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C_4 photosynthesis and the economic spectra of leaf and root traits independently influence growth rates in grasses

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Abstract

1. Photosynthetic pathway is an important cause of growth rate variation between species such that the enhanced carbon uptake of C_4 species leads to faster growth than their C_3 counterparts. Leaf traits that promote rapid resource acquisition may further enhance the growth capacity of C_4 species. However, how root economic traits interact with leaf traits, and the different growth strategies adopted by plants with C_3 and C_4 photosynthetic pathways is unclear. Plant economic traits could interact with, or act independently of, photosynthetic pathway in influencing growth rate, or C_3 and C_4 species could segregate out along a common growth rate–trait relationship.
2. We measured leaf and root traits on 100+ grass species grown from seeds in a controlled, common environment to compare with relative growth rates (RGR) during the initial phase of rapid growth, controlling for phylogeny and allometric effects.
3. Photosynthetic pathway acts independently to leaf and root functional traits in causing fast growth. Using C_4 photosynthesis, plants can achieve faster growth than their C_3 counterparts (by an average 0.04 g g^{-1} day^{-1}) for a given suite of functional trait values, with lower investments of leaf and root nitrogen. Leaf and root traits had an additive effect on RGR, with plants achieving fast growth by possessing resource-acquisitive leaf traits (high specific leaf area and low leaf dry matter content) or root traits (high specific root length and area, and low root diameter), but having both leads to an even faster growth rate (by up to 0.06 g g^{-1} day^{-1}). C_4 photosynthesis can provide a greater relative increase in RGR for plants with a 'slow' ecological strategy than in those with fast growth. However, above-ground and below-ground strategies are not coordinated so that species can have any combination of 'slow' or 'fast' leaf and root traits.
4. *Synthesis*. C_4 photosynthesis increases growth rate for a given combination of economic traits, and significantly alters plant nitrogen economy in the leaves and roots. However, leaf and root economic traits act independently to further enhance growth. The fast growth of C_4 grasses promotes a competitive advantage under hot, sunny conditions.

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KEYWORDS
ecological strategy, leaf economic spectrum, plant development and life-history traits, Poaceae, relative growth rate, resource acquisition, resource conservation, root economic spectrum

1 | INTRODUCTION

Photosynthetic pathway is one of the main causes of growth rate variation among plant species (Atkinson et al., 2016). C_4 photosynthesis has allowed plants to grow faster by increasing carbon uptake compared to the ancestral C_3 photosynthetic pathway (Björkman, 1970; Sage, Christin, & Edwards, 2011). By concentrating CO_2 around Rubisco and suppressing photorespiration through a coordinated set of anatomical and biochemical modifications, photosynthetic rate and efficiency can be enhanced at high temperatures and low atmospheric CO_2 in C_4 plants. This results in greater carbon availability for costly physiological processes such as growth (Björkman, 1970; Ehleringer, Sage, Flanagan, & Pearcy, 1991; Long, 1999). However, it is not only the carbon concentrating mechanism that has resulted in faster growth of C_4 species over their C_3 counterparts but also the possession of leaf functional traits associated with ecological strategies of resource acquisition. Such traits promote the allocation of resources to rapid growth, at the expense of resource conservation, storage, maintenance and defence (Grime et al., 1997; Reich et al., 1999).

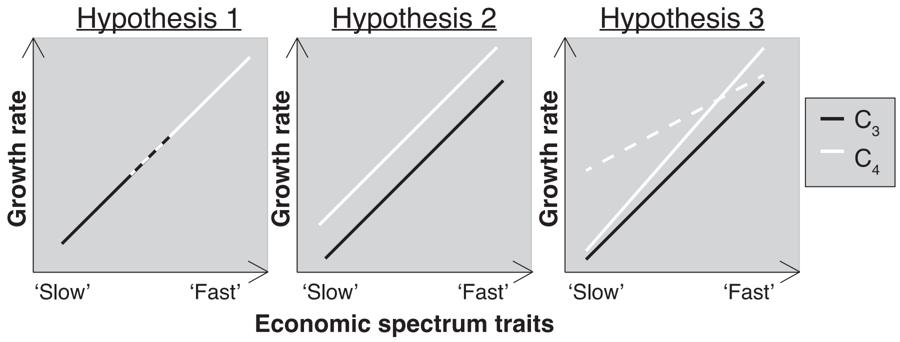
There is a general suite of leaf trait divergences between C_3 and C_4 species, with most evidence coming from the Poaceae family in which C_4 photosynthesis has evolved multiple times independently (Grass Phylogeny Working Group II, 2012). Across a sample of 382 C_3 and C_4 grasses, Atkinson et al. (2016) found that specific leaf area (SLA) was significantly higher in C_4 species than C_3 species, and leaf dry matter content (LDMC) was considerably lower. Together, these traits enable greater energy capture for a given leaf mass investment (Reich et al., 2003; Wright et al., 2004) and cause marked variation in relative growth rate (RGR). These leaf traits, in combination with the higher photosynthetic efficiencies of C_4 species, led to a daily growth advantage of 19%–88% over C_3 species (Atkinson et al., 2016). The C_4 CO_2-concentrating mechanism also ensures that C_4 species fix more carbon for a given investment in photosynthetic proteins, leading to higher nitrogen-use efficiency and, as a consequence, a lower leaf nitrogen requirement (Brown, 1978; Craine, Lee, Bond, Williams, & Johnson, 2005; Ehleringer & Monson, 1993; Ghannoum, Evans, & von Caemmerer, 2011; Reich, Walters, & Ellsworth, 1997).

While the leaf economic spectrum is a well-established axis of adaptive variation in plant functional traits (Diaz et al., 2016; Reich et al., 1997, 2003; Wright et al., 2004), the root economic spectrum is not universal, nor as strongly coordinated as in leaves (Reich, 2014). Root traits are considered to be highly plastic and greatly influenced by resource supply (Eissenstat, Wells, Yanai, & Whitbeck, 2000; Larson & Funk, 2016; Ryser & Eek, 2000) and climate (Freschet et al., 2017). In resource-poor environments, species typically display traits linked with investment in root structure and low root turnover, such as large diameter, low nutrient content and high tissue density, which prolong the retention of resources in roots, but lead to low plant growth rate. In contrast, a strategy of resource acquisition is typically adopted in high-resource environments, with fast-growing species having root traits associated with the rapid uptake of resources such as thin roots with a low tissue density and a large surface area (Eissenstat et al., 2000; Hernández, Vilagrosa, Pausas, & Bellot, 2010; Roumet, Urcelay, & Diaz, 2006; Ryser, 1998; Tjoelker, Craine, Wedin, Reich, & Tilman, 2005).

The study of root economic traits in relation to photosynthetic pathway has largely been confined to field surveys, with traits of both C_3 and C_4 species varying substantially and being aligned closely with resource availability (Angelo & Pau, 2016; Craine et al., 2005). How root traits differ by photosynthetic pathway in a common environment has been little explored. Atkinson et al. (2016) found that C_4 grass species had a higher root mass allocation than C_3 species in a common garden experiment, but did not explore functional root traits. The relationships among the economic spectra in different plant organs are also unclear. In theory, biophysical trade-offs and selection on coordinated strategies of resource-use should favour the convergence of economic traits in leaves and roots (Reich, 2014), but empirical evidence is mixed. Although similar economic scaling relationships have been observed in leaves and roots (Reich, 2014; Reich et al., 2008), direct comparisons of leaf and root strategies within species show that they are not always coordinated (Craine et al., 2005). The influence of photosynthetic pathway on the coordination of leaf and root traits is currently untested.

Here we examine the interactions between the fast–slow plant functional strategy axis and C_3 versus C_4 photosynthetic pathways in a large sample of grass species (100+) grown under controlled environment conditions that simulated the tropics. We measure functional traits above- and below-ground, and investigate how they are associated with growth rate in C_3 and C_4 species while accounting for phylogeny and allometric effects. Controlling for plant size when interpreting the relationships between growth rate and underlying functional traits rates is important because organisms become increasingly inefficient as they grow, due to self-shading, tissue turnover and allocation to structural components, and the allometric growth of roots and shoots means that mass allocation and leaf economic traits change with size (Enquist, West, Charnov, & Brown, 1999; Maranon & Grubb, 1993; Rees et al., 2010). In addition, the positive effect of C_4 photosynthesis on growth is highly size-dependent (Atkinson et al., 2016). For these reasons, correcting for plant size in comparative analysis of growth has led to revised interpretations of life-history trade-offs (e.g. Turnbull et al., 2012). It has already been established

BOX 1 Alternative hypotheses about the influence of photosynthetic pathway and economic traits on growth rate



Hypothesis 1 *Species segregate by photosynthetic pathway along a common RGR–economic trait relationship.*

The growth–trait relationships for C_3 and C_4 species have the same intercept and slope, but C_4 species fall more towards the upper end of this common relationship and C_3 species at the lower end. C_4 species achieve faster growth because they possess more resource-acquisitive traits than C_3 species, but considerable variation exists within C_3 and C_4 species in both traits and growth rates (e.g. Atkinson et al., 2016; Taylor et al., 2010) such that the growth–trait relationship is continuous across photosynthetic pathways.

Hypothesis 2 *Photosynthetic pathway and economic traits independently influence RGR.*

Growth–trait relationships for C_3 and C_4 species have the same slope, but the intercept for C_4 species is higher than for C_3 species. The C_4 carbon-concentrating mechanism functions equally well in leaves of differing construction, meaning that it provides a constant growth enhancement over C_3 species for a given set of economic traits.

Hypothesis 3 *Photosynthetic pathway and economic traits interact to determine RGR.*

Growth–trait relationships for C_3 and C_4 species have different slopes, with C_4 species having steeper (example 1) or flatter (example 2) slopes than C_3 species:

Example 1 (white solid line): Resource-conservative traits limit the growth enhancement provided by C_4 photosynthesis. Previous analysis of C_3 plants has shown that dense, tough leaves constrain the capacity for carbon assimilation through a low mesophyll conductance (Hassiotou, Renton, Ludwig, Evans, & Veneklaas, 2010).

Example 2 (white dashed line): If the growth rate of a species is already very fast, due to resource-acquisitive economic traits, then C_4 photosynthesis cannot increase the growth rate further (e.g. because growth has become more limited by the rate of tissue expansion than carbon acquisition). In contrast, the growth rate of species that possess resource-conservative traits can be increased using C_4 photosynthesis. Therefore, the growth 'boost' provided by C_4 photosynthesis depends upon economic traits.

that C_4 species have faster relative growth rate (RGR) and tend to adopt a more resource-acquisitive leaf strategy than C_3 species under hot, high-light conditions (Atkinson et al., 2016). These observations give rise to three alternative hypotheses for the relationship between growth rates and functional traits in C_3 and C_4 species (Box 1).

2 | MATERIALS AND METHODS

2.1 | Plant growth data

This work utilized plant material described in the study by Atkinson et al. (2016), and detailed methodology on experimental design, plant growth conditions and growth rate modelling can therefore be found there. Briefly, seeds were obtained for 382 grass species, representing a broad sample across the two major Poaceae clades (BOP [Bambusoideae, Oryzoideae and Pooideae] and PACMAD [Panicoideae, Arundinoideae, Chloridoideae, Micrairoideae, Aristidoideae and Danthonioideae]) and including 13 of the 22–24 evolutionary origins of C_4 photosynthesis in grasses along with C_3 sister lineages for comparison (Grass Phylogeny Working Group II, 2012). Seed sources included seed banks (89% of species), commercial suppliers (10% of species; derived from wild, non-cultivated populations) or the wild (1% of species). Seeds of each species were sterilized and germinated, and seedlings were transplanted into 1-L pots containing a 90:10 mix of vermiculite and sand.

Plants were grown under the same environmental conditions, in a controlled environment chamber (MTPS 120, Conviron) to provide a day/night temperature of 30/25°C and 70% relative humidity. Day length in the chamber was 14 hr, with a maximum photosynthetic photon flux density at canopy height of 1,600 μmol m^{-2} s^{-1}. Water and nutrient supply aimed to be non-limiting with a twice daily watering regime and a twice weekly feeding with 50% nitrate-type Long Ashton solution (Hewitt, 1966).

Two individuals of each species were harvested each week for 5 weeks. At harvest, plants were divided into leaf, stem and roots and the weight of the fresh leaves was recorded. Leaf area was measured using the WinDIAS Leaf Image Analysis System (Delta-T Devices), and root length, area and average diameter using the WinRHIZO Root Image Analysis System (Regent Instruments). The constituent parts of the plants were oven dried at 70°C and weighed to calculate total plant biomass.

2.2 | Growth analysis

Total plant dry mass over time was used to model species-specific growth curves using the R language and environment (R Core Team, 2019). Four-parameter logistic models were fitted for each species using the 'nlme' function (Pinheiro, Bates, Debroy, & Sarkar, 2016). These four parameters (described below) were then used to calculate RGR at a specific plant size, following Rees et al. (2010), using the following equation:

$$\mathrm{RGR} = \frac{k\left(A - \ln\left(M_c\right)\right)\left(B - \ln\left(M_c\right)\right)}{\left(A - B\right)}, \tag{1}$$

where M_c is the mass at which RGR is calculated. The four parameters A (minimum mass), B (the asymptotic or maximum mass), t_0 (the time when a plant is midway between A and B) and k (a growth parameter) were fitted as independent, species-specific random effects.

For species average RGR values, RGR was determined for each individual that functional traits were measured on (see the Section 2.3 below for details) using Equation 1 based on their harvested dry mass, and a mean calculated. A small number of individuals (6%) had negative RGR values because their mass exceeded the fitted asymptotic mass for that species. The effect of including these individuals in analyses was tested (see the Section 2.4 for details).

For the size-standardized growth rate, RGR was compared at a plant size of 0.044 g, which is the 60th percentile for total plant dry weight across all species and all harvests. This size encompasses all species, including those with large initial or small final sizes, and represents the plant size at which traits were predicted for the size-standardized analyses (see next section for details). RGR values produced using this method are robust and correspond well to other growth rate metrics (Atkinson et al., 2016).

2.3 | Functional trait data

Functional trait values were calculated for each individual as follows: specific leaf area (SLA = leaf area/leaf dry mass), leaf dry matter content (LDMC = leaf dry mass/leaf fresh mass), specific root area (SRA = root surface area/root dry mass) and specific root length (SRL = total root length/root dry mass). Carbon and nitrogen concentrations of the dry leaf and root material ([C] and [N], respectively) were measured after grinding samples in a ball mill (TissueLyser 2, Qiagen) using a CN analyzer (vario EL cube, Elementar). Only samples with a dry weight of 30 mg or more were analysed, to meet the minimum sample size needed to run the CN analyser, which reduced the number of individuals and species included in further analyses. Leaf data were reduced to 551 individuals belonging to 137 species (31 C_3 and 106 C_4 species) and, for roots, 497 individuals from 123 species (25 C_3 and 98 C_4 species). For the size-standardized analyses, all functional traits were predicted for a common reference size (as in the growth rate analysis: 60th percentile for total plant dry weight across all species and all harvests—0.044 g) by modelling their change with size and predicting a value at a common size (Rees et al., 2010).

2.4 | Comparative analyses

For both leaves and roots, we used principal components analysis (PCA) to reduce dimensionality of the trait data (both average species and size-standardized datasets), as leaf and root economic traits each tend to be tightly correlated, before comparison with RGR. Separate PCAs were performed on leaf trait (SLA, LDMC, [N] and [C]) and root trait (SRA, SRL, [N], [C] and average root diameter) data. All trait data were first log-transformed to improve normality. When the leaf and root data were merged, the dataset was further reduced (388 individuals belonging to 106 species).

The relationships of functional traits and photosynthetic pathway with RGR were determined using a phylogeny of the species and a generalized least squares approach ('pgls' function in the CAPER package; Orme et al., 2018). PGLS models were constructed to test the influence on mean RGR of the mean scores for PCA axes one and two, for leaves, roots or both (leaves and roots), together with photosynthetic pathway, with maximum likelihood methods used to calculate phylogenetic signal (Pagel's λ). An interaction between the explanatory variables (leaf or root PCA scores and photosynthetic pathway) was tested and included in the final analysis if significant. These analyses were repeated but excluding the 6% of individuals that had negative RGR values (due to their mass exceeding the asymptotic mass of the growth curve). We found their inclusion did not alter the outcome of these models (see Table S1 for model outputs using the reduced dataset). The size-corrected analysis was done in the same way but with RGR and trait values predicted at a common size.

To assess the coordination of leaf and root strategies, the relationship between leaf PCA axis one and the root PCA axis one was assessed using a PGLS approach with photosynthetic pathway added as an additional explanatory variable, for both the species average and size-standardized data.

To investigate trade-offs among functional traits, and how these interacted with photosynthetic pathway, pairwise relationships between traits were assessed, using PGLS models. Photosynthetic pathway was added as an additional explanatory variable to evaluate whether trait relationships differed between C_3 and C_4 species. p values were adjusted (using the Bonferroni correction) to account for multiple comparisons.

3 | RESULTS

3.1 | Leaf functional traits

Functional traits show strong covariance within leaves and within roots. For example, SLA and LDMC are inversely related, and SRL and SRA are positively related. Across the leaf trait analyses (species average and size-standardized data), axis one of the PCA was driven by SLA and LDMC in opposing directions, and axis two by leaf [N] and leaf [C] (Figure 1a; Table S2). PC axis one scores correlated negatively with whole-plant biomass ($p < 0.05$; Figure S1) such that larger plants have lower SLA and higher LDMC than smaller plants, demonstrating size dependence in the leaf traits and the importance of trait comparisons at a common size. PC axis one explained 43% of species average leaf trait variation and PC axis two explained an additional 28%. For the size-standardized analysis, these values were 44% and 28%, respectively.

Leaf functional traits and photosynthetic pathway explained a significant proportion of variation in RGR, both for species average ($p < 0.001$) and size-standardized data ($p < 0.001$, Table 1). Across 136 grass species, there was a significant correlation between RGR and the leaf traits represented by PCA axis one ($p = 0.003$). C_4 species had a significantly higher growth rate than C_3 species (by 0.039 g g^{-1} day^{-1}) for a given position along the traits axis one ($p < 0.001$), a difference that was independent of leaf functional traits (PCA axis one). There was therefore no significant interaction between these effects, supporting Hypothesis 2. In the size-standardized dataset, similar significant positive correlations between RGR and PC axis one ($p < 0.001$), and differences between C_3 and C_4 species ($p = 0.01$), were found (Table 1). Leaf PCA axis two was unrelated to growth rate ($p = 0.09$ for species average RGR; $p = 0.42$ for size-standardized RGR; Table S4).

3.2 | Root functional traits

Axis one of the PCA for the root traits of species was driven by high values of SRL and SRA in one direction, and larger average root diameter in the other direction (Figure 1b; Table S4). Axis two was associated with root [N] and leaf [C] in opposite directions. As with leaves, PC axis one for roots was correlated with whole-plant biomass ($p < 0.001$; Figure S2) such that larger plants have lower SRL and SRA and higher average root diameters than smaller plants, showing

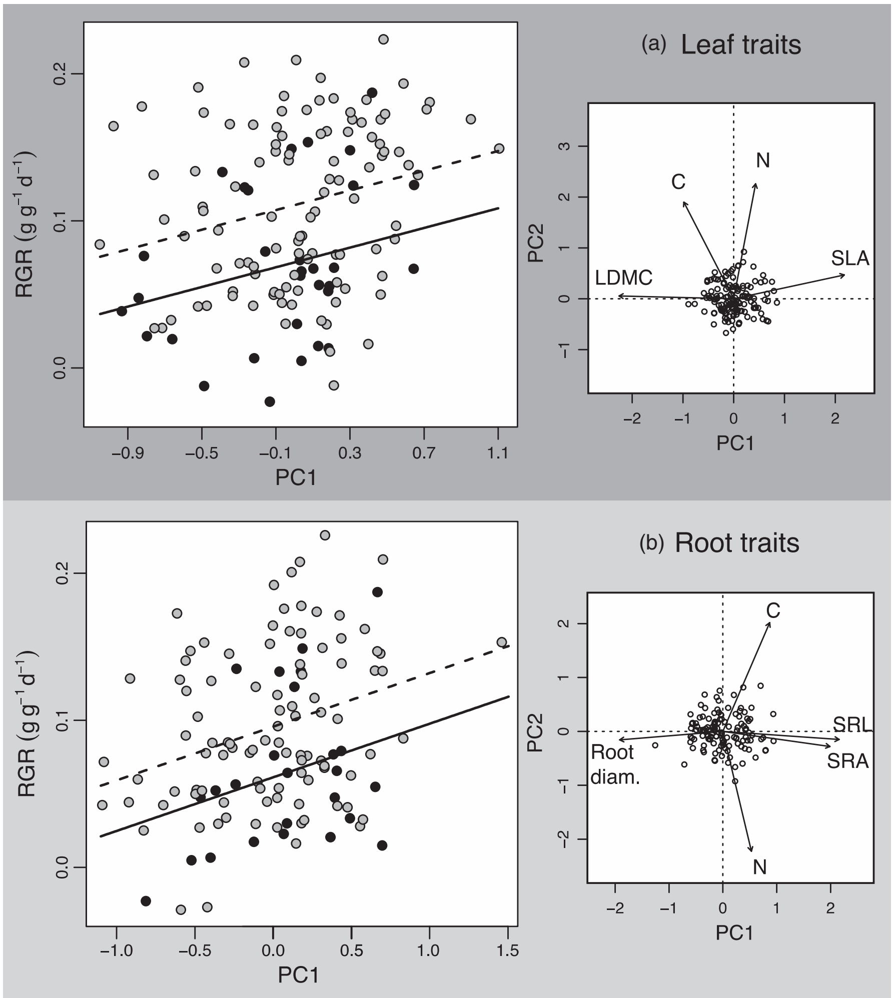


FIGURE 1 Species average relative growth rate (RGR) is positively associated with principal components analysis axis one (PC1) for grass leaf (a) and root economic traits (b). C_4 grass species (grey points, dashed lines) consistently achieve a faster RGR for a given PC1 score over C_3 grass species (black points, solid lines). In all cases, photosynthetic pathway and economic traits acted independently (i.e. no significant interaction) and significantly predicted RGR (leaf traits: $F_{2,133} = 12.1$, $p < 0.001$, $R^2 = 0.14$; root traits: $F_{2,119} = 9.66$, $p < 0.001$, $R^2 = 0.13$; both leaf and root traits: $F_{3,101} = 7.41$, $p < 0.001$, $R^2 = 0.16$). Regression lines are taken from phylogenetic generalized least squares models. The small number of negative RGR values occur where average plant size is above the modelled asymptotic plant size for a species. Trait abbreviations on PCA biplots: C, carbon concentration; diam., diameter; LDMC, leaf dry matter content; N, nitrogen concentration; SLA, specific leaf area; SRA, specific root area; SRL, specific root length

TABLE 1 The relationships between relative growth rate (RGR), plant traits and photosynthetic pathway. The relationships of growth rate (both species average and size-standardized values), photosynthetic pathway and leaf, root and the combination of leaf and root economic traits (represented by trait principal component analysis axis one) were modelled using phylogenetic least squares. Species average RGRs were significantly predicted by photosynthetic pathway in combination with leaf traits ($F_{2,133} = 12.1$, $p < 0.001$, $R^2 = 0.14$, Pagel's $\lambda = 0$), root traits ($F_{2,119} = 9.66$, $p < 0.001$, $R^2 = 0.13$, $\lambda = 0$) and both leaf and root traits ($F_{3,101} = 7.41$, $p < 0.001$, $R^2 = 0.16$, $\lambda = 0$). Species size-standardized RGRs were significantly predicted by photosynthetic pathway and leaf traits ($F_{2,133} = 42.9$, $p < 0.001$, $R^2 = 0.38$, Pagel's $\lambda = 0$) or the combination of leaf and root traits ($F_{3,101} = 20.45$, $p < 0.001$, $R^2 = 0.36$, $\lambda = 0.7$), but not root traits alone ($F_{2,119} = 0.78$, $p = 0.46$, $R^2 = 0.001$, $\lambda = 0$). Intercepts and slopes significantly different from zero are represented by asterisks; *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$

	Average RGR		**Size-standardized RGR**	
	Estimate (±*SE*)	***t* Value**	**Estimate (±*SE*)**	***t* Value**
Leaf				
Intercept	0.072 (0.010)	7.45***	0.233 (0.015)	15.06***
Functional traits	0.034 (0.011)	3.05**	0.080 (0.009)	8.23***
Photosynthetic pathway	0.039 (0.011)	3.50***	0.041 (0.015)	2.84**
Root				
Intercept	0.061 (0.010)	5.92***	0.228 (0.020)	11.38***
Functional traits	0.037 (0.010)	3.50***	0.007 (0.011)	0.62
Photosynthetic pathway	0.034 (0.012)	2.96**	0.023 (0.022)	1.00
Combined				
Intercept	0.068 (0.011)	6.31***	0.259 (0.019)	13.08***
Leaf functional traits	0.031 (0.012)	2.49*	0.080 (0.010)	7.63***
Root functional traits	0.032 (0.011)	2.93**	0.013 (0.009)	1.40
Photosynthetic pathway	0.032 (0.012)	2.62**	0.006 (0.020)	0.28

that it was necessary to compare size-standardized traits and RGR. PC axis one explained 56% of species average root trait variation and axis two explained an additional 26%. For size-standardized analysis, these values were 53% and 21%, respectively.

Species average RGR was significantly explained by root PCA axis one and photosynthetic pathway ($p < 0.001$, Table 1). There was a significant positive correlation between RGR and root PCA axis one ($p < 0.001$) such that species with high SRL and SRA, and thin roots, grew faster. For a given score on axis 1, C_4 species had a significantly higher RGR than C_3 species (by 0.034 g g^{-1} day^{-1}; $p = 0.004$; Figure 1b). However, when size-standardized, RGR was independent of root PCA axis one and photosynthetic pathway (Table 1), demonstrating that root economic associations with growth are driven by size. Root PCA axis two was unrelated to growth rate ($p = 0.71$ for species average RGR; $p = 0.42$ for size-standardized RGR; Table S3).

3.3 | Leaf and root functional traits

The economic spectra of leaf and root traits were not coordinated at the species level. Root and leaf PCA axis scores were independent of each other ($t = 0.26$, $p = 0.80$) and did not differ by photosynthetic pathway ($t = -0.008$, $p = 0.97$; overall PGLS model: $F_{2,102} = 0.04$, $p = 0.97$, $R^2 = 0.02$; Figure 2). This result held when traits were compared at a common plant size: leaf traits PCA axis scores were independent of root scores ($t = 0.41$, $p = 0.67$) and photosynthetic pathway ($t = 1.19$, $p = 0.23$; overall model: $F_{2,102} = 0.74$, $p = 0.47$, $R^2 = 0.001$).

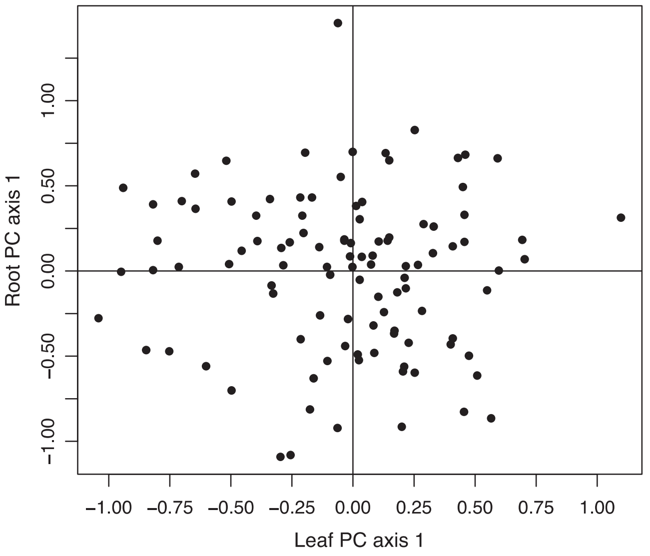


FIGURE 2 The economic spectra of leaf and root traits of grass species are not coordinated. Root and leaf PCA axis scores were independent of each other ($t = 0.26$, $p = 0.80$) and did not differ by photosynthetic pathway ($t = -0.008$, $p = 0.97$). The plot has points in each of the four quadrants, which shows that a species can adopt any combination of fast/slow leaf and root strategies

Species average RGR was significantly explained by the combination of photosynthetic pathway and PCA axis one values for both leaf and root traits (Table 1). Axes one for leaf and root traits were independently and positively correlated with RGR ($p = 0.01$ and $p = 0.004$

TABLE 2 Pairwise trait correlations for (a) species mean and (b) size-standardized data. Values are *t* values from PGLS models ($n = 105$). The direction and significance of differences in trait relationships between C_3 and C_4 individuals is indicated in brackets (non-significant results are not shown). Arrows represent how the coefficient of C_4 individuals differs relative to C_3 individuals (i.e ↑ indicates a higher coefficient for C_4, while ↓ indicates a lower coefficient for C_4). *p* values are adjusted using the Bonferroni correction to account for multiple comparisons. Slopes significantly different from zero are represented by asterisks; ***$p < 0.001$; **$p < 0.01$; *$p < 0.05$

	SLA	LDMC	Leaf N	Leaf C	Root N	Root C	SRL	SRA
(a)								
LDMC	**−7.97*** (↑4.46***)**							
Leaf N	**4.84***	**−3.62***						
Leaf C	−1.45 **(↑4.04**)**	2.71	2.26 **(↓4.76***)**					
Root N	**2.18***	−0.95	**7.67***	0.74				
Root C	1.63	0.50	−1.25 **(↓3.24*)**	−0.30	−2.16 **(↓4.27**)**			
SRL	1.04	1.50	2.67 **(↓2.76**)**	0.82	1.83 **(↓4.65***)**	**3.71** (↑3.20*)**		
SRA	0.77	1.25	2.41	0.90	1.50 **(↓4.56***)**	**3.27* (↑3.26*)**	**31.01***	
Root diameter	−1.31	−1.45	−1.86	−0.36	−1.86 **(↓4.91***)**	**−3.82****	**−18.82***	**−8.79***
(b)								
LDMC	**−7.95***							
Leaf N	0.17	0.43						
Leaf C	−1.67	**2.01***	0.39					
Root N	−1.58	1.89	1.50	1.81				
Root C	0.52	0.82	0.28	2.59	−0.22			
SRL	0.35	0.49	−0.21	1.51	−0.19	1.60		
SRA	**2.88***	−0.72	−0.97	−0.58	−1.34	1.85	**18.43***	
Root diameter	0.41	−1.76	−0.65	−1.95	−1.15	−0.88	**−17.65***	**−6.51***

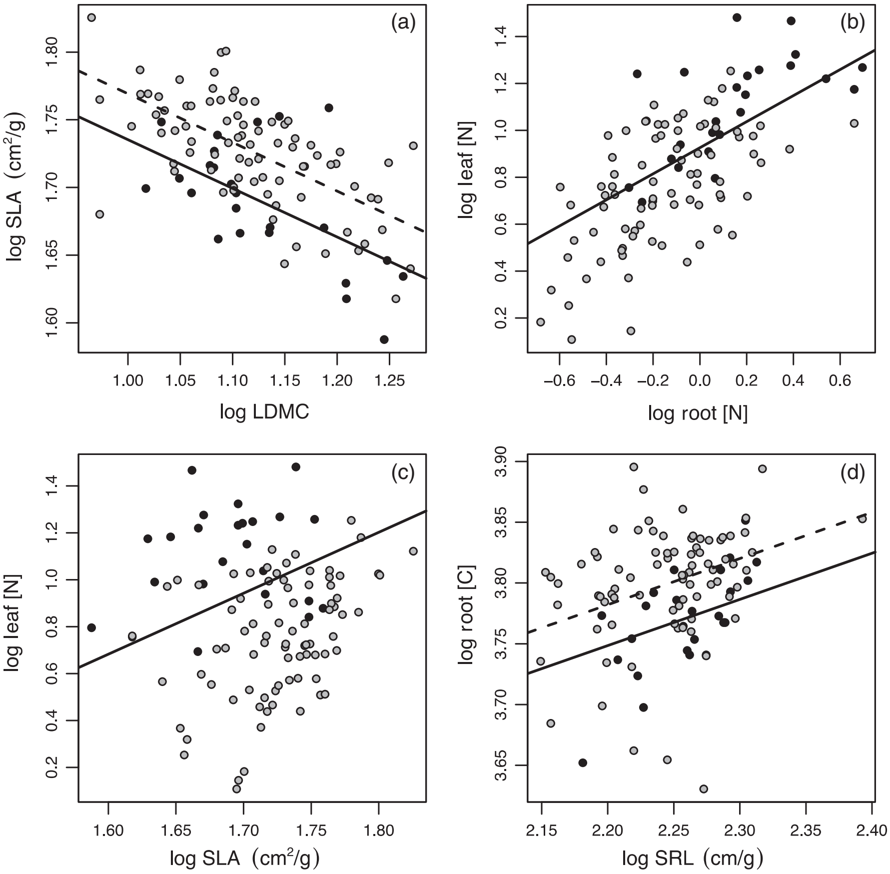


FIGURE 3 Functional trait relationships between species average data of (a) leaf dry matter content (LDMC) and specific leaf area (SLA), (b) root and leaf [N], (c) leaf [N] and SLA, and (d) root [C] and specific root length (SRL), for C_4 (grey circles; $n = 81$) and C_3 (black circles; $n = 24$) species. For (b) and (c), the relationships did not differ between C_4 and C_3 plants, and a common relationship is shown, whereas for (a) and (d), significantly different relationships are shown by the dashed (C_4) and solid (C_3) lines. All regression lines are significant ($p < 0.05$) and taken from the phylogenetic generalized least squares models

for leaves and roots, respectively), with the possession of 'fast' leaf and 'fast' root traits each increasing RGR by >0.03 g g^{-1} day^{-1} (i.e. having both would increase RGR by 0.063 g g^{-1} day^{-1}). There was a C_4 versus C_3 difference caused by a greater RGR in C_4 than C_3 plants of 0.03 g g^{-1} day^{-1} for a given combination of leaf and roots traits ($p = 0.009$). However, when size-standardized, RGR was only significantly associated with the leaf economic spectrum ($p < 0.001$) consistently, whereas average RGR variation was well explained by photosynthetic pathway and PCA axis one for both leaf and root traits ($p < 0.001$, $R^2 = 0.36$; Table 1). Adding leaf and root PCA axis two scores into the combined model explained little more RGR variation as neither were significantly associated with measures of RGR (Table S5).

3.4 | Trait relationships

We investigated the specific relationships among functional traits, and asked whether these differed between C_3 and C_4 species. For the species average data, approximately a third of the pairwise trait comparisons showed significant relationships (11/36 comparisons), with the same number showing significant C_3/C_4 differences (Table 2; Figure 2). Photosynthetic pathway differences were particularly associated with leaf [N], root [N], root [C] and root diameter. Once the traits were size-standardized however, far fewer pairwise comparisons showed significant relationships (6/36) and none showed significant C_3/C_4 differences, emphasizing the role of plant size in determining trait relationships (Table 2; Figure 3). In the size-standardized dataset, there were significant negative relationships between LDMC and SLA, and between SRA/SRL and root diameter, and positive relationships between SRA and SRL, LDMC and leaf [C], and SLA and SRA.

4 | DISCUSSION

We comprehensively investigate the interactions between the fast–slow plant functional strategy axis and alternative photosynthetic pathways, by controlling for environment, phylogeny and plant size, and sampling a large number of species. We find that, for a given suite of leaf and root economic traits, C_4 plants have a higher average RGR than C_3 plants under hot, high-light conditions. This difference arises because the growth–trait relationship for C_4 species has a higher intercept than C_3 species (Hypothesis 2) rather than there being slope differences (Hypothesis 3) or C_4 species occurring at the higher end of a common growth–trait relationship (Hypothesis 1). There is no interaction between photosynthetic pathway and economic traits, which suggests that leaf and root construction do not impact on physiological function (as in Hypothesis 3, example 1), nor is there a growth rate 'ceiling' that limits the C_4 growth advantage in species that possess resource-acquisitive traits (Hypothesis 3, example 2). Instead, the growth boost associated with resource-acquiring traits benefits C_3 and C_4 species to the same extent. C_4 species therefore have a significant growth advantage over C_3 species across the range of the fast–slow trait continuum, provided that the traits under study adequately capture the resource-acquisition potential in the growth conditions used.

Leaf functional traits are a better descriptor than root traits of a plant's ecological strategy along the fast–slow continuum, since they correlate with growth rate in both species average and size-standardized comparisons. For a given combination of leaf functional traits, C_4 species grow consistently faster than C_3 species under high temperature and light. Therefore, if all else is equal in hot, sunny habitats that filter leaf trait values to a particular range, C_4 species can grow faster and have a competitive advantage over their C_3 counterparts. Interestingly, since there is no interaction between the effects of photosynthetic pathway and leaf functional traits on RGR, C_4 photosynthesis has a greater proportional effect on the growth of species at the 'slow' end of the fast–slow continuum—that is, the total RGR increase is the same regardless of position on the continuum, but this represents a larger proportional increase when growth rate is already low. For example, RGR at the 'slow' end of the fast–slow continuum characterized by low values of SLA (210 cm^2 g^{-1}) is 66% higher in C_4 (e.g. *Bouteloua gracilis*) than C_3 (e.g. *Koeleria macrantha*) species. In contrast, RGR at the 'fast' end of the fast–slow continuum characterized by an SLA of 341 cm^2 g^{-1} is 39% higher for C_4 (e.g. *Digitaria seriata*) than C_3 (e.g. *Hymenachne amplexicaulis*) species.

Leaf economic traits are especially important to growth rate once it is size-standardized. In general, C_4 species have more resource-acquisitive leaves than C_3 species, with SLA values being 33%–39% higher in C_4 species, and LDMC lower (Atkinson et al., 2016). Changes in foliar anatomy preceded the emergence of the C_4 syndrome in grasses (Christin et al., 2013), and may have lowered leaf tissue densities in comparison to C_3 plants (e.g. through reductions in the proportions of cell wall or mesophyll investment in C_3 cycle enzymes; Atkinson et al., 2016). Alternatively, reduced tissue densities may have evolved after the C_4 carbon concentrating mechanism, representing an adaptation to the C_4 pathway (Christin & Osborne, 2014). Higher SLA has allowed C_4 plants to produce more leaf area per unit mass, which has resulted, in part, in faster growth under tropical conditions.

Although root traits correlate well with RGR for species average values, this relationship breaks down when comparisons are made at a common size, showing that the effects of these traits on growth depend on size. Plants with faster growth rates have higher SRA and SRL, and lower average root diameter, consistent with previous studies (Fort, Jouany, & Cruz, 2013). However, these trait values also corresponded with a lower plant biomass. This suggests that the decline in RGR with increasing size within each species arises partly from size-related decreases in SRL and SRA, which are probably caused by increasing investment in structural components as root systems grow larger. The results therefore suggest a decoupling between above-ground and below-ground strategies when species are compared at common sizes during the initial phase of rapid growth under laboratory conditions. In previous studies, a link has been made between high SRA/SRL, low average root diameter and fertile soils (Fort et al., 2013; Roumet et al., 2006). These results may arise from the high root turnover rate which is also associated with plants with a fast strategy (McCormack, Adams, Smithwick, & Eissenstat, 2012), and would mean a larger proportion of young roots, which by inference from our study would have a high SRA/SRL and low average diameter.

The additive effects of leaf traits and root traits on average RGR suggest that a plant can achieve rapid growth by producing either fast leaves or fast roots, but having both drives an even faster growth rate. However, leaf and root economic spectra were not coordinated at the species level such that a species can have fast leaves, fast roots, both or neither. The distribution of species across all four quadrants of the functional trait PCA axis space suggests that a species can have a mixture of strategies above-ground and below-ground, as found in field surveys (Craine et al., 2005; Tjoelker et al., 2005). Our work shows that this mixture of strategies persists under common environmental conditions. This suggests that the leaf and root traits of grasses adapt to different dimensions of the environment, rather than being coordinated in resource-acquisitive versus resource-conservative ecological strategies as previously predicted (Reich, 2014) and observed in the leaves and roots of higher land plants (Reich et al., 2008). One potential explanation for uncoordinated strategies found here is water availability; traits conferring a slow root system can also be efficient at extracting water from deep soil layers (Fort et al., 2013).

The C_4 CO_2-concentrating mechanism reduces requirements for photosynthetic proteins in leaves, especially Rubisco, and these account for a large fraction of leaf nitrogen in C_3 plants (Long, 1999). Our findings were consistent with this, and furthermore, we uncovered coordination in above-ground and below-ground [N] traits such that C_4 plants have less nitrogen in both their leaves and roots than C_3 plants. Similar to previous work (Craine et al., 2005), we found the positive correlation between leaf [N] and root [N] differed between C_3 and C_4 plants (although only marginally so, once *p* values were adjusted for multiple comparisons) such that C_4 plants had lower root [N] as well as leaf [N]. The lower requirement for nitrogen in C_4 leaves may also explain the difference in root [N]. High root [N] correlates with high nutrient uptake, metabolic activity and respiration rate (Atkinson, Hellicar, Fitter, & Atkin, 2007; Comas, Bouma, & Eissenstat, 2002; Reich et al., 2008), and C_3 species have a higher root respiration rate than C_4 species, which can be explained by differences in root [N] (Tjoelker et al., 2005). The higher root to shoot ratio (Atkinson et al., 2016) and lower leaf [N] of C_4 than C_3 plants imply that they require a lower nutrient uptake rate per unit root length for a given above-ground biomass. Conversely, C_3 species may have to invest more into root nutrient acquisition to fulfil the greater nitrogen requirement of their leaves. Variation in leaf [N] that is independent of leaf structure (caused by the C_4 effect) may account for why SLA and leaf [N] are largely independent in the PCA, and not tightly linked as is generally observed in the leaf economics spectrum (Wright et al., 2004).

By controlling for plant size in our analyses, we could explore the biological differences between species that do not arise solely from differences in size. Comparisons made at a common size considerably reduced the number of significant pairwise correlations between traits and removed all the photosynthetic pathway differences. In particular, correlations involving leaf and root [N] and root [C] lost significance and C_3/C_4 differences when they were size-standardized. For example, the relationship between SLA and leaf [N] was significant and positive and, for a given SLA, C_3 plants had higher leaf [N] (although only marginally so), which has been reported before (Taylor, Ripley, Woodward, & Osborne, 2011). However, the positive relationship disappeared in the size-standardized analysis. Therefore, these trait relationships are caused by size, whereby small plants have higher leaf [N], root [N] and root [C]. Differences between C_3 and C_4 species in these trait relationships are therefore due to plant size differences between photosynthetic types, rather than photosynthetic type per se.

In conclusion, across a large number of grass species, photosynthetic pathway acts independently to leaf and root functional traits in driving fast growth. Plants using C_4 photosynthesis achieve faster growth than their C_3 counterparts, but growth rate can be further boosted through the possession of resource-acquisitive root and leaf traits. C_4 species differ consistently from C_3 species in certain traits, particularly those relating to nutrient investment. However, some of these differences, and their resulting influence on growth rate, are a product of size variation between plants of differing photosynthetic type and not due to physiology.

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AUTHORS' CONTRIBUTIONS

C.P.O., R.P.F., K.T. and M.R. conceived the project; R.R.L.A., C.B., R.P.F., K.T., M.R. and C.P.O. designed the experiments; C.B., R.R.L.A., E.J.M., K.J.S. and S.M. carried out the experiments and compiled the data; K.J.S., C.B. and M.R. analysed the data; K.J.S., C.B., M.R. and C.P.O. wrote the manuscript with all authors contributing critically to drafts.

DATA AVAILABILITY STATEMENT

Data available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.xwdbrv1b1 (Simpson et al., 2020).

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