-level photosynthesis (Ainsworth & Long, 2005; Ali et al., 2016; Byeon et al., 2021). Thus, we can quantitatively assess photosynthetic capacity via three leaf traits, i.e., N<sub>m</sub>, LMA, and PNUE. However, this leaf nitrogen-based approach is necessarily empirical, yet it is unclear if the responses of these traits are reliable to predict PAC across species.

Understanding the long-term acclimation of photosynthetic capacity is crucial to assess the adaptation and survival of plants under climate change (Stotz et al., 2021). Evidence for phylogenetic trends in photosynthesis parameters among land plants has been widely reported, especially remarkable differences between gymnosperms and angiosperms (Flexas & Carríqui, 2020; Gago et al., 2019). Therefore, the acclimation of photosynthetic traits to

climate changes is expected to be constrained by evolutionary history (Valladares et al., 2007). Numerous studies have investigated phylogenetic constraints on the magnitude of trait acclimation. Some studies provide evidence for phylogenetic signals in trait acclimation (Kembel & Cahill, 2005; Shao et al., 2019), whereas others indicate phylogenetic-independent trait responses (Liu et al., 2022; Stotz et al., 2021; Sweeney et al., 2021). However, a sufficiently broad database has not systematically evaluated whether photosynthetic CO<sub>2</sub> acclimation is constrained by evolutionary history.

Nowadays, an emerging pool of photosynthetic traits is widening our knowledge of how leaf photosynthesis acclimates to rising CO<sub>2</sub> and its mechanisms vary across major clades of land plants (gymnosperms vs. angiosperms). Here, we combined an updated PAC model and a global dataset to quantify the mechanistically limiting factors underlying PAC. This global dataset fully considers leaf traits responsible for photosynthetic acclimation, including measurements of A<sub>sat</sub>, N<sub>m</sub>, LMA, and PNUE for the same species. The updated trait-based PAC framework allows us to quantify the relative contributions of N<sub>m</sub>, LMA, and PNUE to photosynthetic acclimation. Variations in these three leaf traits reveal different PAC mechanisms, including nitrogen acquisition, leaf mass investment, and nitrogen utilization. Our primary goal was to examine three major questions: (1) if the photosynthetic CO<sub>2</sub> acclimation of plants is phylogenetically dependent; (2) if these photosynthetic traits are reliable to predict PAC across species; (3) whether the dominant trait driving photosynthetic CO<sub>2</sub> acclimation differ between gymnosperms and angiosperms.

## 2 | MATERIALS AND METHODS

### 2.1 | A trait-based PAC framework

Plants invest a large amount of leaf nitrogen in the photosynthetic machinery, resulting in a strong coupling of leaf nitrogen content and photosynthetic capacity (A<sub>sat</sub> ∝ N<sub>a</sub>, Kattge et al., 2009). Therefore, a traditional PAC model assumes that net changes in N<sub>m</sub> and LMA can interact to affect photosynthetic capacity (Luo et al., 1994; Peterson, Ball, Luo, Field, Curtis, et al., 1999):

$$A_{\text{sat}} \propto N_m \times \text{LMA}. \quad (1)$$

The ratio of photosynthetic capacity to leaf nitrogen content has been defined as PNUE, another important biochemical trait to constrain PAC (Onoda et al., 2017). Then, the traditional PAC model can be quantitatively updated as:

$$A_{\text{sat}} = N_m \times \text{LMA} \times \text{PNUE}, \quad (2)$$

where N<sub>m</sub> and PNUE reflect the biochemical capacity and LMA is an index of leaf morphology. Here, PNUE is added to explain the residue

in the traditional PAC model (Luo et al., 1994). This decomposition of  $A_{\text{sat}}$  allows us to investigate the relative importance of biogeochemical and morphological traits in regulating photosynthetic  $\text{CO}_2$  acclimation.

## 2.2 | Data compilation

We assembled a global dataset from peer-reviewed publications to test the photosynthetic acclimation framework. Therefore, articles that meet the following criteria were included in our analysis: (1) The elevated  $\text{CO}_2$  manipulation experiments should be performed at the species level; (2) To minimize publication bias caused by different data sources, the candidate articles should report measurements in  $A_{\text{sat}}$ ,  $N_m$ , and LMA simultaneously on the same set of species for both ambient and elevated  $\text{CO}_2$  (i.e.,  $a\text{CO}_2$  and  $e\text{CO}_2$ ) grown individuals; (3) The variable  $A_{\text{sat}}$  refers to photosynthetic assimilation measured at saturated light and  $e\text{CO}_2$  for both  $a\text{CO}_2$ - and  $e\text{CO}_2$ -grown plants. According to the above criteria, a dataset of 51 studies examining photosynthetic biochemistry was included in our analysis. For each study, raw data were obtained from tables directly or extracted from figures using GetData Graph Digitizer 2.24. Finally, a total of 73 species belonging to 22 families were recorded.

It has long been recognized that potential limitations of nutrient resources can affect plant photosynthetic acclimation to climate changes (Lee et al., 2001). Here, we also tested the effects of nitrogen availability on the magnitude of PAC and its primary mechanisms by compiling the experiments with both  $e\text{CO}_2$  and nitrogen addition treatments. In total, 22 out of the 73 species are available to explain how plant PAC responds increased nitrogen supply (Table S1).

## 2.3 | Data analyses

PAC is generally determined by comparing the  $A_{\text{sat}}$  of  $a\text{CO}_2$ - and  $e\text{CO}_2$ -grown plants when both are measured at  $e\text{CO}_2$  (Ghildiyal & Sharma-Natu, 2000; Pastore et al., 2019). A decreased  $A_{\text{sat}}$  after long-term exposure to  $e\text{CO}_2$  is called downregulated acclimation, while the opposite response is called upregulated acclimation (Figure S1). Following Walker et al. (2021), we calculate a  $\beta$ -factor for each variable to make the  $e\text{CO}_2$  effects comparable across studies:

$$\beta(x) = \ln(x_e / x_a) / \ln(e\text{CO}_2 / a\text{CO}_2), \quad (3)$$

where  $x_a$  and  $x_e$  are leaf traits for  $a\text{CO}_2$  and  $e\text{CO}_2$ -grown plants, and  $\beta(x)$  refers to the acclimation strength of a trait  $x$ . Therefore, Equation 2 can be transformed to

$$\beta(A_{\text{sat}}) = \beta(N_m) + \beta(\text{LMA}) + \beta(\text{PNUE}). \quad (4)$$

This equation assumes that relative changes in  $\beta(N_m)$ ,  $\beta(\text{LMA})$ , and  $\beta(\text{PNUE})$  have additive effects on  $\beta(A_{\text{sat}})$ . In addition, the relative contributions of these three traits to explain photosynthetic  $\text{CO}_2$  acclimation are calculated by normalizing their responses

$$R(x_i) = \frac{|\beta(x_i)|}{\sum_{i=1}^3 |\beta(x_i)|}, \quad (5)$$

where  $R(x_i)$  refers to the relative contribution of a trait  $x_i$  to PAC.

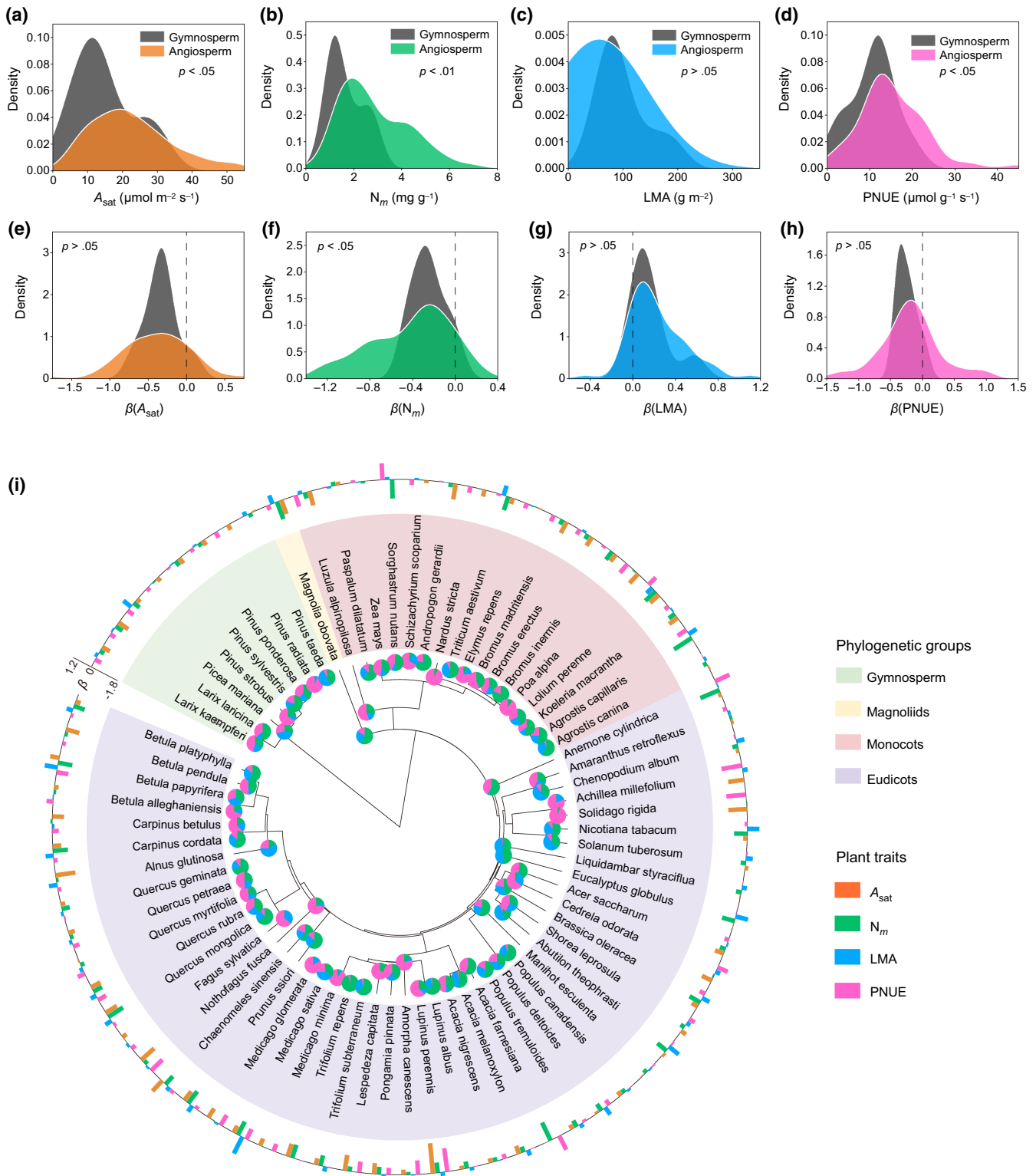
In addition, we generated phylogenetic trees for the 73 species using *v.phyloMaker*, an available R package that can generate phylogenies for vascular plants (Jin & Qian, 2019). The mega-tree used in *v.phyloMaker* is the largest dated phylogeny for vascular plants. To explore the influence of evolutionary history on photosynthetic  $\text{CO}_2$  acclimation, we tested the phylogenetic signal in the  $\beta$ -factor of leaf traits by estimating both Pagel's  $\lambda$  and Blomberg's  $K$ . Pagel's  $\lambda$  varies from 0 to 1, where  $\lambda=0$  indicates no phylogenetic signal and  $\lambda=1$  implies strong phylogenetic dependence (Pagel, 1999).  $K$  measures the extent to which a variable displays a phylogenetic signal, where  $K=0$  indicates no phylogenetic dependence and  $K>1$  indicates a stronger phylogenetic signal than expected under Brownian motion (Blomberg et al., 2003). Pagel's  $\lambda$  and Blomberg's  $K$  were calculated by using "phylosig" function in the R package *phytools* (Revell, 2012).

## 3 | RESULTS

### 3.1 | Acclimation of leaf photosynthetic capacity to $e\text{CO}_2$

Across all assessed species, we found that  $A_{\text{sat}}$  increased significantly from gymnosperms to angiosperms. Larger  $N_m$  and PNUE mainly lead to maximized  $A_{\text{sat}}$  along the plant phylogenetic continuum in angiosperms ( $p < .05$ , Figure 1a-d). In addition, photosynthetic acclimation was generally accompanied by a reduction in  $N_m$  and PNUE as well as an increase in LMA. However, there was no significant difference in photosynthetic  $\text{CO}_2$  acclimation between gymnosperms and angiosperms ( $p > .05$ , Figure 1e-h). As shown in the phylogenetic tree, downregulation of photosynthetic capacity under  $e\text{CO}_2$  was found in 60 species, while other 13 species showed upregulated acclimation (Figure 1i). Specifically,  $\beta(A_{\text{sat}})$  ranged widely from  $-1.79$  (*Lupinus perennis*) to  $0.84$  (*Cedrela odorata*), and the average acclimation of  $N_m$ , LMA, and PNUE were  $-0.40$ ,  $0.22$ , and  $-0.19$ , respectively. By considering the interaction between  $\text{CO}_2$  concentration and soil nitrogen availability, we found that the magnitude of plant PAC was not significantly affected by nitrogen addition (Figure S2).

To further determine if evolutionary history affects photosynthetic  $\text{CO}_2$  acclimation, we examined the phylogenetic signal for trait acclimation by estimating Blomberg's  $K$  and Pagel's  $\lambda$  (Table S2). As a result, we found no significant phylogenetic signal in the effect of  $e\text{CO}_2$  on  $A_{\text{sat}}$ ,  $N_m$ , LMA, and PNUE ( $K=0.06-0.1$ , all  $p > .05$ ). In addition, the signals for Pagel's  $\lambda$  were very similar to those for Blomberg's  $K$ , and the results of these two metrics of phylogenetic signal together indicated that photosynthetic  $\text{CO}_2$  acclimation was phylogenetically independent.



**FIGURE 1** Acclimation of photosynthetic capacity to  $e\text{CO}_2$  and its limiting traits. (a–h), Density curves representing the distribution of  $e\text{CO}_2$  effect on leaf photosynthetic traits for gymnosperms and angiosperms. (i) Phylogenetic tree showing  $\beta$  value of  $N_m$ , LMA, and PNUE as well as their relative contributions to photosynthetic  $\text{CO}_2$  acclimation. Phylogenetic groups are highlighted (gymnosperms, magnoliids, monocots, eudicots) with corresponding  $\beta$  values of  $N_m$ , LMA, and PNUE. The mini-bar charts are  $\beta$  values of  $N_m$ , LMA, and PNUE. The pie chart shows the relative contributions of  $N_m$ , LMA, and PNUE to photosynthetic  $\text{CO}_2$  acclimation.  $A_{sat}$ , leaf photosynthetic capacity; LMA, leaf dry mass per area;  $N_m$ , leaf nitrogen concentration; PNUE, photosynthetic nitrogen-use efficiency.  $\beta$ -value refers to the acclimation strength of these traits.

### 3.2 | Major mechanisms underlying photosynthetic CO<sub>2</sub> acclimation

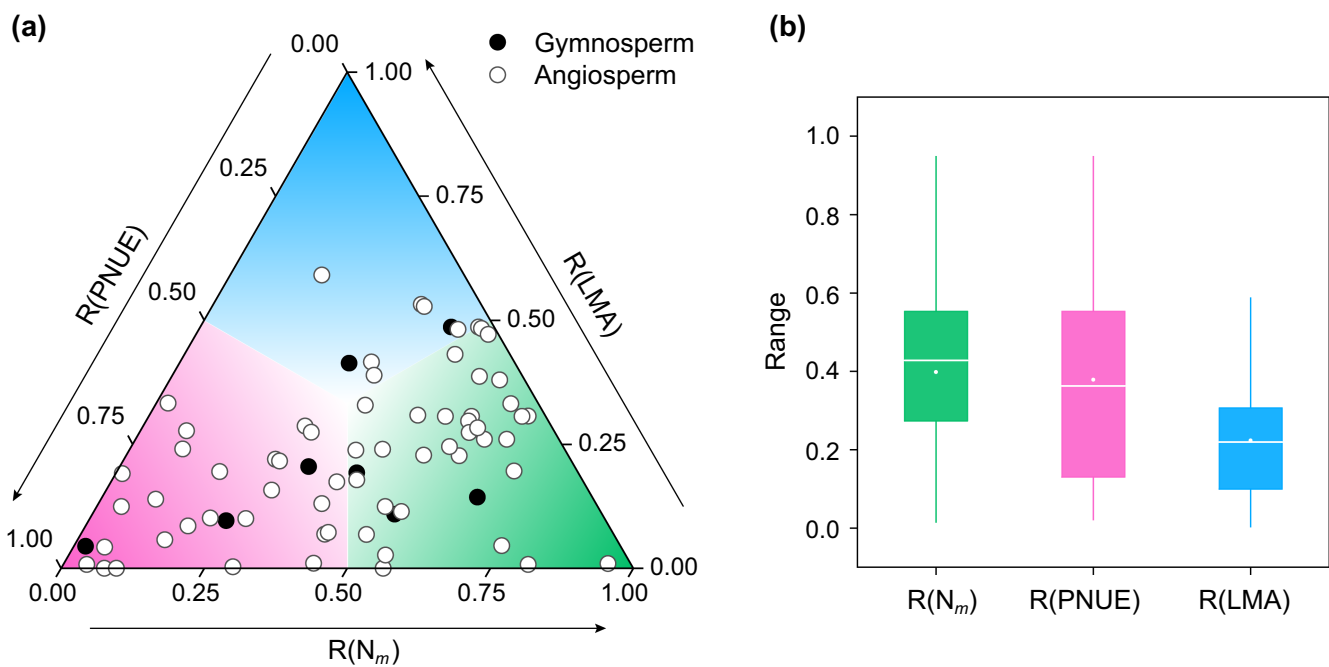
To better understand the mechanisms responsible for plant photosynthetic acclimation, we further quantified the relative contributions of  $N_m$ , LMA, and PNUE to photosynthetic CO<sub>2</sub> acclimation for each species. For example, the photosynthetic acclimation of *Bromus*, *Trifolium*, and *Agrostis* was consistently driven by  $N_m$ , while PNUE dominated the photosynthetic acclimation of Asteraceae. By comparison, the photosynthetic acclimation of Solanaceae was mainly driven by plasticity in LMA (Figure 1i). As shown in the ternary plot, acclimation of  $A_{\text{sat}}$  was dominated by  $N_m$ , LMA, and PNUE in 36, 8, and 29 species, respectively (Figure 2a). The above pattern indicated that the effect of LMA changes on most species was negligible. Meanwhile, there was no apparent difference in photosynthetic acclimation mechanisms across major evolutionary clades, with nearly 75% of gymnosperms and 92% of angiosperms regulated by the combination of  $N_m$  and PNUE. The averaged contributions of  $N_m$ , LMA, and PNUE across species were 0.40, 0.22, and 0.38, respectively (Figure 2b). Furthermore, it was worth noting that the primary mechanism responsible for PAC would be influenced by increased nitrogen supply and gradually become dominated by nitrogen utilization (Figure S3).

Further, we found that the relative contributions of  $N_m$  and PNUE were negatively related to one another (Figure 3a,  $R^2 = .68$ ,  $p < .01$ ). This emergent linear relationship indicates a trade-off

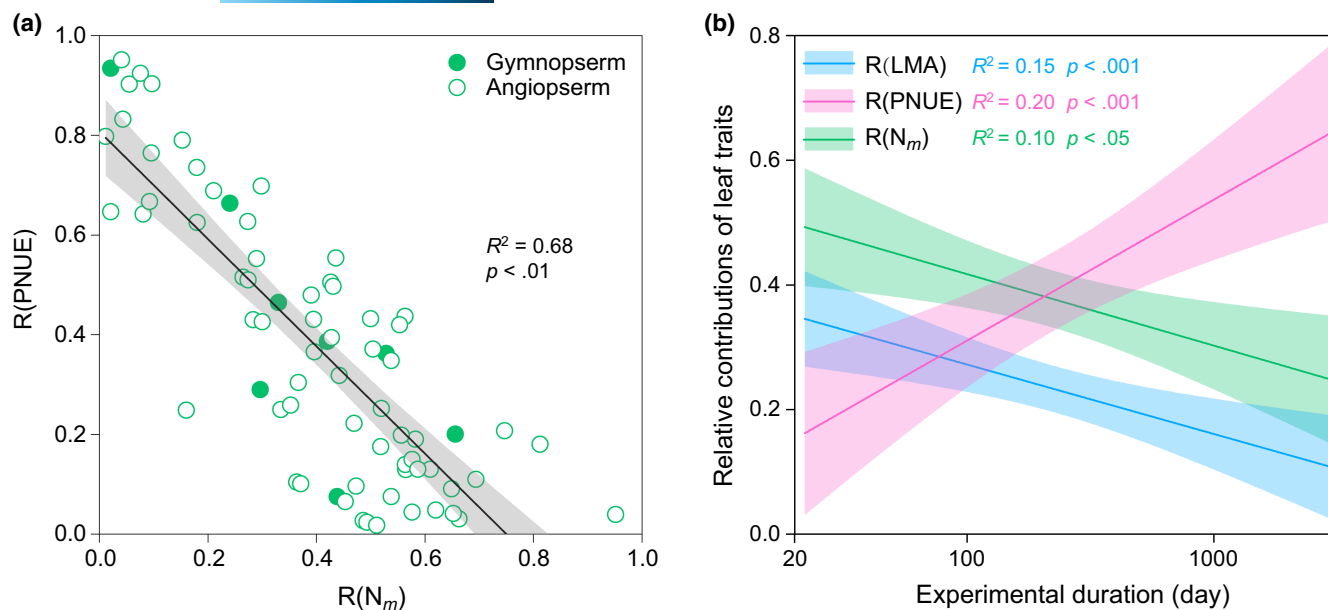
between plant nitrogen acquisition and utilization in driving PAC across species. In addition, the relative contributions of leaf traits were significantly affected by experimental duration (Figure 3b). The contributions of  $N_m$  ( $R^2 = .10$ ,  $p < .05$ ) and LMA ( $R^2 = .15$ ,  $p < .001$ ) decreased with increasing experimental duration. In contrast, the contributions of PNUE ( $R^2 = .20$ ,  $p < .001$ ) significantly increased with experimental duration and gradually became the dominant trait. These results highlighted the importance of nitrogen-use efficiency in driving photosynthetic CO<sub>2</sub> acclimation, especially for long-term CO<sub>2</sub> fertilization.

### 3.3 | Nitrogen-use efficiency drives interspecific differences in photosynthetic CO<sub>2</sub> acclimation

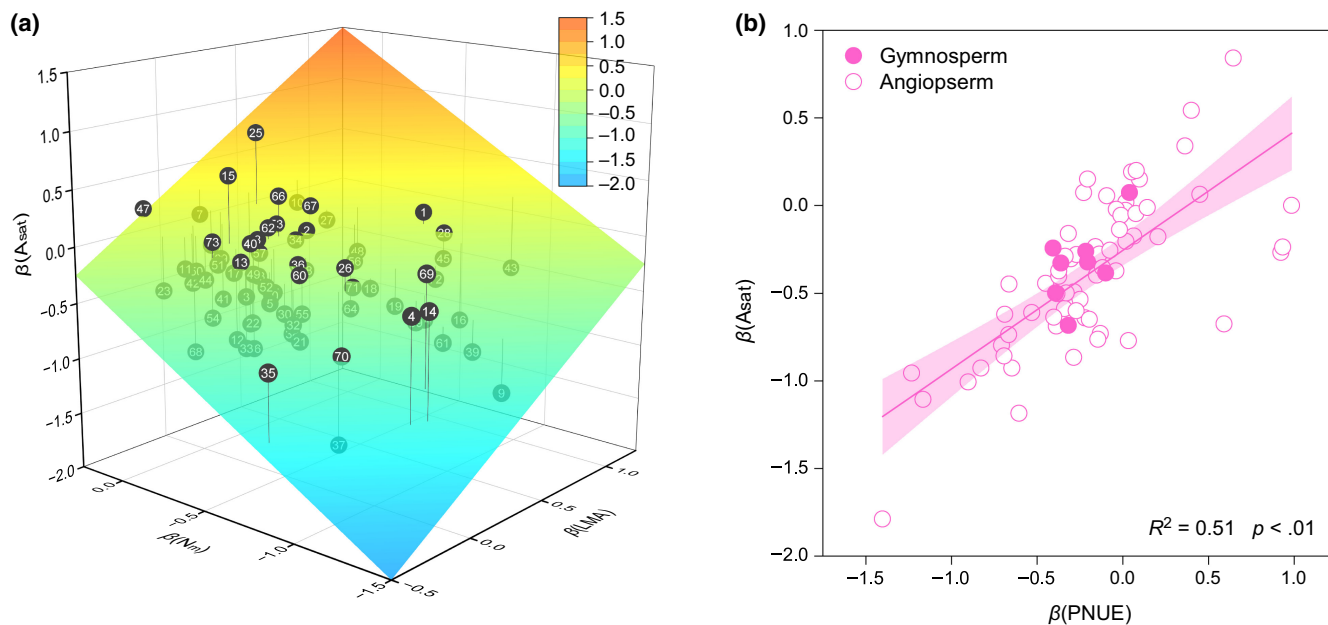
According to the previous PAC model, acclimation of interspecific differences in photosynthetic capacity was proportional to  $N_d$  and was, therefore, jointly controlled by relative changes in  $N_m$  and LMA. Here we showed that the magnitude of PAC was not always fitted to the response surface predicted by changes in  $N_m$  and LMA (Figure 4a). In the three-dimensional space, there were diverse deviations between the observed and predicted acclimation of photosynthetic capacity among species. Such a mismatch could be attributed to the dramatic plasticity in PNUE induced by eCO<sub>2</sub>, with  $\beta(\text{PNUE})$  varied from  $-1.40$  (*Lupinus perennis*) to  $0.99$  (*Sorghastrum nutans*; Figure 1i).



**FIGURE 2** Relative contributions of  $N_m$ , LMA, and PNUE to photosynthetic CO<sub>2</sub> acclimation. (a) Ternary diagram shows the contributions of  $N_m$ , LMA, and PNUE to acclimation of photosynthetic capacity for 73 species. Gymnosperms and angiosperms are differentiated by closed and open circles. (b) Distribution of the contributions of  $N_m$ , LMA, and PNUE to photosynthetic CO<sub>2</sub> acclimation. For the boxplots: vertical bars are data ranges defined as  $1.5 \times$  the inter-quartile range; horizontal lines within the boxes are median values; and the upper and lower bounds of the boxes are the third and first quartiles, respectively. LMA, leaf dry mass per area;  $N_m$ , leaf nitrogen concentration; PNUE, photosynthetic nitrogen-use efficiency.  $R$ -value refers to the relative contribution of these traits.



**FIGURE 3** The trade-off between nitrogen acquisition and utilization in plant photosynthetic acclimation to  $\text{CO}_2$ . (a) Relationships between contributions of  $N_m$  and PNUE to photosynthetic  $\text{CO}_2$  acclimation. Gymnosperms and angiosperms are differentiated by closed and open circles. (b) Relative contributions of leaf traits depend on the duration of the experiments. LMA, leaf dry mass per area;  $N_m$ , leaf nitrogen concentration; PNUE, photosynthetic nitrogen-use efficiency. R-value refers to the relative contribution of these traits.



**FIGURE 4** A framework for quantifying trait controls on interspecific differences in photosynthetic  $\text{CO}_2$  acclimation. (a) Joint control of interspecific difference in  $A_{\text{sat}}$  by  $N_m$  and LMA according to the previous PAC model (Luo et al., 1994). The 3D response surface reflects changes in photosynthetic capacity predicted from relative changes in  $N_m$  and LMA, where  $\beta(A_{\text{sat}}) = \beta(N_m) + \beta(\text{LMA})$ . Vertical lines are added to show the difference between the observed and predicted response of photosynthetic capacity. The label for the circle corresponds to the serial number of species in Table S1. (b) Relationships between acclimation of  $A_{\text{sat}}$  and PNUE across the assessed 73 species. Gymnosperms and angiosperms are differentiated by closed and open circles.  $A_{\text{sat}}$ , leaf photosynthetic capacity; LMA, leaf dry mass per area;  $N_m$ , leaf nitrogen concentration; PNUE, photosynthetic nitrogen-use efficiency.  $\beta$ -value refers to the acclimation strength of these traits.

To illustrate the importance of PNUE in driving interspecific differences in photosynthetic  $\text{CO}_2$  acclimation, we further quantified the relationships between the acclimation of  $A_{\text{sat}}$  and PNUE. As shown in Figure 4b, the response of leaf PNUE explained most of the

differences in photosynthetic  $\text{CO}_2$  acclimation across the phylogeny ( $R^2 = .51, p < .01$ ). Specifically, LMA and  $N_m$  could jointly explain 60% of the variation in  $A_{\text{sat}}$  across species, while interspecific differences in acclimation of  $A_{\text{sat}}$  were dominated by PNUE (68%, Figure S4).

## 4 | DISCUSSION

### 4.1 | Equally important of nitrogen acquisition and utilization for PAC

Plant nitrogen metabolism (e.g., acquisition and utilization) is an essential determinant of photosynthetic acclimation to  $eCO_2$ . The nitrogen limitation hypothesis contends that depletion of nitrogen availability after long-term exposure to  $eCO_2$  constrains photosynthetic responses (Hungate et al., 2003; Luo et al., 2004). This nitrogen-acquisition-driven photosynthetic  $CO_2$  acclimation has been interpreted by the consistent reduction in leaf nitrogen concentration and photosynthetic capacity at both leaf and ecosystem levels (Ainsworth & Long, 2005; Wang et al., 2020). In contrast, the least-cost optimality theory states that nitrogen utilization in leaves may be more important for regulating photosynthetic capacity under  $eCO_2$  than leaf nitrogen concentration (Byeon et al., 2021; Smith & Keenan, 2020). The above hypotheses are usually considered independently and challenged by conflicting experimental evidence. To our knowledge, no study has examined the relative importance of nitrogen acquisition and utilization to photosynthetic acclimation in the same set of species.

Based on the updated PAC model (Luo et al., 1994), this study demonstrated that leaf nitrogen acquisition and utilization strategies are equally crucial for photosynthetic  $CO_2$  acclimation (Figures 2 and 3). The trade-off between the relative contributions of  $N_m$  and PNUE can perfectly explain the reported contrasting acclimation responses. For example, many FACE experiments concluded that the down-regulation of photosynthetic capacity was governed by nitrogen-acquisition efficiency for low nitrogen availability plants (Terrer et al., 2018). However, responses of nitrogen-fixing bacteria associating species and fertilized species were well predicted by optimal nitrogen allocation theory, indicating a PNUE-driven photosynthetic acclimation (Smith & Keenan, 2020). Our findings suggest a need to revisit the mechanisms whereby leaf nitrogen economy drives photosynthetic acclimation in carbon-nitrogen coupled models.

### 4.2 | Trade-off between nitrogen acquisition and utilization along the duration of $CO_2$ fertilization

The study shows a lack of phylogenetic signal in leaf trait acclimation across the assessed species. This finding indicates that environmental factors are critical in determining photosynthetic acclimation mechanisms. Plant growth environment (nutrient resources) and experimental treatment (duration) are two critical factors affecting photosynthetic response (Cui et al., 2020). In this study, we found that short-term photosynthetic acclimation was mainly driven by a decrease in  $N_m$ , while long-term photosynthetic acclimation was largely dependent on PNUE adjustment (Figure 3b). The trade-off between nitrogen acquisition and utilization mechanisms in explaining photosynthetic  $CO_2$  acclimation should be attributed to long-term biochemical feedback. At the initial stage of  $CO_2$  fertilization,

leaf photosynthetic rates are often observed to be stimulated quickly (Ainsworth & Rogers, 2007). This response increases the plant's demand for nitrogen and stimulates the sequestration of nitrogen into long-lived plant tissues. The above processes can decrease soil nitrogen availability and serve as the core mechanisms limiting nitrogen acquisition, and further induce photosynthetic acclimation (Luo et al., 2004). In addition, nitrogen limitation often results in the accumulation of leaf nitrogen reallocation and subsequent adjustment of PNUE (Liberloo et al., 2007). Therefore, long-term photosynthetic acclimation would instead depend on such biochemical feedback.

By comparing results across different levels of nitrogen supply ( $eCO_2$  and  $eCO_2$  + nitrogen addition), we found that nitrogen addition did modestly alleviate the decrease of  $N_m$  while the magnitude of photosynthetic  $CO_2$  acclimation was independent of nitrogen supply (Figure S2). Some long-term FACE experiments that support this finding have provided the following mechanisms: photosynthetic acclimation may be more limited by other nutrients (e.g. phosphorous, potassium, or magnesium) than nitrogen; plants are already adapted to low nitrogen and therefore are not as responsive to nitrogen addition (Lee et al., 2011; Pastore et al., 2019). More importantly, we found that nitrogen utilization would become the primary mechanism responsible for PAC with increased soil nitrogen availability (Figure S3). These additional results imply that the primary mechanism responsible for PAC can change with nutrient conditions and experimental durations.

### 4.3 | Implications for PNUE-driven photosynthetic acclimation

Plant PNUE is considered to be a vital leaf trait to characterize photosynthetic capacity. A nearly 40-fold interspecific difference in leaf PNUE has been reported in the Glopnet dataset (Wright et al., 2004). Here, we first quantified the acclimation of PNUE to  $eCO_2$ , which also varied dramatically in direction and magnitude among species. We then demonstrated that the leaf PNUE acclimation drives interspecific differences in photosynthetic  $CO_2$  acclimation (Figure 4). This evidence argues for increased focus on the mechanisms responsible for PNUE acclimation. Variation in PNUE can be attributed to mesophyll conductance, stomatal conductance and the fraction of nitrogen allocated to Rubisco (Hikosaka, 2004; Onoda et al., 2017). In our study, we provided overwhelming evidence of a decline in PNUE (Figure 1). The previous studies are mainly focused on linking photosynthesis and leaf nitrogen allocation under  $eCO_2$  (Sharwood et al., 2017). After systematic investigation, we indeed found a lower fraction of nitrogen allocated to Rubisco for  $eCO_2$ -grown plants (Figure S5), which indicates a reallocation of leaf nitrogen from photosynthetic to non-photosynthetic functions (Choi et al., 2017; Lei et al., 2012). However, the contributions of mesophyll conductance and stomatal conductance to  $eCO_2$ -induced decline in PNUE remain to be further explored.

Our finding of PNUE-driven photosynthetic  $CO_2$  acclimation forces us to pay attention to more mechanistic processes that

influence leaf photosynthetic biochemistry. First, leaf nitrogen allocation between photosynthetic and non-photosynthetic components as well as among photosynthetic apparatus should be considered to better represent leaf nitrogen utilization across species (Luo et al., 2021). Second, eCO<sub>2</sub>-induced variation in PNUe would motivate the application of a flexible nitrogen allocation strategy, which is commonly set as fixed parameters in many ecosystem models (Ali et al., 2016). Third, except for leaf nitrogen, environmentally regulated biophysical constraints and light availability are especially important for photosynthetic capacity (Smith et al., 2019). Therefore, more theoretical frameworks that consider environmental and plant morphological influences on photosynthetic traits rather than simple empirical relationships should be used to reform new photosynthetic models (Wang et al., 2017).

## 5 | CONCLUSIONS

Our results demonstrate a nitrogen-driven photosynthetic CO<sub>2</sub> acclimation across terrestrial plants, which is phylogenetically independent. The nitrogen-driven PAC is characterized by a trade-off between nitrogen acquisition via the change in N<sub>m</sub> and utilization as indicated by PNUe. These findings have several implications for understanding and modeling PAC for terrestrial plants. First, the lack of phylogenetic signal in PAC across species indicates that the acclimation of leaf traits driving photosynthesis is largely shaped by environmental constraints rather than genetic differences. The robustness of this finding needs to be further validated with other available datasets, although some previous studies have reached similar conclusions (Liu et al., 2022; Stotz et al., 2021). Second, the physiological causes for the conceptual parameter PNUe in acclimation to rising atmospheric CO<sub>2</sub> are still controversial, due to challenges associated with its direct measurement. Recent advances in plant trait compilation, isotope labeling technique, and high-resolution remote sensing data provide a unique opportunity to explore within-leaf nitrogen allocation that determines PNUe for a wide range of species (Li et al., 2022; Luo et al., 2021). Third, the differential response of plants under nitrogen addition indicates the urgent need to investigate the role of plant-available nitrogen in determining the primary PAC mechanisms, especially considering the widespread declining nitrogen availability in terrestrial ecosystems (Mason et al., 2022). Fourth, multiple processes have been developed to determine plant photosynthetic capacity acclimation in Earth system models (Smith & Dukes, 2013). The models that use either the nitrogen limitation hypothesis (Luo et al., 2004) or the optimal nitrogen allocation theory (Thomas et al., 2015; Wieder et al., 2019) should further consider the trade-off between nitrogen acquisition and utilization in explaining PAC mechanisms across plant species. Lastly, our updated PAC model can be used to explore the mechanisms responsible for photosynthetic acclimation to other global environmental changes (e.g., warming and drought), which are equally important for predicting future

biosphere-atmosphere feedback. Overall, our results provide quantitative insights into the mechanisms influencing PAC, and suggest a high priority to explore nitrogen utilization strategies of global vegetation.

## AUTHOR CONTRIBUTIONS

Erqian Cui and Jianyang Xia devised and conducted the analysis. Erqian Cui collected the dataset and drafted the first manuscript. Yiqi Luo and Jianyang Xia provided critical suggestions on the method and results. All authors contributed to the writing and revising of the manuscript.

## ACKNOWLEDGMENTS

This work was financially supported by the Fellowship of China Postdoctoral Science Foundation (2022T150216), Shanghai Pilot Program for Basic Research (TQ20220102), and Shanghai Key Lab for Urban Ecological Processes and Eco-Restoration (SHUES2021B03).

## CONFLICT OF INTEREST STATEMENT

The authors declare no competing interests.

## DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in figshare at <https://doi.org/10.6084/m9.figshare.22297978>.

## ORCID

Erqian Cui  <https://orcid.org/0000-0002-2639-5069>

Jianyang Xia  <https://orcid.org/0000-0001-5923-6665>

Yiqi Luo  <https://orcid.org/0000-0002-4556-0218>

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## SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

**How to cite this article:** Cui, E., Xia, J., & Luo, Y. (2023).

Nitrogen use strategy drives interspecific differences in plant photosynthetic CO<sub>2</sub> acclimation. *Global Change Biology*, 29, 3667–3677. <https://doi.org/10.1111/gcb.16706>