



RESEARCH PAPER

Leaf economics spectrum in rice: leaf anatomical, biochemical, and physiological trait trade-offs

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Abstract

The leaf economics spectrum (LES) is an ecophysiological concept describing the trade-offs of leaf structural and physiological traits, and has been widely investigated on multiple scales. However, the effects of the breeding process on the LES in crops, as well as the mechanisms of the trait trade-offs underlying the LES, have not been thoroughly elucidated to date. In this study, a dataset that included leaf anatomical, biochemical, and functional traits was constructed to evaluate the trait covariations and trade-offs in domesticated species, namely rice (*Oryza* species). The slopes and intercepts of the major bivariate correlations of the leaf traits in rice were significantly different from the global LES dataset (Gloptnet), which is based on multiple non-crop species in natural ecosystems, although the general patterns were similar. The photosynthetic traits responded differently to leaf structural and biochemical changes, and mesophyll conductance was the most sensitive to leaf nitrogen (N) status. A further analysis revealed that the relative limitation of mesophyll conductance declined with leaf N content; however, the limitation of the biochemistry increased relative to leaf N content. These findings indicate that breeding selection and high-resource agricultural environments lead crops to deviate from the leaf trait covariation in wild species, and future breeding to increase the photosynthesis of rice should primarily focus on improvement of the efficiency of photosynthetic enzymes.

Keywords: Leaf economics spectrum, mesophyll conductance, mesophyll structure, nitrogen, photosynthesis, photosynthetic limitation.

Introduction

The global covariation of leaf anatomical, biochemical, and gas exchange traits along with resource availability gradients, which is known as the leaf economics spectrum (LES), has received widespread attention (Wright *et al.*, 2004; Blonder *et al.*, 2011; Sack *et al.*, 2013, 2014; Niinemets, 2015; Onoda *et al.*, 2017). In brief, the global LES describes a continuum leaf spectrum,

ranging from fast-growing species combined with low structural investment, high nutrient investment, quick return, and generally highly photosynthesizing leaves, to slow-growing species combined with high structure investment, low nutrient investment, slow return, and stress-tolerant leaves. The global LES is defined by several core leaf traits, including leaf mass per area

(LMA), nitrogen (N) concentration (N_m), the light-saturated photosynthetic rate per leaf mass (A_m), and leaf lifespan (Wright *et al.*, 2004). However, the LES trait network is typically based on multiple non-crop species in natural ecosystems, and it is unclear whether the trade-offs among the leaf traits of domesticated species in agroecosystems are constrained by similar principles to those of non-crops. Agricultural environments often present strong ecological contrasts with the natural environments of non-crop species. This is because agriculture has historically been undertaken in resource-rich and low-risk areas, since farmers supply sufficient resources, including water and nutrients, and protect their crops from herbivores and pathogens (Meyer *et al.*, 2012; Zeder, 2015). A recent study confirmed that domestication has increased the leaf N and phosphorus (P) concentration by 57% in domesticated crops compared with their wild relatives (Delgado-Baquerizo *et al.*, 2016). Hence, it is logical to expect that selection for desired agronomic traits and breeding in resource-rich and predictable environments potentially shifts leaf trait correlations in domesticated crops from the LES correlations, which are based on non-crop species in natural ecosystems. A detailed investigation is required to fill important gaps in our understanding of the LES.

Despite broad recognition of the LES on multiple scales, the fundamental constraints underlying the LES are still unclear (Blonder *et al.*, 2011; Osnas *et al.*, 2013; Sack *et al.*, 2013; Niinemets, 2015; Onoda *et al.*, 2017). Several previous studies suggest that the core trait relationships of the LES are likely to operate via other traits (Shipley *et al.*, 2006; Sack *et al.*, 2013; Onoda *et al.*, 2017). For instance, Shipley *et al.* (2006) proposed that the ratio of the cell volume to cell wall volume was responsible for generating the LES. More recently, Onoda *et al.* (2017) highlighted the fundamental role of cell wall thickness and the proportion of N allocation to the cell wall in mediating the trait correlations in the LES. These studies basically focused on the role of N investment in the content of photosynthetic enzymes, especially Rubisco. However, in C_3 plants, the area-based light-saturated photosynthetic rate (A) is limited by stomatal conductance (g_s), mesophyll conductance to CO_2 (g_m), and/or the biochemistry of photosynthesis (Flexas, 2016). Under a given ambient condition, g_s relates to the leaf water status, which is largely determined by plant hydraulic conductance. Indeed, the coupling of LES traits and leaf hydraulic traits, including vein density (vein length per unit area; VLA) and leaf hydraulic conductance (K_{leaf}), has been proposed (Blonder *et al.*, 2011; Sack *et al.*, 2013; Reich, 2014), while Li *et al.* (2015) observed that leaf vein traits are decoupled from LES traits. Recently, g_m was identified as an important photosynthetic limiting factor and was related to leaf structure and biochemical traits (Flexas *et al.*, 2012; Giuliani *et al.*, 2013; Tomás *et al.*, 2013; Xiong *et al.*, 2017a). Chloroplast size, number, and arrangement, mesophyll cell wall thickness (T_{cw}), and the permeability of membranes are suggested as the major traits restricting g_m . The biochemical limitations include the amount and activities of enzymes and metabolites involved in photosynthesis and the components of the thylakoid electron transport chains. Any change in N allocations within the leaf structure may potentially change the photosynthetic limitation processes and subsequently affect A .

Rice (*Oryza sativa*) is one of the most important crops worldwide, and enhancing A is considered a primary approach to improve grain yield (Long *et al.*, 2006; Zhu *et al.*, 2010; Long *et al.*, 2015). One ambitious approach is to convert current rice from C_3 to C_4 photosynthesis by introducing the CO_2 concentrating mechanism (CCM) because the radiation use efficiency in C_4 plants is higher than in C_3 plants. However, introducing the CCM pathway requires many changes in both leaf anatomy and biochemical enzymes. In consideration of the elusive mechanistic basis of some of the photosynthetic traits and the methodological bottlenecks in certain aspects of biotechnology, simultaneous alterations of all of the limiting factors of photosynthesis, achieved by manipulating multiple genes, are unlikely to be accomplished in the near future (Zhu *et al.*, 2010; Flexas, 2016). Altering Rubisco kinetics is another approach for genetic engineering to improve the photosynthetic efficiency in crops. However, the complex assembly pathway of Rubisco and the apparent trade-offs in its kinetic parameters (Tcherkez *et al.*, 2006) indicate that creating a 'better Rubisco' is not likely, at least in the near future (Whitney *et al.*, 2011). Therefore, an efficient way to improve photosynthesis would be to exploit existing genetic variations in photosynthetic traits and the coordination among these traits in the existing genotypes. In fact, the genetic variations of leaf functional, anatomical, and biochemical traits have been observed in many crop species, including rice (Giuliani *et al.*, 2013; Gu *et al.*, 2014; Xiong *et al.*, 2017a). However, the coordination and/or trade-offs of intra-species leaf functional, anatomical, and biochemical traits (i.e. LES) has not been fully revealed.

In this study, we constructed a database that included leaf functional, anatomical, and biochemical traits of the most important cereal crop species, rice, to elucidate: (i) whether the concept of the LES can be applied within a domesticated crop and (ii) the roles of g_s and g_m in LES trait correlations. Based on these analyses, we then discuss the implications for improving photosynthesis and source use efficiency of rice along the LES.

Materials and methods

To test the correlations among the leaf traits, a database of rice leaf functional, biochemical, and structural traits of 263 genotypes growing in multiple conditions was compiled from the literature (see [Supplementary data](#) at [JXB](#) online). Because the aim of this study was to identify the potential effects of anatomical and biochemical traits on leaf function, the articles that reported one or more gas exchange traits and at least one structural or biochemical trait or leaf hydraulic conductance (K_{leaf}) were included in the database. Leaf traits vary among plant species, growth conditions, and genotypes, and to extend the rice leaf spectrum as widely as possible, we included field, outdoor pot, greenhouse, and growth chamber studies (Fig. 1). Studies of short-term treatments, including light, CO_2 , temperature, and vapour-pressure deficit (VPD), were excluded. However, studies of long-term nutrient treatments were included in the database. In this study, we considered gas exchange parameters, including A , g_s , and g_m , and leaf anatomical and structural traits including the leaf mass per leaf area (LMA), leaf vein length per area (VLA), leaf thickness (LT), leaf density (LD), volume fraction of intercellular air space (f_{IAS}), cell wall thickness (T_{cw}), mesophyll surface area exposed to the intercellular air space per leaf area (S_m), and mesophyll cell surface area occupied by chloroplasts exposed to the intercellular air space per leaf area (S_c). The LD values were calculated as LMA/LT in the case of papers only reporting the LMA and LT values. The biochemical traits included the N content and

Rubisco content. K_{leaf} was estimated using the evaporative flux method and its components, K_{leaf} inside the xylem (K_c) and outside the xylem (K_{ox}), were measured using the cutting method (e.g. Stiller *et al.*, 2003; Xiong *et al.*, 2017a). All of the data were extracted directly from the tables, text, and supplementary information in the original papers or indirectly from the figures, and all of the data were later converted to their standard units. Other information, if available, such as temperature, the maximum rate of carboxylation (V_{cmax}), and the maximum rate of electron transport (J_{max}), were also extracted for further analysis.

Many approaches have been developed to estimate g_m . In the current database, three major common methods were used: (i) the online carbon isotope discrimination method (Evans *et al.*, 1986), (ii) the combined chlorophyll fluorescence and gas exchange method (Harley *et al.*, 1992), and (iii) the curve-fitting method (Ethier & Livingston, 2004). In all of the studies providing g_m values from the curve-fitting method, parallel estimates were provided by using the combined chlorophyll fluorescence and gas exchange method, and the g_m values from these two methods are quite similar in rice (Xiong *et al.*, 2015b) as well as across other species (Carriqui *et al.*, 2015). Thus, for these studies, only the g_m values from the combined chlorophyll fluorescence and gas exchange method were used to analyse the relationship of g_m with the other traits. Several studies have compared the g_m values from the online carbon isotope discrimination method and from the combined chlorophyll fluorescence and gas exchange method, and noted a remarkable similarity in the values of g_m from these two methods (Kodama *et al.*, 2011).

The effect of environmental factors (i.e. light, CO_2 , and temperature) on the instantaneous gas exchange has been verified by many studies (Bernacchi *et al.*, 2002; Yamori *et al.*, 2011; Walker *et al.*, 2013; Xiong *et al.*, 2015a). In the current database, the gas exchange measurements were performed under light-saturated conditions, and the light was not considered to affect the inter-study comparisons. Although almost all of the studies claimed that gas exchange measurements were performed under ambient CO_2 conditions, the actual CO_2 concentrations ranged between 350 and 420 ppm with a mean of 379 ppm (Supplementary Fig. S1). Overall, the first quartile (Q1) of the CO_2 concentration was 374 ppm and the third quartile (Q3) was 391 ppm. This result indicated that across all of the studies, the CO_2 concentrations in most of the studies were very similar (~380 ppm). Leaf temperature is another important factor influencing photosynthesis, and in the current database, the leaf temperature ranged from 25.0 to 30.6 °C, with an average of 28.2 °C. This temperature range may potentially affect the rice gas exchange. However, previous studies show the variations in photosynthesis in this temperature range are quite small and the photosynthetic optical temperature of rice is around 30 °C (Yamori *et al.*, 2011; Scafaro *et al.*, 2012; von Caemmerer & Evans, 2015).

The relationships between the leaf N content (N_a) and the physiological traits (A , g_s , g_m , V_{cmax} , and J_{max}) were fitted by a logistic model (Sinclair & Horie, 1989; Rotundo & Cipriotti, 2017) as:

$$y = \alpha \left[\frac{2}{1 + e^{-\beta(N_a - \gamma)}} - 1 \right]$$

where y represents A , g_s , g_m , V_{cmax} , or J_{max} , α is the asymptotic y at high leaf N content, β is the curvature of the response, and γ is the leaf N content at which y is zero. Next, the photosynthetic N use efficiency (PNUE) was calculated as:

$$\text{PNUE} = \frac{\partial A}{\partial N_a}$$

A photosynthetic limitation analysis is a helpful tool to quantify the relative limitation of g_s , g_m , and the photosynthetic biochemistry on A (Grassi & Magnani, 2005; Buckley & Diaz-Espejo, 2015), and it has been widely used recently, especially under stress conditions (Flexas *et al.*, 2009; Galle *et al.*, 2009; Tosens *et al.*, 2016; Wang *et al.*, 2018). In this study, the photosynthetic limitations, including the relative stomatal (l_s), mesophyll (l_m), and biochemical (l_b) limitations at different N_a , were calculated using fitted A , g_s , g_m , and V_{cmax} values according to Grassi and Magnani (2005):

$$l_s = \frac{g_t/g_{sc} \times \partial A/\partial C_c}{g_t + \partial A/\partial C_c}$$

$$l_m = \frac{g_t/g_m \times \partial A/\partial C_c}{g_t + \partial A/\partial C_c}$$

$$l_b = \frac{g_t}{g_t + \partial A/\partial C_c}$$

where g_t is the total conductance, which is calculated as:

$$g_t = \frac{1}{\frac{1}{g_s} + \frac{1}{g_m}}$$

C_c is the CO_2 concentration in the chloroplasts, which is calculated as:

$$C_c = C_a - \frac{A}{g_t}$$

where C_a is the ambient CO_2 concentration, and 400 μbar was used in this study.

The differences in the slope and intercept of the bivariate relationships between rice and the global dataset (Gloptnet) were tested by using standardized major axis tests with the R package, SMATR 3v (Warton *et al.*, 2012). All of the analyses in this study were performed in R v3.4.4 (R Core Team, 2018).

Results

Variation of leaf traits in rice

Most of the leaf functional, biochemical, and anatomical traits of rice in the current dataset showed considerable variability (Fig. 1). A and g_s showed the widest variation, which was 59-fold between the highest and the lowest, and the cell wall thickness (T_{cw}) showed the narrowest variation, which was only 1.9-fold between the highest and the lowest. A varied from 0.66 to 38.8 $\mu\text{mol m}^{-2} \text{s}^{-1}$ with a median of 20.8 $\mu\text{mol m}^{-2} \text{s}^{-1}$, N_a varied from 0.47 to 2.83 g m^{-2} with a median of 1.20 g m^{-2} , and LMA varied from 24.6 to 73.8 g m^{-2} with a median of 48.7 g m^{-2} . The variation in g_m , the Rubisco concentration per leaf area, and the Rubisco concentration per leaf mass was more than 20-fold, and the variation in the mass-based light-saturated photosynthetic rate (A_m), the mass-based stomatal conductance (g_{sm}), the mass-based mesophyll conductance (g_{mm}), and the leaf density was more than 10-fold.

Coordination of leaf traits

The leaf economic trait correlations in rice were observed in this study. As shown in Fig. 2, the bivariate correlations among LMA, A_m , and N_m in the domesticated rice were generally consistent with the correlations for the natural species in Gloptnet (Wright *et al.*, 2004). However, we observed significant differences in the slopes and intercepts of those correlations between rice and Gloptnet (Fig. 2). Compared with the global database, rice tended to have a higher value of A_m at a given LMA or N_m , but the decline in N_m with increasing

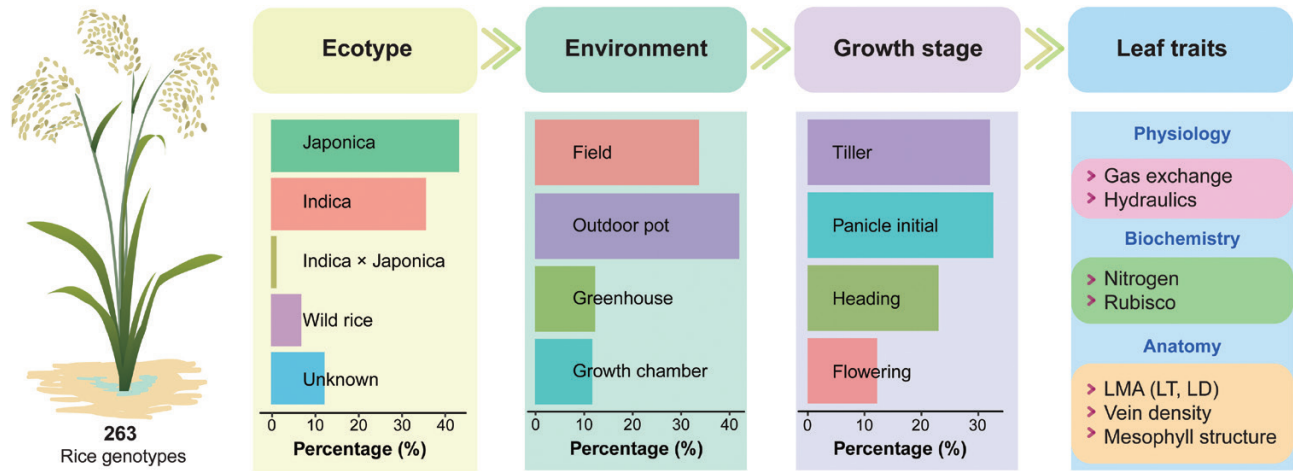


Fig. 1. Summary of rice ecotypes, growth conditions (environment), plant developmental stage, and the leaf traits used for the analysis. (This figure is available in color at JXB online.)

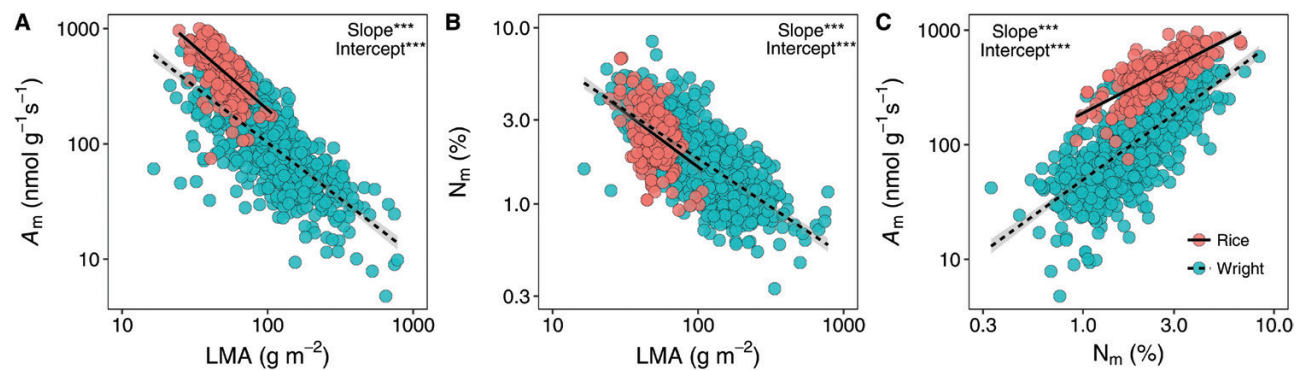


Fig. 2. Relationships among the leaf nitrogen concentration (N_m), the leaf mass per area (LMA), and the light-saturated photosynthetic rate per mass (A_m). The circles represent data from Glopnet (Wright *et al.*, 2004) and rice as shown. The gray shaded area indicates the 95% confidence interval. Solid lines are standardized major axis (SMA) lines fitted to the rice dataset, and dashed lines SMA lines fitted to the Glopnet dataset. *** $P < 0.001$. (This figure is available in color at JXB online.)

LMA in rice was large. In rice, A was positively correlated with both g_s ($r^2=0.51$, $P<0.001$) and g_m ($r^2=0.44$, $P<0.001$; Fig. 3A), and the correlations were stronger when expressed on a mass basis (Fig. 3B). However, A ($r^2=0.03$, $P=0.43$), g_s ($r^2=0.00$, $P=0.97$), and g_m ($r^2=0.04$, $P=0.053$) were independent of LMA in rice (Fig. 4; Supplementary Fig. S2). Otherwise, A and g_s increased with the leaf thickness, and decreased with leaf density. In contrast, g_m was independent of leaf thickness, as well as leaf density (Fig. 4). In this study, we also observed significant effects of the leaf anatomical and mesophyll structural traits on the leaf physiological traits (Fig. 4). A was positively correlated with the mesophyll (S_m) and chloroplast (S_c) surface exposed to the intercellular airspace but was negatively correlated with the cell wall thickness (T_{cw}). We also found a significant effect of LMA, LT, and LD on leaf hydraulic conductance (K_{leaf}). All of the estimated functional traits were independent of the leaf vein length per area (Fig. 4). Moreover, we observed significant interactions among the leaf structural traits (Fig. 4). For instance, the intercellular air space proportion (f_{IAS}), S_m , and S_c were positively correlated with LT, and f_{IAS} was negatively correlated with LD. In addition, S_m and S_c were tightly correlated.

The leaf N content was correlated with almost all of the leaf structural, functional, and biochemical traits (Fig. 4). The light-saturated photosynthetic rate (area base: $r^2=0.43$, $P<0.001$; mass base: $r^2=0.50$, $P<0.001$) the stomatal conductance (area base: $r^2=0.24$, $P<0.001$; mass base: $r^2=0.34$, $P<0.001$) and the mesophyll conductance (area base: $r^2=0.44$, $P<0.001$; mass base: $r^2=0.58$, $P<0.001$) were strongly correlated with the leaf N concentration on the area and mass base (Fig. 5). Basically, Rubisco concentration was linearly correlated with leaf N concentration, although the correlation differed strongly depending on the Rubisco estimation method (Fig. 6A). Moreover, A increased linearly with increasing Rubisco concentration at relatively low Rubisco concentrations and later levelled off (Fig. 6B). Typically, A ($r^2=0.65$; $P<0.001$), g_s ($r^2=0.55$; $P<0.001$), and g_m ($r^2=0.63$; $P<0.001$) linearly increased with K_{leaf} . However, the correlations were weaker for the mass than for the area base (Supplementary Fig. S3).

Photosynthetic N use efficiency

The photosynthetic N use efficiency (PNUE) varied 10-fold across the database (Table 1). The PNUE was strongly correlated

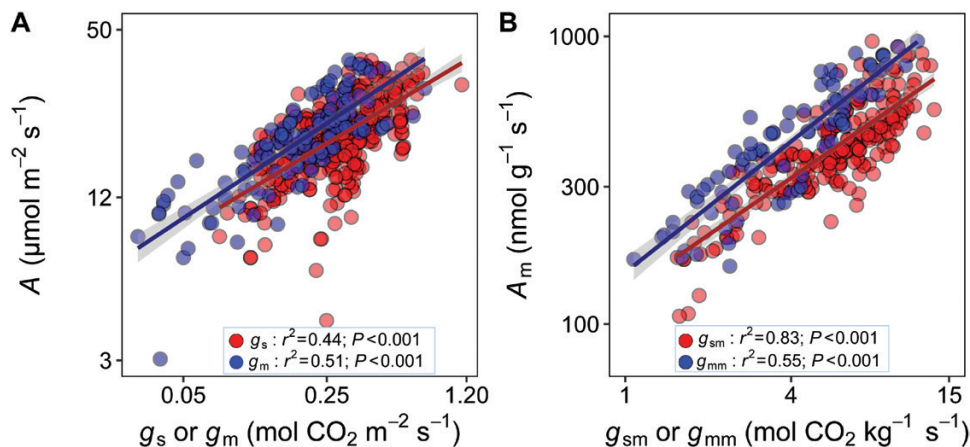


Fig. 3. Correlations of the light-saturated photosynthetic rate (A) to the stomatal conductance (g_s) and the mesophyll conductance (g_m). (A) area-based correlations; (B) mass-based correlations. The gray shaded area indicates the 95% confidence interval. (This figure is available in color at JXB online.)

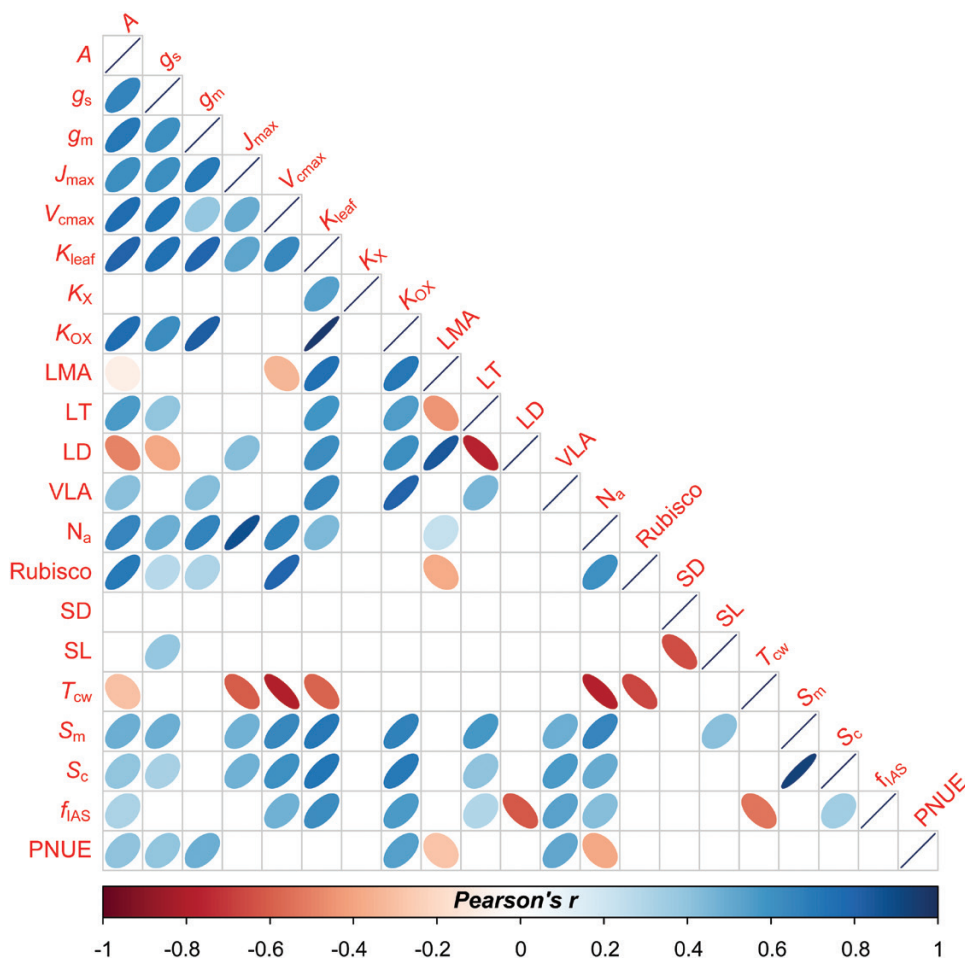


Fig. 4. Correlations between the leaf traits (area base). The full name and units of the traits are shown in Supplementary Table S1. The correlations were estimated by the linear model and the 95% confidence level was used to draw the ellipses. The significant correlations are shown ($P<0.05$). (This figure is available in color at JXB online.)

with the N_a , VLA , and LMA but not with the other leaf anatomical and biochemical traits (Fig. 4). As shown in Fig. 6 and Supplementary Fig. S4, $PNUE$ increased with A ($r^2=0.16$, $P<0.001$) and A_m ($r^2=0.22$, $P<0.001$) but decreased with LMA ($r^2=0.10$, $P<0.001$) and N_a ($r^2=0.76$, $P<0.001$). Compared with

the global species database, rice tended to have a high $PNUE$ under a given LMA , N_a , N_m , or A_m (Fig. 7; Supplementary Figs S4, S5). Moreover, as shown in Fig. 8, $PNUE$ increased with increasing g_s ($r^2=0.16$, $P<0.001$), g_{sm} ($r^2=0.28$, $P<0.001$), g_m ($r^2=0.23$, $P<0.001$), and g_{mm} ($r^2=0.13$, $P<0.001$).

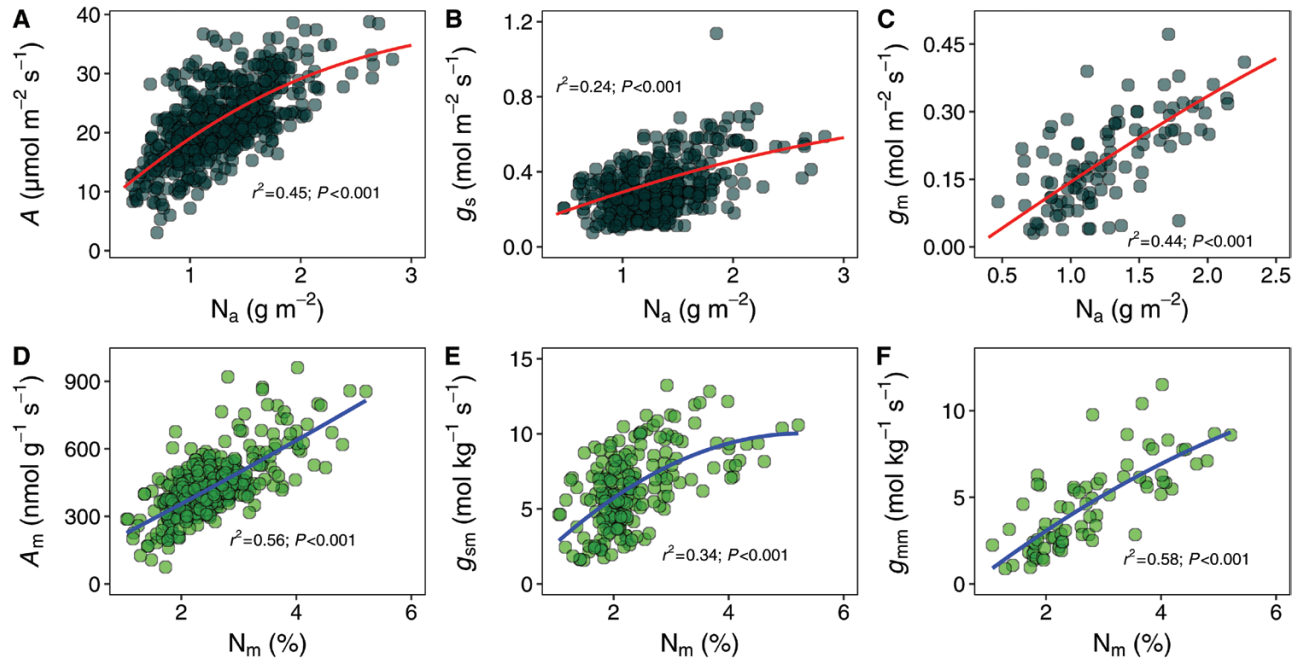


Fig. 5. Effects of leaf nitrogen (N) concentration on the light-saturated photosynthetic rate (A) (A, D), the stomatal conductance (g_s) (B, E), and the mesophyll conductance (g_m) (C, F). (A–C) Area-based correlations; (D–F) mass-based correlations. The correlations were fitted by the logistic model as described in ‘Materials and methods’. (This figure is available in color at JXB online.)

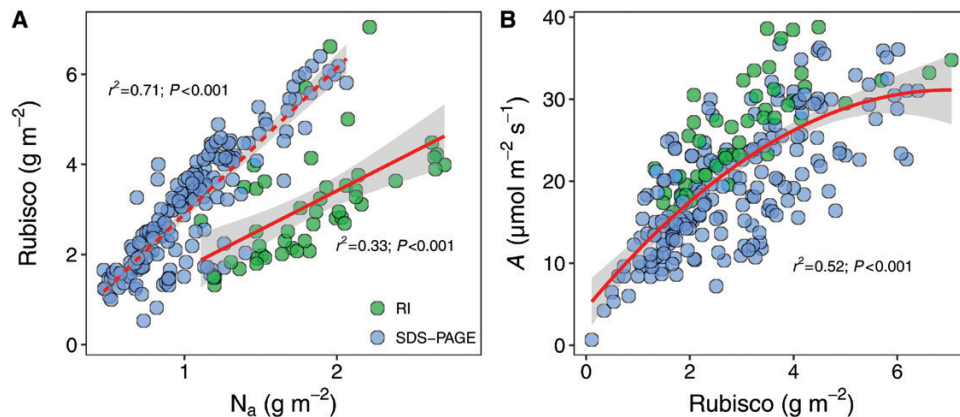


Fig. 6. (A) Correlation between the N concentration per area (N_a) and the Rubisco concentration, and (B) the correlation between the light-saturated photosynthetic rate (A) and the Rubisco concentration. RI, Rubisco concentration estimated by the radial immunodiffusion method; SDS-PAGE, Rubisco concentration estimated by SDS-PAGE. The gray shaded area indicates the 95% confidence interval. (This figure is available in color at JXB online.)

Relative photosynthetic limitation

In this study, A , g_s , g_m , V_{cmax} , and J_{max} were modeled using the logistic model with the input of the leaf N content per area (N_a), and the outputs shown in Table 2. The curvature (β) of the relationship between A and N_a was the highest, and the value of β of the relationship between g_m and N_a was the lowest. To estimate the sensitivity of the photosynthetic traits to N_a , the values of A , g_s , g_m , V_{cmax} , and J_{max} were normalized by their values at 1.0 g m⁻² of N_a (Fig. 9). The results showed that g_m was the most N-sensitive trait, and V_{cmax} was the most N-insensitive trait. We analysed the relative photosynthetic limitation based on the modeled photosynthetic traits. The leaf N status had a strong influence on the relative stomatal (l_s), mesophyll (l_m), and biochemical (l_b), limitations in rice (Fig. 9). Overall, the biochemical limitation was the major

photosynthetic limiting factor, which contributed more than 60% of the relative photosynthetic limitations, while l_s contributed than 10%. l_m strongly declined with N_a , but l_b increased with N_a .

Discussion

Leaf economics spectrum

For a given species, leaf traits vary depending on (i) the genotype, (ii) the growth environment and crop management, and (iii) the leaf and plant age (Wright *et al.*, 2004; Niinemets, 2015). In this study, 263 rice genotypes, which covered all of the ecotypes of rice growing under field, outdoor, greenhouse and/or growth chamber conditions, were included in our dataset (Fig. 1). Moreover, we also included multiple N

management, growth stage, and leaf age variables. Considering that it would be almost impossible to estimate the leaf spectrum traits of all existing rice genotypes at all growth stages under all possible growth conditions, our dataset is a reasonable subset to represent the rice leaf spectrum.

Table 1. Functional, biochemical, and anatomical trait variations in rice

Trait	Min.	Q1	Median	Mean	Q3	Max.
Function						
A	0.66	16.3	20.8	21.2	25.4	38.8
g_s	0.075	0.219	0.290	0.313	0.383	1.138
g_m	0.03	0.129	0.210	0.218	0.295	0.75
K_{leaf}	3.31	4.99	7.20	7.77	10.72	13.46
V_{cmax}	41.2	84.0	100.0	103.1	118.8	163.0
J_{max}	59.4	111.4	140.9	149.3	170.3	308.3
A_m	74.77	337.7	417.7	456.8	543.6	998.5
g_{sm}	1.57	4.45	6.27	6.39	8.14	19.75
g_{mm}	0.87	2.66	4.38	4.67	6.17	11.51
K_{leafm}	0.122	0.135	0.199	0.199	0.263	0.307
Biochemistry						
N_a	0.47	0.97	1.20	1.25	1.49	2.83
N_m	0.92	1.94	2.24	2.47	2.81	6.73
Rubisco	0.11	1.74	2.68	2.90	3.74	7.05
Rubisco _m	0.032	0.058	0.087	0.094	0.126	0.207
Anatomy						
LMA	24.6	42.8	48.7	49.2	56.1	73.8
VLA	2.98	3.98	4.34	4.62	5.28	6.73
VLA _m	0.073	0.095	0.112	0.118	0.140	0.192
LT	0.058	0.084	0.132	0.148	0.216	0.282
LD	0.039	0.098	0.136	0.210	0.213	0.654
f_{PAS}	7.7	16.7	19.5	19.3	23.1	28.8
T_{cw}	0.125	0.155	0.167	0.168	0.183	0.235
S_m	6.27	11.24	15.72	16.48	19.26	31.20
S_c	6.43	11.54	15.72	15.81	18.04	30.59
Efficiency						
PNUE	4.33	14.52	17.31	18.12	21.1	44.4

The full name and units of the traits are shown in Supplementary Table S1. Min, minimum value; Q1, the first quartile; Q3, the third quartile; Max., Maximum value.

Contrasting with two recent studies that indicated that the leaf trait correlations within a species may differ from the correlations among species (Anderegg *et al.*, 2018; Osnas *et al.*, 2018), the patterns of the leaf functional and anatomical trait correlations of rice were generally consistent with previously reported correlations in global (Wright *et al.*, 2004) and intraspecies LESs (Gagliardi *et al.*, 2015; Niinemets, 2015; Martin *et al.*, 2017). The significant trait covariations observed in this study suggested that the fundamental ecophysiological trade-off applies not only to natural species but also to domesticated species. This raises another question of whether human selection for traits in domesticated species has modified the leaf trait trade-offs from the global economic spectrum.

The light-saturated photosynthetic rate of domesticated rice was significantly higher than that in natural species in the Glopnet dataset at a given leaf N level, which indicated that the PNUE was improved by human selection in rice. In fact, an enhancement in A during breeding progress was observed in previous studies (Fischer *et al.*, 1998; Koester *et al.*, 2016). Although more information is needed to reveal the mechanism of PNUE enhancement in rice, greater N allocation to Rubisco may be one. The Rubisco concentration in rice is almost twice as high as in the natural species under a given leaf N level (Supplementary Fig. S6). A further difference from the Glopnet dataset was that both A_m and N_m decline faster with LMA in rice, suggesting that the influence of leaf structure on function were stronger in rice than in the native species in the Glopnet. Several recent studies demonstrated that correlations in the global LES arise, in part, by mathematical necessity (Lloyd *et al.*, 2013; Osnas *et al.*, 2013). However, these correlations reflect the physical effects of leaf structure on physiology (Sack *et al.*, 2013). In the current study, the different trait correlations between the two datasets was caused by biological factors because the same mathematical method was used. Moreover, the area-based bivariate correlations also support that the effects of leaf structure on physiological processes are stronger in rice (Fig. 4).

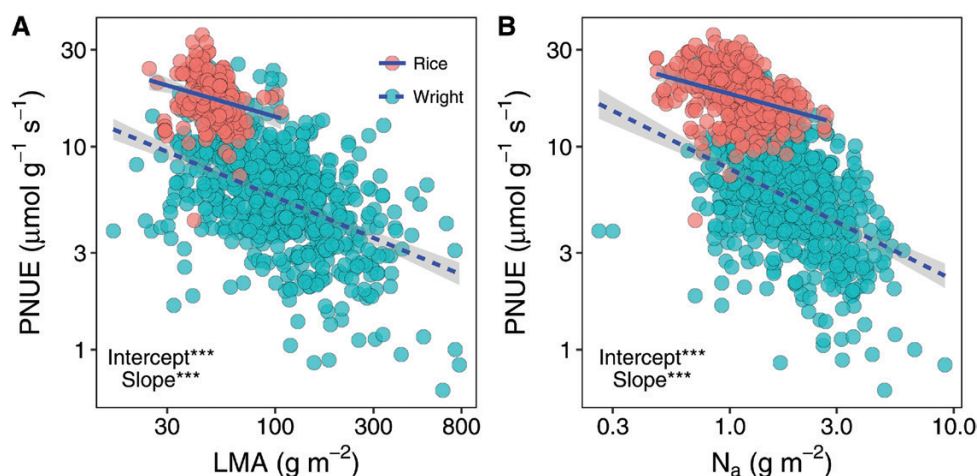


Fig. 7. Effects of leaf mass per area (LMA) (A) and N concentration per leaf area (N_a) (B) on photosynthetic N use efficiency (PNUE). The gray shaded area indicates the 95% confidence interval. Solid lines are standardized major axis (SMA) lines fitted to the rice dataset, and dashed lines SMA lines fitted to the Glopnet dataset. *** $P < 0.001$. (This figure is available in color at JXB online.)

Photosynthetic limitations in rice

The photosynthetic limitation factors in C_3 plants have been widely investigated and three have been identified, namely g_s , g_m , and the biochemistry of photosynthesis, which includes the enzymes and metabolites involved in photosynthesis and the components of the thylakoid electron transport chain (Flexas, 2016). The positive relationships between photosynthetic rate and CO_2 diffusion conductance, including the stomatal and mesophyll conductance, on both the area and mass base, indicated important limiting roles of g_s and g_m on rice photosynthesis (Fig. 3). Moreover, A was also tightly correlated with V_{cmax} and J_{max} , which supports a biochemical limitation in photosynthesis. In fact, previous studies suggested that the major photosynthetic limiting factors in C_3 plant leaves are modified by slight changes in the growing conditions (Yamori *et al.*, 2011; Wang *et al.*, 2018). Therefore, the LES trait trade-offs may relate to the photosynthetic limitations, shifting the cause to leaf anatomy and/or biochemical changes.

g_s is dependent on both the stomatal features, namely density and size and stomatal opening status. In our dataset, g_s of rice is independent of stomatal density and size (Fig. 4), which agreed with previous studies, suggesting that stomatal density and size mainly determine the maximum theoretical stomatal conductance, rather than the operational g_s (Franks & Beerling, 2009; Bartlett *et al.*, 2016; Xiong *et al.*, 2017a). The regulation of stomatal opening is complex, and many factors, including leaf water potential and abscisic acid concentration, are involved. In addition, under a given ambient condition, stable

Table 2. Fitted parameters relating light-saturated photosynthetic rate (A), stomatal conductance (g_s), mesophyll conductance (g_m), maximum carboxylation rate (V_{cmax}), and maximum electron transport rate (J_{max}) in responding to leaf N content per area (N_a)

Trait	Fitted parameters			Value at $N_a=1.0$
	α	β	γ	
A	40.04	0.8117	-0.2737	19.03
g_s	0.8044	0.5350	-0.4183	0.2913
g_m	0.8623	0.4813	0.3003	0.1438
V_{cmax}	182.6	0.7052	-0.5763	92.17
J_{max}	300.6	0.5600	-0.3988	116.7

g_s is predominantly determined by the water supplement of the plant. In this study, the strong positive correlation between g_s and K_{leaf} supported a mechanistic relationship between the carbon assimilation and plant hydraulics (Brodribb *et al.*, 2005, 2007; Xiong *et al.*, 2015b, 2017a; Scoffoni *et al.*, 2016).

In C_3 plants, photosynthesis and respiration primarily occur in the mesophyll; therefore, the trade-offs among the mesophyll structural, biochemical, and physiological traits are largely represented the LES (Onoda *et al.*, 2017). As one of the photosynthetic limiting factors, g_m is proposed to be related to both mesophyll structure and biochemistry (Evans *et al.*, 2009; Flexas *et al.*, 2012; Xiong *et al.*, 2015a, 2017b). Contrary to several previous studies (Tomás *et al.*, 2013; Tosens *et al.*, 2016; Xiong *et al.*, 2017a), g_m was independent of mesophyll structural traits in the current study. However, our results are consistent with the results obtained by Giuliani *et al.* (2013), who observed no correlation between g_m and mesophyll structural features across 24 genotypes of rice. Considering the important role of mesophyll structures in g_m , the lack of correlation in this instance may be partly attributable to the narrow range of structural traits in the current dataset (Table 1). Our results indicated that g_m in rice might be predominantly regulated by biochemical factors. In fact, g_m was positively correlated with N_a , which is one of the most important leaf biochemical traits in determining leaf functions. However, a further analysis showed that the effects of N_a on g_m might be mediated by both biochemical factors and mesophyll structure. On one hand, a high N_a may promote aquaporin gene expression (Hacke *et al.*, 2010; Ding *et al.*, 2016) and the subsequent accumulation of aquaporins would enhance g_m by improving the permeability of biological membrane (Flexas *et al.*, 2006; Uehlein *et al.*, 2012; Mori *et al.*, 2014). Conversely, there were tight correlations between N_a and leaf structural traits, including S_c and T_{cw} (Fig. 4). A leaf with a high N_a tends to have a large size and/or number of chloroplasts, and hence a large S_m and S_c (Xiong *et al.*, 2015a). A negative relationship was observed between N_a and T_{cw} and may indicate that chloroplasts in leaves with low photosynthetic capacity may require thick and/or flexible cell walls to avoid excess light energy absorption, and the cell wall is known to restrict the diffusion of CO_2 in the mesophyll.

According to the Farquhar–Berry–von Caemmerer model (Farquhar *et al.*, 1980), the biochemical limitation largely

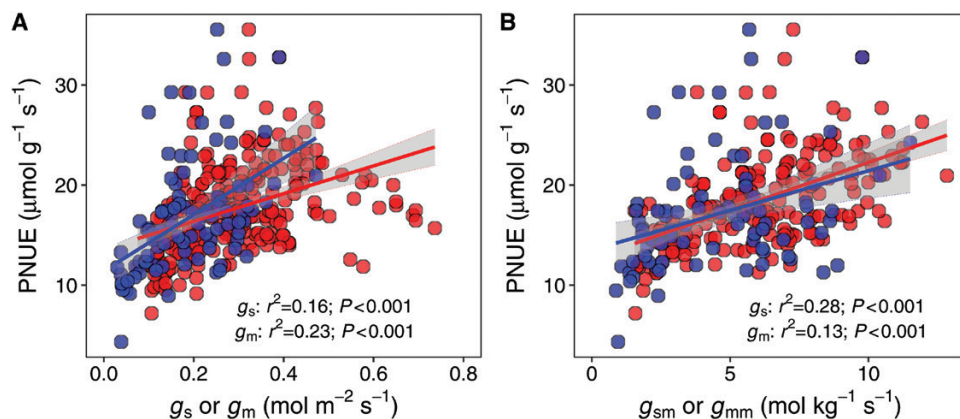


Fig. 8. Contributions of the area- (A) and mass-based (B) stomatal conductance and mesophyll conductance to photosynthetic N use efficiency (PNUe). The gray shaded area indicates the 95% confidence interval. (This figure is available in color at JXB online.)

relates to the carboxylation capacity of Rubisco and the ribulose 1,5-bisphosphate regeneration rate, which is represented by V_{cmax} and J_{max} , respectively. It is clear that V_{cmax} and J_{max} are highly dependent on the Calvin–Benson cycle and the amounts and activities of electron transport proteins. The allocation of leaf N to those photosynthetic proteins, especially Rubisco, is suggested to be determined by the species position in the LES (Onoda *et al.*, 2017). In this study, the Rubisco content per area increased linearly with N_a in rice, indicating a relatively constant proportion of N allocation to Rubisco (Fig. 6). However, the non-linear correlation between N_a and V_{cmax} indicated that Rubisco activity might vary with N_a . Indeed, a reduction of Rubisco activation states (the ratio of the initial activity and the total activity) with an increase in N_a has been observed in previous studies (Cheng & Fuchigami, 2000; Warren & Adams, 2001). This result suggests that a V_{cmax} estimate based only on the Rubisco content (e.g. Buckley & Warren, 2014) may need to be calibrated by the Rubisco activation states. The mechanism of the low Rubisco activation state in the high N_a leaf is unclear, although the Rubisco activase activity, the ATP supplement capacity, and the storage function of Rubisco are suspected to cause the deactivation of Rubisco in high N leaves (Cheng & Fuchigami, 2000; Yamori *et al.*, 2006, 2012).

The photosynthetic limitation factors are apparently influenced by the leaf N concentration due to the profound effects of N on both leaf structural and leaf biochemical traits (Fig. 3). Therefore, the responses of the photosynthetic limitation factors to N_a may potentially exploit the mechanisms of the curvilinear correlation between the A and N_a . In fact, g_s , g_m , and V_{cmax} responded differently to N_a in rice, and g_m was the most N-sensitive trait. The dramatic responses of g_m to N_a might be primarily caused by the enlargement of the chloroplasts and, to a lesser extent, the increasing S_c in the high N_a leaf (Xiong *et al.*, 2015a). More importantly, the relative limitation analysis suggested that g_m contributed the major relative limitation in A in the low N_a leaf. However, due to the dramatic increase in g_m with N_a , photosynthetic biochemistry (i.e. V_{cmax} and J_{max}) contributed the largest relative photosynthetic limitation in the high N_a leaf (Fig. 9). The results suggest that more attention should be paid to photosynthetic biochemistry for future A , as well as PNUE improvement in rice. However, we note that there are substantial variations in g_s and g_m , and also biochemical limitations across genotypes, and therefore the photosynthetic limiting factors are actually genotype specific.

In summary, this study focused on the leaf structural, biochemical, and physiological trait variations and trade-offs in domesticated rice. The major bivariate correlations, including A_m vs. N_m , A_m vs. LMA, and N_m vs. LMA, of the LES traits in rice were shifted in comparison with the global LES dataset. A_m was higher in rice than in the natural species in the Glopnet at a given N_a , and the breeding process in the past has therefore improved the PNUE of rice. The photosynthetic traits, including g_s , g_m , V_{cmax} , and J_{max} , were sensitive to the leaf structural and biochemical traits, and all of these traits increased with N_a in rice. Due to the asynchronous responses of the photosynthetic traits to the changes in N_a , the major photosynthetic limitation steps were altered dramatically with N_a , and biochemistry was the major limiting factor at an N_a above 1.0 g m^{-2} . The leaf trait

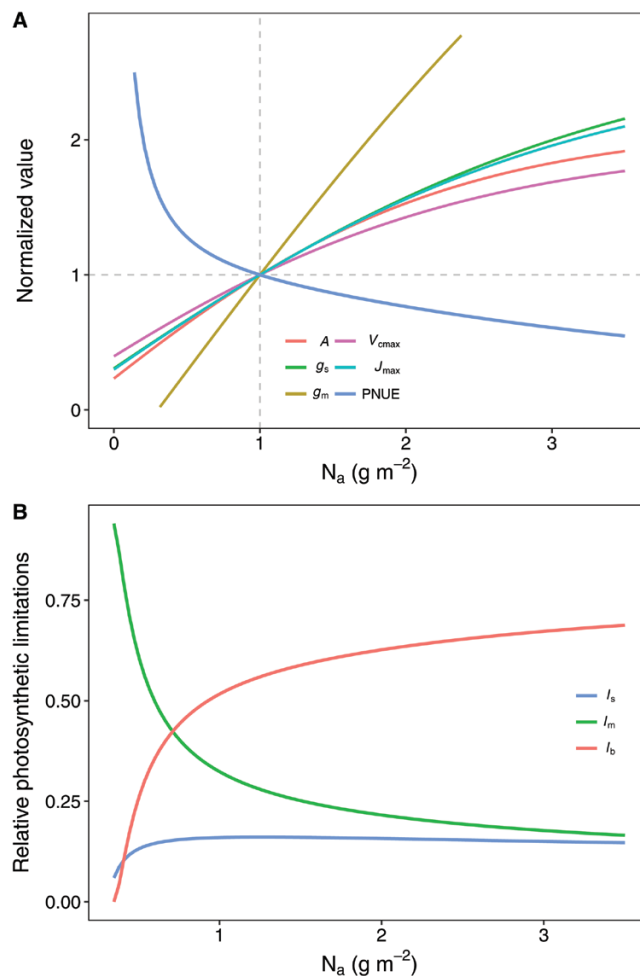


Fig. 9. (A) Responses of light-saturated photosynthetic rate (A), stomatal conductance (g_s), mesophyll conductance (g_m), maximum carboxylation rate (V_{cmax}), maximum electron transport rate (J_{max}), and photosynthetic N use efficiency (PNUE) to leaf N content per area (N_a). (B) Changes in the relative photosynthetic limitation of stomatal conductance (l_s), mesophyll conductance (l_m), and biochemistry (l_b) with N_a . In (A), the parameters were normalized by dividing their values by 1.0 g m^{-2} of N_a .

trade-offs underlying the general LES should be considered for future photosynthetic improvement in crops.

Supplementary data

Supplementary data are available at *JXB* online.

Table S1. List of leaf traits in this study, and symbols and units adopted.

Fig. S1. Leaf temperature and the reference CO_2 concentration inside the cuvette for gas exchange measurements.

Fig. S2. Influences of the leaf mass per area (LMA) on the light-saturated photosynthetic rate (A), the stomatal conductance (g_s) and the mesophyll conductance (g_m).

Fig. S3. Correlations of the leaf hydraulic conductance (K_{leaf}) to the light-saturated photosynthetic rate (A), the stomatal conductance (g_s), and the mesophyll conductance (g_m) in rice.

Fig. S4. Correlations between the area-based light-saturated photosynthetic rate (A) and the photosynthetic N use efficiency (PNUE), and between the mass-based light-saturated photosynthetic rate (A_m) and the PNUE.

Fig. S5. Effects of the N concentration per leaf mass (N_m) and the mass-based light-saturated photosynthetic rate (A_m) on the photosynthetic N use efficiency (PNUE).

Fig. S6. The correlation between Rubisco content and leaf N content within rice or among natural species (data from Onoda *et al.*, 2017).

Data deposition

The data for the results presented here are available at the Dryad Digital Repository: <https://doi.org/10.5061/dryad.6060q21> (Xiong and Flexas, 2018).

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Author contributions

DX planned and designed the research and collected data, and DX and JF analysed the data and wrote the manuscript.

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