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# $C_4$ photosynthesis, trait spectra, and the fast-efficient phenotype

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## Summary

It has been 60 years since the discovery of $C_4$ photosynthesis, an event that rewrote our understanding of plant adaptation, ecosystem responses to global change, and global food security. Despite six decades of research, one aspect of $C_4$ photosynthesis that remains poorly understood is how the pathway fits into the broader context of adaptive trait spectra, which form our modern view of functional trait ecology. The $C_4$ $CO_2$-concentrating mechanism supports a general $C_4$ plant phenotype capable of fast growth and high resource-use efficiencies. The fast-efficient $C_4$ phenotype has the potential to operate at high productivity rates, while allowing for less biomass allocation to root production and nutrient acquisition, thereby providing opportunities for the evolution of novel trait covariances and the exploitation of new ecological niches. We propose the placement of the $C_4$ fast-efficient phenotype near the acquisitive pole of the world-wide leaf economic spectrum, but with a pathway-specific span of trait space, wherein selection shapes both acquisitive and conservative adaptive strategies. A trait-based perspective of $C_4$ photosynthesis will open new paths to crop improvement, global biogeochemical modeling, the management of invasive species, and the restoration of disturbed ecosystems, particularly in grasslands.

## I. Introduction

The $C_3$ photosynthetic pathway is the ancestral means by which all autotrophic terrestrial plants capture solar energy, drive oxidation–reduction reactions, and synthesize sugars from assimilated atmospheric $CO_2$ (Sharkey *et al*., 2012). In $C_3$ plants, the biochemical rate constants and physiological constraints associated with photosynthesis are highly dependent on atmospheric composition, especially $CO_2$ and $O_2$ partial pressures, as well as leaf temperature (Dusenge *et al*., 2019). As $CO_2$ and $O_2$ partial pressures changed during past geological periods, limitations appeared in the carboxylation efficiency of ribulose 1,5-bisphosphate carboxylase/oxygenase (Rubisco), the enzyme catalyzing the first reaction of $C_3$ photosynthesis (Andersson, 2008; Erb & Zarzycki, 2018). The evolution of Rubisco isoforms that are able to completely overcome these limitations has not occurred. Rather, natural selection has more often favored changes in the cellular systems that deliver $CO_2$ to Rubisco. One of the most well-studied systems of this type is the $C_4$ $CO_2$-concentrating mechanism (CCM) – a metabolic accessory to $C_3$ photosynthesis (Ehleringer & Monson, 1993; Christin & Osborne, 2014; Schlüter & Weber, 2020). Major abbreviations used in this paper are defined in Box 1.

Although $C_4$ plants are only a small fraction (< 5%) of terrestrial species, they contribute up to 25% to global gross primary productivity (GPP; Still *et al*., 2003; Yan *et al*., 2015; Luo *et al*., 2024), rendering them highly relevant to economic and ecological sustainability (Furbank, 1998). For example, most $C_4$ plants are grasses and sedges native to tropical and subtropical savannas and semi-arid steppes (Kellogg & Campbell, 1987; Sage *et al*., 1999; Edwards *et al*., 2010), giving them an important role in the productivity of grazed rangelands (Bodner & Robles, 2017; Uddin & Kebreab, 2020). Furthermore, species with the $C_4$ pathway include some of the world's most productive crops (e.g. maize, sorghum, and sugarcane), as well as some of the most pernicious weeds in both managed and natural ecosystems (Rolim *et al*., 2015; Korres *et al*., 2016; Catford *et al*., 2020). All of the services and challenges provided by $C_4$ species are susceptible to future shifts in the Earth system, including changes in climate, land use, and the influences of invasive species. Being able to understand how these changes affect the function of global photosynthesis and vegetation productivity will greatly assist

**Box 1.** Abbreviations used in the text and boxes

| | |
|---|---|
| *A* | leaf net photosynthesis rate (μmol $m^{-2}$ $s^{-1}$) |
| $A_{area}$ | net photosynthesis rate specified per unit leaf area (μmol $m^{-2}$ $s^{-1}$) |
| $A_{mass}$ | net photosynthesis rate specified per unit leaf mass (μmol $g^{-1}$ $s^{-1}$) |
| BSC | bundle sheath cell |
| CCM | $CO_2$-concentrating mechanism |
| $c_i$ | intercellular $CO_2$ concentration (μmol $mol^{-1}$) |
| *E* | leaf transpiration rate (mmol $m^{-2}$ $s^{-1}$) |
| $g_s$ | stomatal conductance (mol $m^{-2}$ $s^{-1}$) |
| G3P | glyceraldehyde 3-P |
| iWUE | intrinsic water use efficiency ($A_{area}/g_s$) |
| IVD | leaf interveinal distance (mm) |
| LFT | lineage functional type |
| LLS | leaf life span (months) |
| LMA | leaf mass per unit area (g $m^{-2}$) |
| MC | mesophyll cell |
| $N_{mass}$ | nitrogen concentration per unit leaf mass (g $g^{-1}$) |
| PEPc | phosphoenolpyruvate carboxylase |
| PFT | plant functional type |
| PLUE | photosynthetic light-use efficiency (mol $CO_2$ $mol^{-1}$ photons absorbed) |
| $P_{mass}$ | phosphorus concentration per unit leaf mass (g $g^{-1}$) |
| PNUE | photosynthetic nitrogen-use efficiency (mol $CO_2$ $mol^{-1}$ leaf N) |
| PWUE | photosynthetic water-use efficiency (mol $CO_2$ assimilated $mol^{-1}$ $H_2O$ transpired) |
| RGR | relative growth rate (g biomass $g^{-1}$ $d^{-1}$) |
| $R_{mass}$ | respiration rate per unit leaf mass (μmol $g^{-1}$ $s^{-1}$) |
| Rubisco | ribulose 1,5-bisphosphate carboxylase/oxygenase |
| RuBP | ribulose 1,5-bisphosphate |
| TRY | trait database |
| WLES | world-wide leaf economic spectrum |

people in their efforts to manage and conserve Earth's natural capital for the benefit of future generations.

Our ability to predict patterns of $C_4$ plant productivity and responses to future environmental change is limited by a lack of theory, data, and models that link the $C_4$ CCM to a broader set of plant traits, especially those that determine whole-plant growth rates, lifetime resource-use efficiencies, and ultimately fitness. Shortly after the discovery of the $C_4$ pathway, the potential adaptive advantages of the CCM regarding higher leaf $CO_2$ assimilation rates (*A*) and photosynthetic water-use efficiencies (PWUE; *A*/*E*, where *E* is leaf transpiration rate), were recognized (Björkman, 1971; Downton, 1971). Since that time, our understanding of $C_4$ biochemistry has progressed and the $C_4$ CCM has been incorporated into leaf-scale models providing a detailed understanding and predictive capability of the $C_4$ $CO_2$ assimilation rate and its limitations due to light, temperature, and $CO_2$ concentrations (Edwards *et al.*, 1985; von Caemmerer & Furbank, 2003; von Caemmerer, 2021). However, we still do not fully understand how the evolution of the $C_4$ pathway has shaped the synergies and trade-offs among other plant traits, many of which have come under recent scrutiny within the context of world-wide trait economic spectra (Wright *et al.*, 2004; Reich, 2014; Díaz *et al.*, 2016; Liu *et al.*, 2019). This deficiency has not only narrowed our perspectives on the adaptive advantages of $C_4$ photosynthesis, but it has also prevented us from improving models for predicting how the distribution of $C_4$ plants will respond to future climate change (Sage & Kubien, 2003; Monson & Collatz, 2012; Fox *et al.*, 2018; Still *et al.*, 2019). Furthermore, it has hampered our ability to design new strategies for improving the performance of $C_4$ crops (Gong *et al.*, 2015; Leakey *et al.*, 2019; Jobe *et al.*, 2020) or engineering the $C_4$ pathway into $C_3$ crops (Mitchell & Sheehy, 2006; Ermakova *et al.*, 2019).

Our aims in writing this synthesis were to place $C_4$ photosynthesis more directly within the scope of past leaf economic spectra and to explore the potential for the $C_4$ CCM to produce novel forms of trait covariances. We have addressed three primary questions: (1) What are the principal trait correlations in $C_4$ species and how do they differ from $C_3$ species? (2) How do $C_4$ leaf trait covariances shape the adaptive strategies of fast or slow growth and high or low resource-use efficiency? (3) Where are $C_4$ trait combinations best placed within the $C_3$-dominated perspectives of the world-wide leaf economic spectrum (WLES)?

## II. The $C_4$ CCM influences plant traits by altering the dependence of photosynthesis on light, nitrogen, and water

Evolution of the CCM in $C_4$ lineages has fundamentally altered photosynthetic nitrogen-use efficiency (PNUE), PWUE, and photosynthetic light-use efficiency (PLUE), relative to the $C_3$ ancestral state, providing adaptive advantages under specific environmental conditions (Ehleringer & Monson, 1993; Ehleringer *et al.*, 1997; Christin & Osborne, 2014). The $C_4$ advantage is a consequence of the CCM and its capacity to enhance the catalytic efficiency of Rubisco. The $C_4$ CCM works as a biochemical pump that actively transports $CO_2$ against a diffusive gradient (from low to high $CO_2$ concentration) and thereby concentrating it at the active sites of Rubisco. This allows $C_4$ leaves to achieve higher $CO_2$ assimilation rates (*A*) at lower intercellular $CO_2$ concentrations ($c_i$). A brief description of the biochemical features of the $C_4$ CCM is provided in Box 2. For a more complete recent review of the biochemistry and anatomy of $C_4$ photosynthesis, see Schlüter & Weber (2020).

Operation of the CCM in $C_4$ leaves requires energy beyond that utilized by the photosynthetic and photorespiratory pathways in $C_3$ leaves. This is observed as a lower PLUE in $C_4$ leaves at leaf temperatures below 20–25°C and high $CO_2$ concentrations, wherein $C_3$ photorespiration rates are relatively low. At low light, PLUE is quantified as the net $CO_2$ assimilation rate per mole of photons absorbed and is equivalent to the photosynthetic quantum yield (Ehleringer *et al.*, 1997). The principal limitations to $CO_2$ assimilation in $C_3$ leaves at low light intensities are the rate at which ribulose 1,5-bisphosphate (RuBP) can be regenerated by the thylakoid electron transport processes, and the rate at which photorespiratory metabolites are recycled. In $C_4$ leaves at low light, *A* is determined by the rates at which both RuBP and phosphoenolpyruvate (PEP) are regenerated. At leaf temperatures above 20–25°C and lower $CO_2$ concentrations, the higher energetic cost of the $C_4$ CCM is more than offset by the even higher energetic cost of recycling photorespiratory intermediates in $C_3$ leaves, leading to a $C_4$ advantage (Ehleringer *et al.*, 1997).

At high light intensities, limitations to *A* due to thylakoid electron transport processes are relaxed in both $C_3$ and $C_4$ leaves. In this case, *A* is most limited by the catalytic activity of Rubisco in $C_3$ leaves, and the catalytic activities of phosphoenolpyruvate carboxylase (PEPc) and Rubisco in $C_4$ leaves. In $C_4$ leaves at high light intensities, higher $CO_2$ concentrations at the active sites of Rubisco drive higher RuBP utilization rates, leading to higher *A* per unit of Rubisco protein than that in $C_3$ leaves. In the absence of a CCM, the only way for a $C_3$ species to achieve $CO_2$ assimilation rates that would approach those of a $C_4$ species would be for the $C_3$ species to allocate a higher fraction of its available nitrogen (N) to the photosynthetic apparatus, thereby increasing the leaf's carboxylation capacity (Sage *et al.*, 1987; Masle *et al.*, 1993; Sharkey *et al.*, 2012). However, in the absence of a CCM, and assuming no increase in $g_s$, each mole of N devoted to photosynthetic proteins in a $C_3$ leaf would operate at a lower $c_i$, thereby decreasing leaf PNUE. The decrease in PNUE could be mitigated by an increase in $g_s$, and therefore $c_i$, but only at the cost of a lower PWUE. In essence, in $C_3$ species, an increase in carboxylation capacity to achieve a higher value of *A* requires a trade-off to a lower PNUE or a lower PWUE, compared with $C_4$ species. The trade-offs affecting *A*, PNUE, and PWUE are weakened in $C_4$ leaves because of the presence of the CCM, allowing $C_4$ species to achieve higher *A* at lower leaf N concentrations and without increasing $g_s$.

In one comparative analysis, Sage *et al.* (1987) observed that 25% of the leaf N was allocated to Rubisco in the $C_3$ dicot species, *Chenopodium album*, compared with 9% in the $C_4$ dicot species, *Amaranthus retroflexus*, despite similar photosynthetic capacities. It was estimated that if Rubisco in the $C_3$ species were able to operate

**Box 2.** Rubisco and the $C_4$ CO2-concentrating mechanism (CCM)

A key evolutionary innovation in $C_4$ photosynthesis is the substitution of Rubisco for the enzyme phosphoenolpyruvate carboxylase (PEPc) to catalyze the fixation of atmospheric $CO_2$ in leaf mesophyll cells (MCs). In the MCs of $C_4$ leaves, the assimilation of atmospheric $CO_2$ occurs as $HCO_3^-$, which allows PEPc to operate catalytically within an inorganic carbon pool that is *c.* 100 times larger than the enzyme's $K_m$ affinity for that carbon. This is different from Rubisco in $C_3$ leaves, which in today's atmosphere operates at $CO_2$ concentrations near its $K_m$ for $CO_2$, which consequently imposes greater affinity limitations on its catalytic activity. Furthermore, under normal conditions, the turnover number for each active site of PEPc is *c.* 10 times higher than that for Rubisco, providing a considerable advantage in the velocity at which $CO_2$ is moved from the gas phase of leaf intercellular air spaces to the pool of organic compounds used in photosynthesis.

In $C_4$ plants, Rubisco is isolated to bundle sheath cells (BSCs), which are located at the interior of the leaf and have low exposure to the leaf's intercellular air spaces. In $C_4$ leaves, carbon fixed by PEPc is transferred from MCs to BSCs. The serial rates of PEPc and Rubisco carboxylation reach a steady state only when the $CO_2$ concentration of the BSCs is *c.* 10 times higher than ambient. This system of $CO_2$ transfer and accumulation in BSCs provides the opportunity to isolate Rubisco and creates a cellular microenvironment that better resembles the high-$CO_2$ atmosphere of past geological periods, during which Rubisco evolved. The principal advantages of $C_4$ photosynthesis are twofold, including high rates of carboxylation and low rates of competitive oxygenation at the active site of Rubisco. Higher rates of leaf $CO_2$ assimilation in $C_4$ species influence patterns of trait selection, including those controlling whole-plant growth rate and resource use efficiency (Fig. B2).

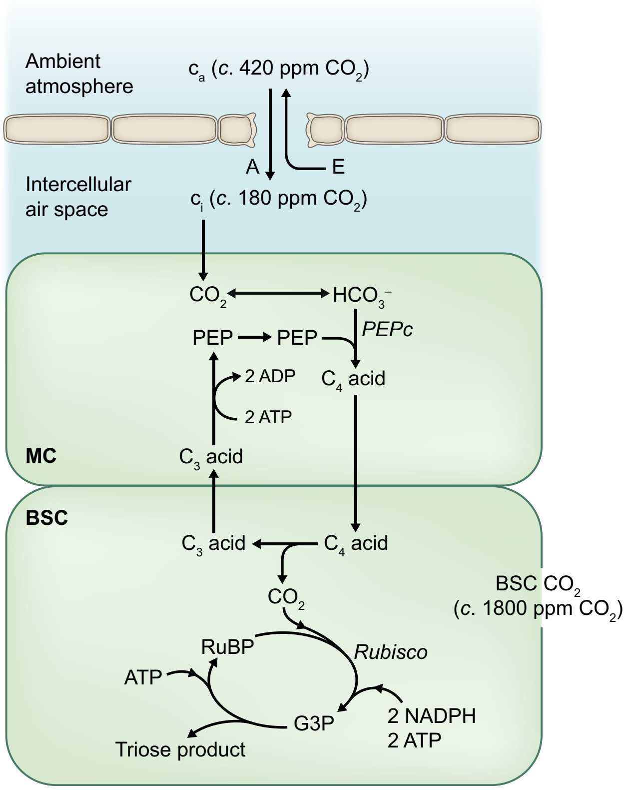

**Fig. B2** $C_4$ $CO_2$-concentrating mechanism (CCM). Diffusion of $CO_2$ from the ambient atmosphere through stomatal pores determines the intercellular $CO_2$ concentration of the leaf ($c_i$). The catalytic equilibrium between $CO_2$ and $HCO_3^-$ in the mesophyll cell (MC) cytosol determines the $HCO_3^-$ concentration available for fixation by phosphoenolpyruvate carboxylase (PEPc). The diffusive transfer of fixed carbon from MCs to bundle sheath cells (BSCs) occurs in the form of $C_4$ organic acids through plasmodesmata connections. Decarboxylation of the acids releases $CO_2$ within BSCs, which accumulates to a steady-state concentration *c.* 10 times higher than that of $c_i$. $C_3$ acids produced by the decarboxylation reactions diffuse back to the MC where they are converted to phosphoenolpyruvate (PEP), the organic substrate for PEPc, and the cycle begins again. Variants on this general scheme, known as $C_4$ subtypes, have evolved among $C_4$ taxa.

at the same high $CO_2$ concentration as found in $C_4$ bundle sheath cells (BSCs), it could increase its PNUE by 23%. The amount of N required to produce the proteins that drive the $C_4$ CCM will increase in a $C_4$ leaf and thus offset in part the overall PNUE advantage; however, the cost of $C_4$ cycle proteins like PEPc is modest, being estimated at 3–5% of the total leaf N content. Furthermore, $C_4$ species are likely to reduce their metabolic N costs even further by investing less in photorespiratory enzymes (Ueno

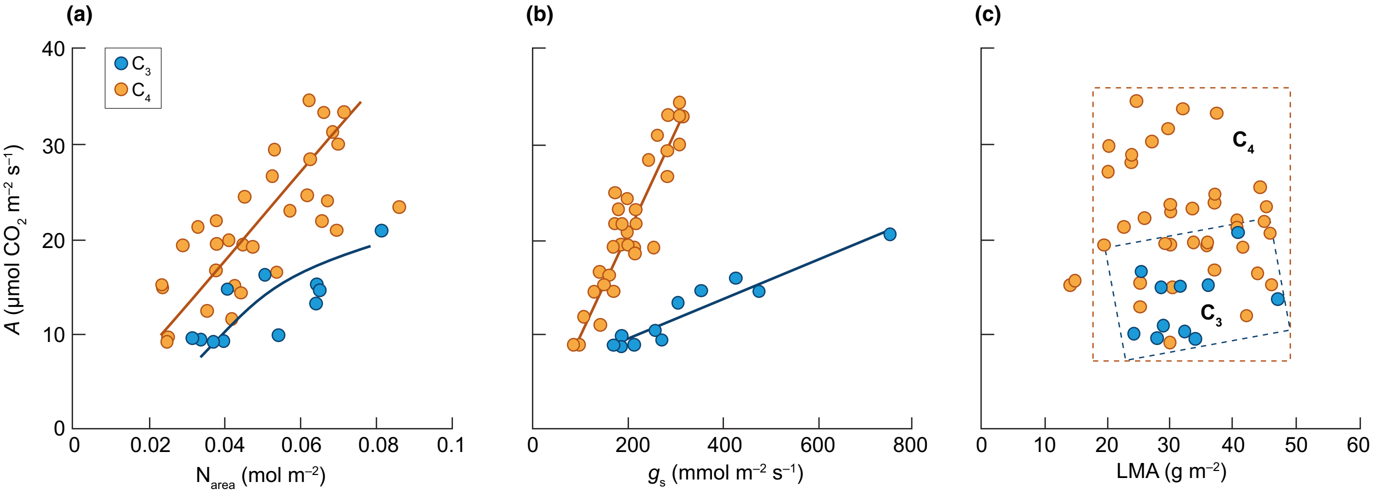

**Fig. 1** Relationships between $CO_2$ assimilation rate ($A$) and changes in nitrogen content per unit leaf area ($N_{area}$, a), stomatal conductance ($g_s$, b), and leaf mass to area ratio (LMA, c) from experiments with $C_3$ and $C_4$ grass species. The slope of the $A$ vs $g_s$ response reflects intrinsic water use efficiency (iWUE), and the slope of the $A$ vs $N_{area}$ response reflects photosynthetic nitrogen-use efficiency (PNUE). The rectangular domains shown in (c) represent the respective ranges for $C_3$ and $C_4$ species in the relationships between A and LMA (except for two outlier $C_4$ species), showing the much larger range of photosynthetic trait space allowed by the evolution of the $C_4$ pathway. Adapted from Togawa-Urakoshi & Ueno (2021).

*et al.*, 2005), which can be substantial in warm climates. The higher PNUE of $C_4$ leaves has been observed as a steeper slope and lower *x*-intercept in plots of $A$ vs N per unit of leaf area ($A_{area}$ and $N_{area}$, respectively; Fig. 1a).

The capacity to reach higher values of $A$ at lower $c_i$ because of the CCM also allows $C_4$ leaves to operate at higher PWUEs (Taylor *et al.*, 2010; Cano *et al.*, 2019). By facilitating high rates of $CO_2$ transfer from intercellular air spaces to BSCs, the CCM permits relaxation of the diffusive demand placed on stomata to supply atmospheric $CO_2$ to Rubisco. This advantage is observed as higher $A$ at any given $g_s$ (Fig. 1b). The higher PWUE of $C_4$ plants provides them with an advantage under water-limited conditions, in that the amount of photosynthate available for growth is increased per unit of $H_2O$ transpired ($E$). Thus, the CCM allows $C_4$ leaves to weaken an important trait trade-off that is strong in $C_3$ leaves, in this case between $CO_2$ acquisition and $H_2O$ utilization.

One of the leaf traits that has proven most useful in predicting adaptive strategies in $C_3$ species, particularly within the context of the WLES, is leaf mass per unit area (LMA; Wright *et al.*, 2004; Poorter *et al.*, 2009). Among $C_3$ species, when assessed across diverse phylogenetic groups, those with high LMA tend to exhibit a longer leaf lifespan (LLS), a greater allocation of resources to defense against herbivores, and a slower relative growth rate (RGR; Reich *et al.*, 2003). These same correlations with LMA do not always hold up in direct comparisons between $C_3$ and $C_4$ species within a common growth form. As shown in the example of Fig. 1(c), significant correlations have not been observed between $A$ and LMA across 22 $C_3$ and $C_4$ grass species. This is consistent with past analyses showing that area-based correlations between maximum observed $A$ and LMA are weak within species and growth forms, but strong across broader taxonomic comparisons (Osnas *et al.*, 2018). In the study portrayed in Fig. 1(c), we see a similar range of LMA values for $C_3$ and $C_4$ species, although the $C_4$ species are able to express higher $A$ at any given LMA. The multivariate complexities of interpreting correlations between an area-based measure, such as $A$, and mass-normalized measures, such as LMA, have been discussed previously (Field & Mooney, 1983; Osnas *et al.*, 2013, 2018), showing that as we expand the view of trait correlations beyond those directly involving $A$, nuanced studies of $C_3$ and $C_4$ trait interactions are required.

## III. Trait spectra and $C_4$ photosynthesis

Trait-based ecological theory has focused on 'functional traits', which are considered 'any attributes that have the potential to influence establishment, survival, and fitness' (Reich *et al.*, 2003), and 'which impact fitness indirectly via their effects on growth, reproduction and survival' (Violle *et al.*, 2007). Although studies throughout the 1970s, 1980s, and 1990s laid the groundwork for understanding functional traits and their mutual covariances (Lerdau *et al.*, 2023), the global significance of the functional-trait concept was not fully developed until 2004 with the first publication describing the WLES (Wright *et al.*, 2004; see Box 3).

The original description of the WLES by Wright *et al.* (2004) was based on the Glopnet global trait dataset, which at that time only included 22 $C_4$ trait records out of a total of 2559 trait records. There was no effort to explicitly study differences in trait covariance between $C_3$ and $C_4$ species. However, the same three traits, photosynthesis rate per unit mass ($A_{mass}$), LMA, and nitrogen concentration per unit mass ($N_{mass}$), that individually explained 84–90% of the trait variation in all plants, were also observed to explain 71–87% of the variation in $C_4$ species that were included in the database. What is largely missing from the studies to date is an understanding of how and to what degree $C_4$ species have diverged from their $C_3$ ancestors in patterns of trait trade-offs and the quantitative details of trait correlations (although see Simpson

**Box 3.** The world-wide leaf economic spectrum

Within the world-wide leaf economic spectrum (WLES), leaf traits are assumed to follow whole-plant growth patterns according to a spectrum bounded by two contrasting growth strategies (Wright *et al*., 2004; Reich, 2014). The fast-growth strategy is described as exploitative, with leaf traits specialized for rapid resource acquisition, including high photosynthesis rates and rapid leaf turnover. By nature, many exploitative leaf traits in $C_3$ species are inconsistent with high resource-use efficiencies, for example, the inherent inefficiencies associated with rapid leaf turnover and short mean residence times for leaf nitrogen (N) reduce plant N use efficiencies (Hirose, 2012). The slow-growth strategy includes positive covariances among low metabolic rates, slow leaf turnover, long leaf residence times for N, and high resource-use efficiencies. Leaves produced by plants at the fast-inefficient pole of the WLES are constructed at lower carbon and energy costs compared with plants at the slow-efficient pole. Most support for the existence of the WLES has been derived from trait databases focused on $C_3$ species.

The higher rates of carbon assimilation facilitated by the $C_4$ $CO_2$-concentrating mechanism (CCM; see Box 1) provide a means for $C_4$ species to grow faster per unit of N allocated to Rubisco and per unit of stomatal conductance. These advantages result in high whole-plant relative growth rates (Supporting Information Notes S2) at lower $N_{mass}$ and $P_{mass}$ values, as well as lower leaf transpiration rates. This provides the basis for the fast growth, high resource-use efficiency phenotype in $C_4$ species when compared to $C_3$ species. Whereas the $C_4$ CCM is most easily understood within species exhibiting acquisitive growth strategies, adaptive radiation within $C_4$ taxa has resulted in some species specialized in resource-poor habitats, including those with saline or infertile soils and frequent droughts. This has resulted in a $C_4$-specific plant economic spectrum with trait covariances like those at the slow-growth pole of the WLES, which have been sparsely studied.

*et al*., 2020). The same traits appear to be important in species with both photosynthetic pathways, but the ways by which those traits are related to one another, particularly within the scope of the WLES, remain largely unknown, particularly among plants grown in their natural field settings.

Two past analyses have addressed aspects of $C_3$ vs $C_4$ trait syndromes. In an analysis focused on leaf photosynthetic traits, Ghannoum *et al*. (2011) examined 41 $C_4$ grass species, including the $C_4$ species listed in the original Glopnet database from Wright *et al*. (2004) and some additional species from Australia. The results revealed higher *A* on both leaf area and leaf mass bases, as well as higher PNUE and PWUE in $C_4$ species, than in $C_3$ species. The $C_4$ advantages occurred despite similar leaf N concentrations. From this, it was concluded that the higher PNUEs in the Australian $C_4$ grasses were indeed directly due to the higher catalytic activity of Rubisco carboxylase, which in turn was due to the presence of the $C_4$ CCM. A recent analysis by Li *et al*. (2022) showed that trait correlations for a $C_4$ grass (*Miscanthus* × *giganteus*) exist at the upper boundary for photosynthetic rates reported in the Glopnet trait database. The largest boundary effects were seen for correlations involving $A_{mass}$ as the dependent trait variable, especially when assessed against $N_{mass}$, phosphorus concentration per unit mass ($P_{mass}$), and LMA as independent trait variables.

## IV. A new case study of $C_3$ and $C_4$ grasses within the context of the WLES

To take the studies of $C_4$ trait correlations further, we analyzed data from two sources that focused on field-grown plants, like those used in the original WLES analysis. We narrowed our analysis to grasses to provide consistency in growth form. The first data source included 77 grass species, including 47 $C_4$ species and 30 $C_3$ species, growing at the Konza Prairie Biological Station located in northeastern Kansas (see Donnelly *et al*., 2023). Within this dataset, we focused on LMA, $N_{mass}$, and leaf gas exchange data. The second data source included 150 $C_4$ grass species and 2160 $C_3$ grass species contained within a sub-database (no. 45; see Garnier *et al*., 2007) of the much larger TRY trait database (Kattge *et al*., 2020). We filtered the analysis to include species that allowed two-way correlations of at least two of the following traits: LMA, LLS, $N_{mass}$, $P_{mass}$, $A_{mass}$, and respiration rate per unit mass ($R_{mass}$). This left us with between 15 and 89 $C_4$ species and between 163 and 1444 $C_3$ species, depending on each specific trait correlation.

Using the data of all 77 species from the Konza Prairie experiments, we examined trait contrasts from $C_4$ and $C_3$ leaves. As shown in Fig. 2(a), $C_4$ species share a similar mean value of LMA with $C_3$ species (57.3 vs 55.2 g $m^{-2}$). The values of $A_{area}$ and $A_{mass}$ were higher for $C_4$ species (Fig. 2b,c), which were attained with lower $N_{mass}$ values (Fig. 2d), revealing a higher $C_4$ PNUE. Observed gas exchange values for $g_s$ and $c_i$ were lower in $C_4$ grasses than in their $C_3$ counterparts (Fig. 2e,f). As a result, $C_4$ grasses showed a higher intrinsic water use efficiency (iWUE = $A_{area}/g_s$) than $C_3$ species (Fig. 2g). These results collectively demonstrate that with a similar structural investment in LMA, the $C_4$ CCM enables faster $A_{mass}$ and $A_{area}$, as well as higher PWUE and PNUE, than $C_3$ leaves.

The pairwise correlations that showed significant differences between $C_3$ and $C_4$ species for the TRY database are shown in Fig. 3. The trend equations are presented in Supporting Information Table S1 for all correlations. In Fig. 3(a), a clear downward trend in $A_{mass}$ as a function of increasing LMA is observed for the leaves of $C_3$ grasses. A downward trend in the $A_{mass}$ vs LMA correlation might be expected from statistical effects alone (Lloyd *et al*., 2013). This is because the conversion of $A_{area}$ to $A_{mass}$ (whereby $A_{area}$/LMA = $A_{mass}$) in large datasets with randomly distributed variance in LMA has the effect of enhancing a positive covariance between $A_{area}$ and $A_{mass}$ and a negative covariance between $A_{mass}$ and LMA (Osnas *et al*., 2013). However, it is also possible that LMA is not randomly distributed with respect to $A_{mass}$, but rather that their covariance has been shaped directly or indirectly by natural selection (Field & Mooney, 1983; Poorter *et al*., 2013).

We tested one possible cause of a nonrandom correlation between $A_{mass}$ and LMA. We hypothesized that associations between the two traits are the result of lineage-specific modifications to leaf anatomy. To test this hypothesis, we partitioned the correlations of Fig. 3 according to $C_3$ BOP, $C_3$ PACMAD, and $C_4$ PACMAD clades in the TRY trait database (Notes S1). The BOP clade (Bambusoideae, Oryzoideae, and Pooideae subfamilies) consists entirely of $C_3$ species, whereas the PACMAD clade

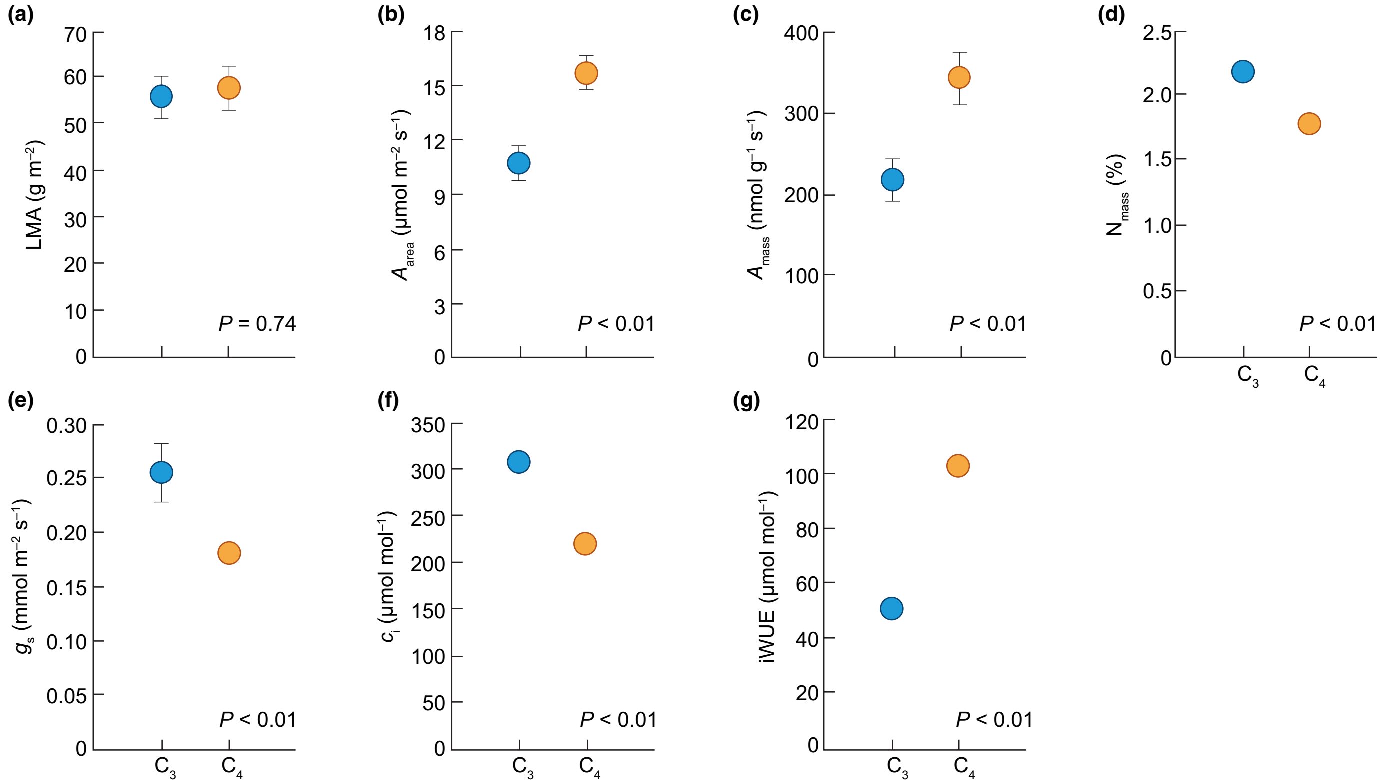

**Fig. 2** Gas exchange traits for $C_3$ (blue) and $C_4$ (orange) grasses measured in the field at the Konza Prairie, Kansas, USA. For (a, c), the sample sizes were 47 $C_4$ species and 30 $C_3$ species; for all other panels, the sample sizes were 46 $C_4$ species and 29 $C_3$ species. Where shown, vertical bars represent ±SE; otherwise, the calculated SEs were smaller than the symbol size, from data presented in Donnelly *et al*. (2023). LMA, leaf mass per unit area (a); $A_{area}$, net photosynthesis per unit leaf area (b); $A_{mass}$, net photosynthesis per unit leaf mass (c); $N_{mass}$, nitrogen per unit leaf mass (d); $g_s$, stomatal conductance (e); $c_i$, intercellular $CO_2$ concentration (f); iWUE, intrinsic water use efficiency (g).

(Panicoideae, Aristidoideae, Chloridoideae, Micraioideae, Arundinoideae, Danthonioideae subfamilies) includes both $C_3$ and $C_4$ species. In the $C_3$ BOP clade, BSC sizes have been reduced during past selection, compared with both $C_3$ and $C_4$ species in the PACMAD clade (Ueno *et al*., 2006; Christin *et al*., 2013; Griffiths *et al*., 2013). According to our hypothesis, the smaller BSCs of the $C_3$ BOP species, compared with $C_3$ PACMAD species, would translate to $A_{mass}$ values distributed within a range of lower LMAs (anchoring the left side of the correlation in Fig. 3a). The larger BSCs observed for $C_3$ PACMAD species would likely translate to $A_{mass}$ values that are distributed within a range of higher LMAs (anchoring the right side of the correlation in Fig. 3a). Support for the hypothesis would mean that the correlations should disappear or at least become muted when constructed separately within each clade. However, when the correlations were partitioned according to clade, significant downward trends remained, causing us to reject the hypothesis (Notes S1: Figs A, B). There is clearly a need for further phylogenetically guided data collection on the $A_{mass}$ vs LMA relationship within grasses.

Fig. 3(b) shows the correlations between $N_{mass}$ and LMA. In both $C_3$ and $C_4$ grasses, the negative correlations indicate that grasses with higher LMA have higher C : N ratios and likely contain more sclerenchyma tissue and cellulose fibers, which enhance leaf durability and defense against herbivores. In this case, the negative correlations between $N_{mass}$ and LMA would be due to greater or lesser C dilution of leaf N. Fig. 3 also shows that $N_{mass}$ in $C_4$ leaves occurs within a narrower range of LMAs than $C_3$ $N_{mass}$. This could be due to evolutionary canalization, in which selection has favored specific anatomical forms in $C_4$ leaves that are required for the efficient function of the CCM. This hypothesis is supported by two lines of evidence – one associated with studies on the evolutionary path of $C_2$ phenotypes (sometimes called $C_3$–$C_4$ intermediates) to $C_4$ phenotypes in a single species (Alenazi *et al*., 2023) and another associated with comparative studies of anatomical traits across a broad range of $C_3$ and $C_4$ species (Griffiths *et al*., 2013).

In the first line of studies, the evolution of fully expressed $C_4$ phenotypes (sometimes called ‘strong $C_4$’ phenotypes) in the grass species *Alloteropsis semialata* has been linked to a progressive shift in Rubisco expression from principally mesophyll cells (MCs) in $C_3$ and $C_2$ phenotypes to principally BSCs in $C_4$ phenotypes (Alenazi *et al*., 2023). The increased investment in BSC Rubisco in $C_4$ phenotypes appears to have been preceded by anatomical modifications to leaf anatomy that likely affected LMA. In *A. semialata* phenotypes, these changes include an increase in BSC size and a decrease in leaf interveinal distance (IVD) between the bundle sheath strands. Both of these modifications are required for an efficient operation of the $C_4$ CCM and include a means for the

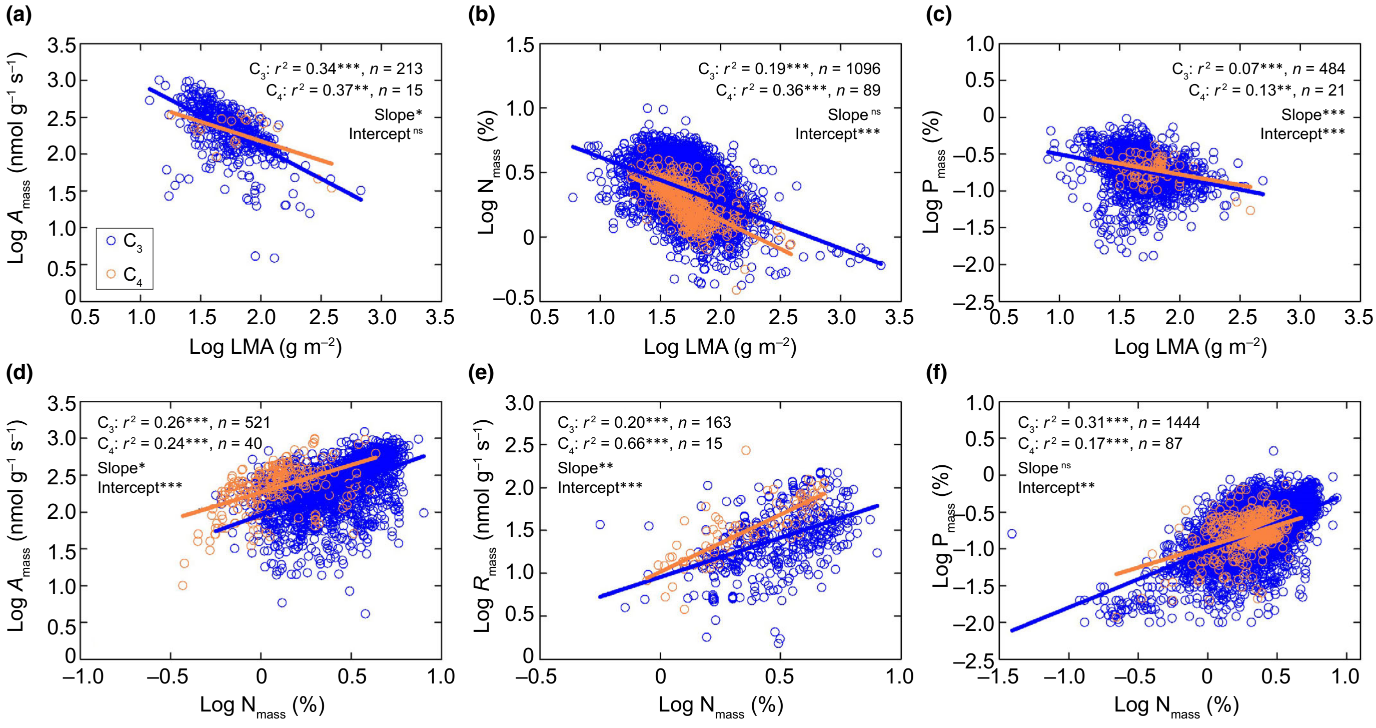

**Fig. 3** Log–log plots of significant trait relationships within $C_4$ (in orange) and $C_3$ (in blue) grass species. (a) Net $CO_2$ assimilation rate per unit of leaf mass ($A_{mass}$) vs leaf mass per unit area (LMA). (b) Leaf nitrogen concentration per unit leaf mass ($N_{mass}$) vs LMA. (c) Leaf phosphorus concentrations per unit leaf mass ($P_{mass}$) vs LMA. (d) $A_{mass}$ vs $N_{mass}$. (e) Leaf respiration rate per unit leaf mass ($R_{mass}$) vs $N_{mass}$. (f) $P_{mass}$ vs $N_{mass}$. Differences in slopes and intercepts were compared for all trait values reported between $C_3$ and $C_4$ species by standardized major axis (SMA) using an R script from the SMATR package (Warton *et al*., 2011; https://cran.r-project.org/web/packages/smatr/index.html). A summary of the results of SMA analyses is given in Supporting Information Table S1. The sample numbers (*n*) refer to the total number of species used in each correlation. In all cases: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

effective exchange of photorespiratory metabolites between MCs and BSCs and metabolic partitioning of the activities of PEP carboxylation and C4-acid decarboxylation in the early stages of $C_4$ evolution (Blätke & Bräutigam, 2019; Alenazi *et al*., 2023). Furthermore, both modifications are likely to have required specific $C_3$ leaf phenotypes as precursors to $C_4$ evolution (Christin *et al*., 2013) and to have narrowed the scope of possible LMAs capable of supporting efficient $C_4$ metabolism, contributing to evolutionary canalization in the $N_{mass}$ vs LMA relationship.

In the second set of studies on numerous $C_3$ and $C_4$ species, it was shown that even more generally, past selection has resulted in decreases in both the MC : BSC (cross-sectional) area ratio and the IVD in $C_4$ species (Hattersley, 1984; Griffiths *et al*., 2013). The MC : BSC ratio and IVD average 9 : 1 and 200–300 µm, respectively, in $C_3$ grasses, and 2 : 1 and 100 µm, respectively, in $C_4$ grasses. There is an important distinction here in that the increase in MC : BSC ratio in $C_4$ species is not due to larger BSC volumes, but rather a wider, but shorter, BSC shape (Danila *et al*., 2018; Khoshravesh *et al*., 2020). The significance of the change in BSC shape to CCM function has not yet been established, but here again, it might be associated with an advantage to the diffusive transfer of photorespiratory metabolites between MCs and BSCs in the early stages of $C_4$ evolution (Khoshravesh *et al*., 2020). There are two additional modifications that were noted in earlier studies of a broad range of $C_3$ and $C_4$ grasses. First, $C_4$ grasses are known to have higher frequencies of plasmodesmatal connections between MCs and BSCs which, along with fewer MCs between veins, facilitates increases in the rates of $C_4$ metabolite diffusion (Danila *et al*., 2018; Khoshravesh *et al*., 2020). Second, $C_4$ grasses tend to have thinner leaves with a denser system of small longitudinal and transverse veins (Dengler *et al*., 1994; Ueno *et al*., 2006). Taken together, this collection of studies supports a role for anatomical modifications within clades of $C_4$ species that might have resulted in a narrower, yet canalized, range of LMA values capable of supporting the effective function of the CCM.

In Fig. 3(c), the dependence of $P_{mass}$ on LMA provides evidence of a slightly higher $P_{mass}$ in $C_4$ species at any given LMA, but like $N_{mass}$, within a narrower set of LMA values. The $C_3$ vs $C_4$ difference in $P_{mass}$ at the *y*-intercept is small (1.32 and 1.42% dry mass for $C_3$ and $C_4$ species, respectively), but it is statistically significant. The reason for a slightly higher $P_{mass}$ in $C_4$ leaves is currently unknown. It is possible that this trend relates to thinner leaves in $C_4$ grasses, meaning that per unit mass there is less C dilution of P. As in the case of the $N_{mass}$ vs LMA relationship, there is also evidence of convergence in $P_{mass}$ and LMA values toward a narrower set of $C_4$ phenotypes. Once again, this could reflect directional selection favoring anatomical traits that improve CCM efficiency in $C_4$ species.

In Fig. 3(d,e), the dependencies of $A_{mass}$ and $R_{mass}$ on $N_{mass}$ are distinctly separated for $C_3$ and $C_4$ species. In these two cases, we can

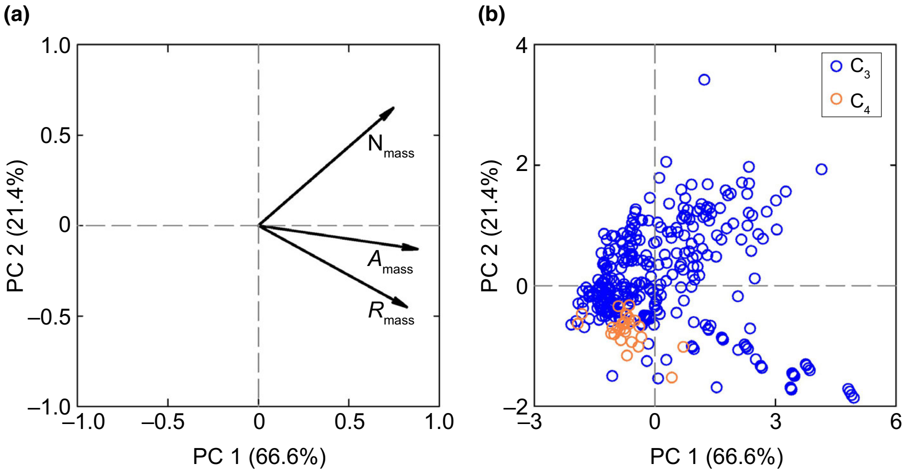

**Fig. 4** Principal components analysis of leaf traits using the dataset extracted from the TRY database. (a) Trait loading plot showing individual traits of mass-based leaf net $CO_2$ assimilation rate ($A_{mass}$), nitrogen concentration ($N_{mass}$), and respiration rate ($R_{mass}$). Arrow tips indicate the loading direction of the traits. (b) Species are grouped based on photosynthetic pathway. In all cases, 148 $C_3$ species with 355 measurements (in blue) and 11 $C_4$ species with 33 measurements (in orange) were included. The TRY trait database can be requested at https://www.try-db.org/.

clearly observe the role of the $C_4$ CCM in enhancing PNUE, and this explains the upward shift in the $A_{mass}$ intercept. The cause of higher $R_{mass}$ at a given $N_{mass}$ in $C_4$ leaves is consistent with past studies (Fan *et al.*, 2024) and might be due to selection for higher maintenance respiration rates and protein turnover rates in support of the higher metabolic capacities of $C_4$ leaves (de Penning Vries, 1975; Reich *et al.*, 2008). In Fig. 3(f), we observed evidence of a combined narrowing in the covariance of $P_{mass}$ and $N_{mass}$ in $C_4$ species, compared with $C_3$ species. Once again, this could reflect requirements for specific anatomical modifications during $C_4$ evolution that improve the efficiency of $C_4$ metabolism and metabolite exchange between MCs and BSCs.

We examined the potential for traits to covary in a way that explains total trait variance using principal components analysis (PCA; Table S2). We were able to obtain sufficient trait data for only three traits, $A_{mass}$, $R_{mass}$, and $N_{mass}$, across a spectrum of $C_3$ and $C_4$ species, but with limited data for the $C_4$ species. The first two principal components together explained 88% of total trait variance (Fig. 4; Table S2). Together, the two principal axes distinguished $C_4$ species as occupying space toward the extreme of higher $A_{mass}$ and $R_{mass}$ at lower $N_{mass}$, whereas $C_3$ species occupied a much broader space but with a main trend toward lower $A_{mass}$ and $R_{mass}$ at higher $N_{mass}$. This pattern is consistent with the role of the CCM in promoting $C_4$ phenotypes with the combination of fast leaf metabolic rates and higher PNUE.

## V. The fast-efficient phenotype of $C_4$ plants and the existence of a $C_4$ trait spectrum

The trends revealed in Fig. 4 support the role of traits, such as $A_{mass}$, $R_{mass}$, and $N_{mass}$, as unique co-varying determinants of the $C_4$ phenotype with the potential to support fast-growth rates and high resource use efficiencies. With this combination of traits, the $C_4$ phenotype is at odds with the fast-growth, low-efficiency *vs* slow-growth, high-efficiency strategies defined in the $C_3$-dominated schemes of the WLES (Box 3). Here, we see that attributes from both poles of the $C_3$ WLES have been coopted during the evolution of $C_4$ photosynthesis. In essence, the $C_4$ CCM provides a means of uncoupling the trade-off between fast, acquisitive growth and high resource-use efficiency that has been observed in $C_3$ species.

How do we explain this conclusion? The fast growth and high PNUE of the $C_4$ photosynthetic phenotype are due to a fundamentally different form of selection than that shaping the growth and resource-use traits of $C_3$ phenotypes. In $C_3$ plants, one of the ways to achieve a higher RGR is through the investment of resources in root N acquisition, which has the potential to increase leaf Rubisco concentration. However, this strategy contains an internal constraint that, in turn, imposes a cost on RGR. That is, increases in plant biomass are driven by autotrophic shoot production, which must be opposed by increases in heterotrophic root production. Assuming that root specific activity for N uptake (N uptake per unit of root biomass) remains constant, as selection favors higher RGR, it must balance the cost of producing more roots to obtain more N. We refer to this as the growth-N uptake conundrum.

In $C_4$ species, the CCM and higher PNUE allow for higher N productivity (g biomass produced $g^{-1}$ N acquired $d^{-1}$) without an increased demand to acquire soil N. Because $C_4$ leaves can operate at lower $N_{mass}$, greater amounts of leaf biomass can be produced and operated at a higher specific activity for carbon uptake (rate of carbon uptake per unit of shoot biomass) at any given amount of acquired N (Sage *et al.*, 1987; Taylor *et al.*, 2010; Simpson *et al.*, 2020). In essence, the higher PNUE of leaves enables the plant to achieve a higher RGR at any given root : shoot biomass fraction, while minimizing the need for fast leaf turnover times and concomitantly fast N turnover times. This enables the fast-growth, resource-efficient phenotype of $C_4$ species. These relationships are developed mathematically within the context of an N *vs* growth optimization model in Notes S2: Fig. A.

The fast-efficient $C_4$ phenotype provides advantages in both high-N and low-N habitats. In high-N habitats, the CCM enables a higher shoot fraction in $C_4$ species and therefore enhances their competitiveness as they shade neighbors and capture aboveground space (Long, 1999). Once again, this is possible because the CCM allows $C_4$ plants to grow faster while relying less on the root biomass fraction to acquire N. Yet, because the C : N acquisition rates are potentially higher in $C_4$ phenotypes, an opportunity exists to

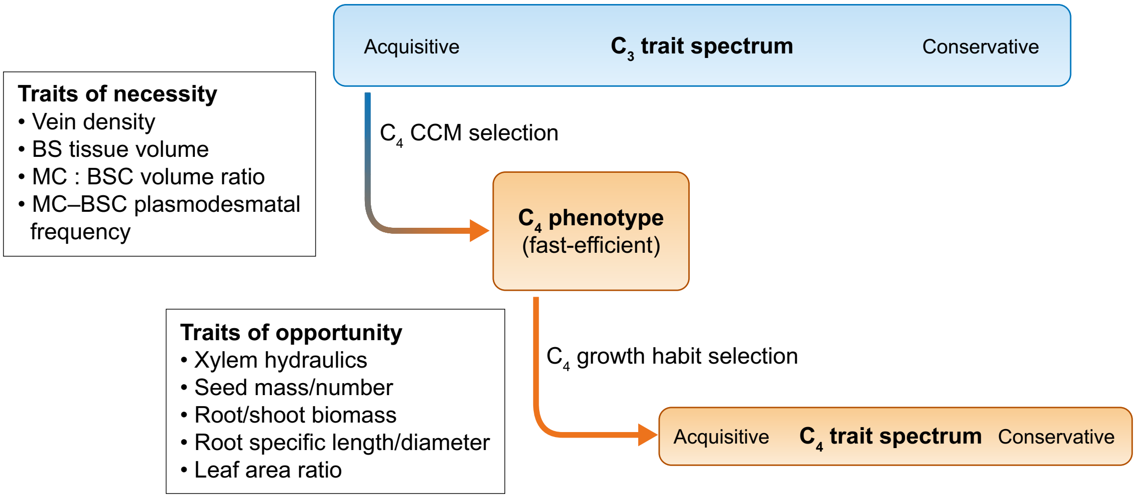

**Fig. 5** Relationships between proposed $C_3$ and $C_4$ trait spectra. Starting from the top, ancestral $C_3$ taxa provide the trait source for the evolution of the $C_4$ $CO_2$-concentrating mechanism (CCM), which provides the basis for the fast-efficient $C_4$ phenotype. During the evolution of the $C_4$ CCM, certain traits of necessity will be favored to facilitate mechanistic efficiency within the CCM. These include leaf anatomical traits, such as bundle sheath cell (BSC) shape, interveinal density, and plasmodesmata frequency between mesophyll cells (MCs) and BSCs. Proceeding down the figure, selection involving $C_4$ plants results in trait sorting along the $C_4$ trait spectrum. This selection is conditioned by niche conservatism, life history, and plant growth habit and is enabled by the opportunities to utilize the increased availability of resources, such as carbon (C), nitrogen (N), and water ($H_2O$), to enhance the expression of non-photosynthetic traits, such as xylem hydraulic efficiency, reproductive output, and plant growth allocation patterns.

allocate more biomass to roots when needed, to competitively coopt N from fertile nutrient patches belowground (Simpson *et al*., 2020). This latter advantage would also provide expanded opportunities for $C_4$ species in low-N habitats, by promoting opportunities for high biomass allocation to root growth, thereby enhancing their competitiveness as they forage for limited sources of soil nutrients (Ripley *et al*., 2008). In both cases, the $C_4$ advantage occurs because the CCM allows $C_4$ plants to rely more on shoot-specific activity and less on shoot biomass fraction to sustain RGR.

We propose that $C_4$ plant traits are best described through a trait spectrum that is aligned with, but separate from, that reflected in the acquisitive pole of the WLES (Fig. 5). Within this view, trait sorting within the $C_4$ spectrum is constrained by both *necessity* and *opportunity*. Necessity would be driven by selection favoring the mechanistic requirements of a fully functioning CCM, potentially including decreased IVD, modifications to the shape and biochemical constituency of leaf BSCs, and increased plasmodesmata frequencies between MCs and BSCs. Opportunity would be due to selection favoring alternative resource allocation opportunities, such as increased fractional allocation to seed production, alterations in root : shoot biomass ratios, and alterations in the efficiency and capacity of whole-plant hydraulic traits. Opportunity selection would be influenced by habitat resource availabilities and would occur independently of $C_4$ CCM selection (see Simpson *et al*., 2020).

## VI. Experimental tests of the adaptive nature of opportunity traits

The fast-efficient $C_4$ phenotype provides opportunities for trait syndromes to form novel adaptive strategies and for $C_4$ taxa to expand their ranges as they radiate into new habitat niches (Lundgren *et al*., 2015). To date, the $C_4$ strategies of faster growth with higher resource-use efficiencies have been most often inferred from leaf gas exchange observations. A few studies have assessed $C_4$ trait syndromes in controlled experiments with whole plants. In one study, Simpson *et al*. (2020) observed seedling growth patterns in over 100 $C_3$ and $C_4$ grass species under controlled growth conditions. They were able to confirm higher RGRs for the $C_4$ species. Furthermore, they showed that the $C_4$ CCM provided an opportunity to devote less $N_{mass}$ to support photosynthesis, permitting a greater allocation of N to new biomass growth. In the $C_4$ species, lower $N_{mass}$ was also observed for roots, which would further contribute to the opportunity for higher shoot growth rates in acquisitive species and higher root growth rates in conservative species (also see Atkinson *et al*., 2016). Overall, the $C_4$ CCM operated independently of leaf and root traits in their effects on RGR. This means that opportunities exist in $C_4$ species for selection to further amplify RGR through the modification of non-photosynthetic traits.

In growth chamber studies using low ambient $CO_2$ concentrations (i.e. at interglacial levels of *c*. 180 ppm) and comparing $C_3$ and $C_4$ subspecies of the grass *A. semialata*, Ripley *et al*. (2008, 2013) observed that when grown with ample N, $C_4$ plants exhibited higher PNUE and growth rates than $C_3$ plants. The higher growth rates were achieved with a reduced allocation of C to leaves and roots. This growth advantage provided the opportunity to allocate more carbon to the production of inflorescences and carbohydrate storage pools in corms despite limited atmospheric $CO_2$ availability. When grown with limited access to N, $C_3$ plants allocated more carbon to roots, at the expense of leaves, while $C_4$ plants reduced inflorescence production. Here, we see that the $C_4$ advantage in PNUE does indeed translate to selection opportunities at the scale of whole-plant resource allocation.

However, rather than channeling the higher PNUE toward enhanced shoot growth and a higher RGR, the $C_4$ sub-species of this grass channeled the advantage toward greater reproduction at high N.

Together, the studies to date have provided evidence that selection favoring the $C_4$ pathway, particularly in grasses, has indeed resulted in higher potential RGRs, while simultaneously providing opportunities for novel C and N allocation patterns. In addition to enhancing reproductive fitness, the assembly of novel trait combinations during $C_4$ evolution likely created new opportunities for $C_4$ radiations into novel niches (Lundgren *et al.*, 2015). Such radiations, in turn, would have facilitated further trait sorting through directional selection or stabilizing selection as determined by local ecological constraints and selection pressures.

## VII. The fast-efficient $C_4$ phenotype and global change

Recognizing that $C_4$ species follow fundamentally different relationships in their mandated trade-off between growth rate and resource-use efficiency, compared with their $C_3$ ancestors, how might species in $C_4$ lineages respond to the global change drivers involving temperature, N, precipitation, and disturbance that are impacting life on Earth today? The responses of $C_4$ photosynthesis to global change have broad ecological ramifications, including the spread of invasive $C_4$ species and the degradation of $C_4$ grassland ecosystems during $C_3$ shrub encroachment. The conventional wisdom is that $C_4$ species have their greatest competitive advantages over $C_3$ species when the atmospheric $CO_2$ concentration is low, and that recent increases in $CO_2$ emissions are likely to reverse those advantages (Ehleringer & Monson, 1993; Ehleringer *et al.*, 1997; Kgope *et al.*, 2010; Monson & Collatz, 2012). That prediction is largely based on the responses of Rubisco to $CO_2$ and $O_2$ and the relative influences of temperature on those responses. However, there is evidence that a broader context for $C_4$ traits, including aspects of plant hydraulics and whole-plant N allocation, can better explain certain aspects of $C_3$ and $C_4$ adaptive strategies and their competitive interactions in the face of global change (Kocacinar & Sage, 2003; Osborne & Sack, 2012; Taylor *et al.*, 2014; Zhou *et al.*, 2018; Baird *et al.*, 2025). Accordingly, several authors have noted a need for caution and caveats in predictions about future changes in $C_4$ *vs* $C_3$ plant abundance (Sage & Kubien, 2003; Monson & Collatz, 2012; Reich *et al.*, 2018; Luo *et al.*, 2024).

Nonetheless, some recent evidence suggests that indeed global $C_3$ abundance has been increasing over the past two decades, and at the expense of $C_4$ abundance. Using a combination of remote sensing, optimality theory, and recorded distributions of $C_3$ and $C_4$ species, Luo *et al.* (2024) showed that the global extent of natural $C_4$ grassland area has decreased slightly from 15.0% to 14.2% between 2001 and 2019. They attributed this decrease to increasing atmospheric $CO_2$ levels and the encroachment of $C_3$ woody species. The observed decreases in $C_4$ coverage are small, which is not surprising given the limited time span considered. But the decreases occur in the direction expected if the competitive advantages of $C_4$ species have been weakened by recent $CO_2$ fertilization. Notably, the optimization model used in this study included consideration of traits beyond the traditional photosynthetic 'temperature cross-over models' of past global analyses (Collatz *et al.*, 1998; Still *et al.*, 2003).

However, even with this recent evidence of global advancement in $C_3$ abundance, Luo *et al.* (2024) observed that in regions where the climate has become warmer and wetter, such as in Western Eurasia and Eastern Africa, $C_4$ species have increased at the expense of $C_3$ species. The more relevant perspective, therefore, might not be where $C_4$ species are ceding dominance to $C_3$ species, but where they are not. Numerous regional and experimental studies have shown the nuances of the $C_4$ advantage. While wetter, warm growing seasons may favor some $C_4$ grass species (Taylor *et al.*, 2011; Jung *et al.*, 2022; Luo *et al.*, 2024), increasing aridity favors others (Edwards & Still, 2008; Taylor *et al.*, 2014; Havrilla *et al.*, 2023). There is also growing evidence that shifts in the seasonality of precipitation, without increases or decreases in total precipitation, can be important in determining $C_4$ species success. Across Australia, the $C_4 : C_3$ grass coverage ratio increases rapidly in response to interannual variability in rainfall seasonality (Xie *et al.*, 2022) and this observation has been replicated experimentally in North America (Hajek *et al.*, 2024). There are multiple mechanisms, whereby the seasonal availability of water may be altered by climate change (Hajek & Knapp, 2022), including influences on precipitation or changes in evapotranspiration, and these types of alterations may become more widespread in the future.

Even for increases in atmospheric $CO_2$ concentrations, we find the potential for nuanced $C_4$ responses. For example, in temperate grasslands, where seasonal droughts are frequent, $C_4$ abundance has not always declined as expected with increased $CO_2$ (Owensby *et al.*, 1993; Morgan *et al.*, 2011). Moreover, in a longer-term $CO_2$ experiment where $C_4$ biomass did initially decline, it subsequently increased after 12 yr, due to slow changes in soil N cycling (Reich *et al.*, 2018). These studies underscore the complexity of predicting net outcomes for future $C_4$ success based on their traits, even when relatively straightforward manipulations of individual global change drivers are assessed.

## VIII. $C_4$ traits and global change models

Global-scale biosphere models cannot easily represent the trait diversity present in plant species, and therefore, researchers have most frequently simplified the models by grouping terrestrial plants with similar traits into categories called plant functional types (PFTs; Harrison *et al.*, 2021). Each model varies in the number and category of PFTs, and typically models will represent global vegetation as < 20 categories of natural vegetation. Through this simplification, $C_4$ photosynthesis is represented in most global models as a single $C_4$ phenotype (typically represented as a generic $C_4$ grass species). Thus, there is little to no variation in $C_4$ traits represented in the current generation of terrestrial biosphere models.

This current situation likely results in several modeling uncertainties (Griffith *et al.*, 2020). Recent evidence suggests that evolutionary history is particularly important for explaining trait variation across a wide range of grasses at the well-studied Konza tallgrass prairie site in

Kansas (Donnelly *et al.*, 2023). Recognition of this pattern presents an opportunity to more accurately represent $C_4$ traits in global models by replacing the standard $C_4$ grass PFT with new lineage-based functional types (LFTs; Griffith *et al.*, 2020). Other studies have shown that broad trait categories, such as life history, can influence the evolution of physiological traits, including hydraulic conductance and stomatal density (Liu *et al.*, 2019; Baird *et al.*, 2025).

## IX. Conclusions

We, as a global change research community, have largely generated predictions about the future of $C_3$ and $C_4$ species on the basis of photosynthetic and photorespiratory traits. Early in our comparative research of $C_3$ and $C_4$ species, we focused on leaf gas exchange processes and have continued in that mode to the present day. However, there is evidence that this approach can only take us so far (Still *et al.*, 2019; Griffith *et al.*, 2020). There is a need to broaden that context to include additional traits associated with other plant attributes, including aspects of plant water use, life history, and evolutionary lineage. We are in an era of rapid global change and unknown biospheric responses. The climate and atmospheric $CO_2$ conditions that originally led to the evolution of the $C_4$ fast-efficient phenotype are changing quickly under the pressures of anthropogenic forcing, with implications for the activity, extent, and success of $C_4$ plants. Broader development of a trait-based approach would help construct hypotheses and studies that could provide insight into regional and global $C_4$ productivity and $C_4$-associated ecological interactions. It would also provide a larger and deeper world-wide database of $C_4$ traits for use in agriculture, including strategies to introduce $C_4$ traits into $C_3$ crop lineages. In light of $C_4$ plants' important role in regulating terrestrial carbon cycling, food production, and the economic and ecological consequences of invasive plants, the building of these questions and frameworks is warranted.

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## Competing interests

None declared.

## Author contributions

RKM conceived the concept for the paper within the discussions provided by the John Wesley Powell Center $C_4$ Working Group. SL conducted the quantitative trait analysis and led its interpretation. All authors developed the perspectives presented in the paper during several plenary discussion sessions. All authors contributed to writing the text. RKM and SL contributed equally to the leadership within this work.

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## Data availability

The original data for Figs 3 and 4 have been deposited in Figshare (https://figshare.com) with doi: 10.6084/m9.figshare.28321865.

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## Supporting Information

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

**Notes S1** Testing the role of independent anatomical evolution as the cause of the $A_{\text{mass}}$ vs LMA correlation.

**Notes S2** A theoretical framework describing the advantages of $C_4$ photosynthesis to the N-limited condition.

**Table S1** Correlation parameters for each two-way correlation shown in Fig. 3 of the main text.

**Table S2** Principal components analysis (PCA) loadings for the three traits shown in Fig. 4 of the main text.