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Article:
Wade, R.N., Seed, P., McLaren, E. et al. (2020) The morphogenesis of fast growth in plants. New Phytologist, 228 (4). pp. 1306-1315. ISSN: 0028-646X

https://doi.org/10.1111/nph.16892

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New Phytologist

Research

The morphogenesis of fast growth in plants

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Received: *27 February 2020*
Accepted: *15 June 2020*

New Phytologist (2020)
doi: 10.1111/nph.16892

Key words: annual, C_4 photosynthesis, development, grasses, growth, morphology, perennial, photosynthetic pathway.

Summary

- Growth rate represents a fundamental axis of life history variation. Faster growth associated with C_4 photosynthesis and annual life history has evolved multiple times, and the resulting diversity in growth is typically explained via resource acquisition and allocation. However, the underlying changes in morphogenesis remain unknown.
- We conducted a phylogenetic comparative experiment with 74 grass species, conceptualising morphogenesis as the branching and growth of repeating modules. We aimed to establish whether faster growth in C_4 and annual grasses, compared with C_3 and perennial grasses, came from the faster growth of individual modules or higher rates of module initiation.
- Morphogenesis produces fast growth in different ways in grasses using C_4 and C_3 photosynthesis, and in annual compared with perennial species. C_4 grasses grow faster than C_3 species through a greater enlargement of shoot modules and quicker secondary branching of roots. However, leaf initiation is slower and there is no change in shoot branching. Conversely, faster growth in annuals than perennials is achieved through greater branching and enlargement of shoots, and possibly faster root branching.
- The morphogenesis of fast growth depends on ecological context, with C_4 grasses tending to promote resource capture under competition, and annuals enhancing branching to increase reproductive potential.

Introduction

Growth is a fundamental process of life and a central determinant of ecological interactions (Vile *et al.*, 2006). Plant species innately differ in their growth rates (Grime & Hunt, 1975), such that faster-growing species can have a short-term competitive advantage due to the rapid occupation of space and acquisition of resources, thereby out-competing slower-growing species (Grime & Hunt, 1975; Poorter, 1990; Rees, 2013). However, growth rate is inversely related to the allocation of internal resources to storage, maintenance and defence (Atkinson *et al.*, 2012; Huot *et al.*, 2014). These relationships lead to growth-survival trade-offs and selection against fast growth under particular ecological conditions (Grime, 1977; Rose *et al.*, 2009).

Maximum growth rate under favourable environmental conditions varies by more than an order of magnitude among plant species (Grime & Hunt, 1975; Atkinson *et al.*, 2016). This interspecific diversity underpins ecological theories that use functional attributes to explain the structure and dynamics of plant communities and the evolution of plant populations (Grime, 1977; Tilman, 1988; Díaz *et al.*, 2015). At larger scales, the growth rates of dominant species in each community influence vegetation productivity, such that spatial turnover in dominant species leads to variation in ecosystem functioning (Grime, 1977; Hooper & Vitousek, 1997).

Plant growth rate is strongly size dependent, and is usually normalised by total mass to give the 'relative growth rate' (RGR) (Grime & Hunt, 1975), or compared at a common plant mass (Turnbull *et al.*, 2012). Explanations for interspecific variation in RGR usually focus on the acquisition and internal allocation of resources. Species particularly differ in the physiological efficiency of nitrogen-use in photosynthesis and growth, the internal allocation of biomass to leaves versus roots, and the deployment of leaf mass as leaf area (i.e. specific leaf area, SLA) (Poorter & Evans, 1998; Weiner, 2004; Taylor *et al.*, 2010; Atkinson *et al.*, 2016). This conceptualisation of plant growth has provided important insights into the causes of functional diversity (Grime & Hunt, 1975; Grime, 1977). However, it does not consider the developmental processes that produce plant forms, that is morphogenesis.

Interspecific differences in morphogenesis give rise to architectural diversity (Reinhardt & Kuhlemeier, 2002), which is critical for differences between functional groups such as forbs vs graminoids (Grime *et al.*, 1997), the ecological adaptations of species (Wright *et al.*, 2017) and niche differentiation (Lynch, 2019). Conversely, growth potential may be limited by allometric relationships that arise from mechanical constraints, such as structural investment in leaves (Li *et al.*, 2008), or functional relationships, such as the dependence of water uptake on root volume and branching (Biondini, 2008). Further constraints may arise from the conservation of developmental mechanisms during the evolution of plant lineages (Watson, 1984). Crucially, rapid growth can only be achieved if plants have the potential to develop sinks for the carbon acquired by photosynthesis (White

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et al., 2016; Hayat *et al.*, 2017). The morphogenesis required for fast growth is therefore of central importance for understanding plant ecological diversification but has not been investigated systematically, and remains largely unknown.

Plants using the C_4 photosynthetic pathway grow faster than those using the ancestral C_3 type through an increased efficiency and rate of photosynthesis, and greater SLA (Black, 1973; Ehleringer & Björkman, 1977; Atkinson *et al.*, 2016). Annual plants also grow faster than closely related perennials, through a greater photosynthetic nitrogen-use efficiency (Garnier, 1992; Garnier & Vancaeyzeele, 1994; Poorter & Evans, 1998), and a higher SLA (Garnier & Laurent, 1994; Garnier *et al.*, 1997). C_4 photosynthesis and annual life history have both evolved multiple times in grasses (Poaceae), an economically and ecologically important plant family. Grasses are a good model system for investigating morphogenesis because they grow in a particularly orderly, predictable and repeated formation, conceptualised as a hierarchical arrangement of modules called phytomers (Gray, 1879). Faster growth in C_4 and annual grasses, compared with C_3 and perennial grasses, could theoretically arise from: (1) faster growth of individual phytomers; or (2) higher rates of phytomer initiation, arising from shorter intervals between organ initiation or branching events.

We used a phylogenetic comparative analysis of 74 species (Fig. 1) grown in a controlled environment to test which of these alternatives explains the contrasting growth rates in C_4, C_3, annual and perennial grasses. Our null hypothesis was that variation in species growth rates would arise equally from changes in the rates of phytomer growth and initiation. However, we predicted that phytomer growth might be more important because of conserved developmental processes that restrict the rate of phytomer initiation, and allometric relationships between phytomer size and costs (e.g. support structures could be cheaper in larger leaves). We also expected that morphogenesis would converge towards a similar pattern in species that have shared ecological strategies, such as the C_3 or C_4 photosynthetic pathway, or an annual or perennial life history.

Materials and Methods

We took a stratified sample of 74 grass species from across the BOP lineage (C_3 species only) and the PACMAD lineage (sister clade to BOP that includes 22–24 independently evolved C_4 lineages and related C_3 sister species) of grasses (Poaceae) (Grass Phylogeny Working Group II, 2012; Soreng *et al.*, 2017). Our strategy was to sample the diversity of C_4 and annual grasses using seeds available from public germplasm collections, covering as many of the independent C_4 grass lineages and their C_3 sister groups as possible. Within each C_4 and C_3 lineage, we also sampled multiple pairs of annual vs perennial groups, making a random draw within each of these lineages, where there was a choice of available seeds. Short-lived perennials (< 3 yr) were coded as annuals for this purpose (Clayton *et al.*, 2006). Overall, we sampled 12 independent lineages of C_4 grasses, and 20 monophyletic groups of annual grasses (Fig. 1), with nine of the annual lineages using C_4 photosynthetic pathway.

Seeds of each species were exposed to pregermination treatments determined from preliminary tests and information published by the Royal Botanic Gardens, Kew (Supplementary Material). The seeds were germinated in Petri dishes (20 seeds per 9 cm diameter Petri dish; Fisher Scientific Ltd, Loughborough, UK) and, once the first true leaf appeared, 20 seedlings for each species were transplanted to 2 litre pots (width, 5 cm; length 5 cm; height, 80 cm) filled with Medium Vermiculite (East Riding Horticulture Ltd, York, UK), and topped with *c.* 5 cm of wet sand, which remains at the top of the pot. There was *c.* 10% mortality at this point, but we were able to replace 30% of the seedlings that died soon after transplanting.

After transplanting, each seedling was assigned a location within controlled environment chambers (Conviron, BDW160 no. 2, S no. 000379), in a randomised block design (the 'block' in each case corresponded to one of the eight trolleys within each chamber). Plants were grown under 14 h daylength at 30°C : 25°C, day : night, with 80% humidity. An average photosynthetic photon flux density (PPFD) of 1056.4 ± 88.3 mol m^{-2} s^{-1} was measured ($N = 87$) using a handheld sensor (Li-Cor 190-R quantum sensor) at pot height. Plants were automatically watered for 30 min twice daily with deionised water using porous piping (LBS Worldwide Ltd, Lancashire, UK), and 100 ml of 50% Long Ashton nitrate-type nutrient solution (Hewitt, 1966) was manually applied to each pot twice a week.

Data were collected from three time-staggered experiments ('experiment' in the statistical analysis). The germination date of each seedling was recorded. Before transplanting seedlings into pots, nondestructive growth measurements were taken of 10 germinated seedlings for each species. The number of root tips and leaves were counted each day until transplanting. After transplanting, the total number of leaves, number of leaves on the main tiller and number of tillers were counted three times a week and main stem height measured once per week.

Four plants of each species were randomly selected and harvested approximately weekly for 5 wk throughout each experiment. Roots were removed, washed and cleaned, and the aboveground plant material detached from the roots. The leaves, stems (including leaf sheaths) and roots were then weighed separately for fresh mass (FM), after dividing stems and roots at soil level. Leaves were detached from the main stem at the ligule and the number of tillers and leaves counted. Image analysis was used to determine root architecture (total root length (cm), total root surface area (cm^2), average root diameter (mm), total number of forks and tips) (WinRhizo 2016; Regent Instruments, Quebec, Canada) and the total leaf area (cm^2) (WinDias 2009 v.3.2; Delta-T Devices, Cambridge, UK). The number of primary roots (roots originating from the embryo) was manually counted using images of the root system. Half of the replicate plants harvested at each time point were dried at 70°C and weighed for biomass (g DM). The number of secondary roots was calculated by subtracting the number of primary roots from the total number of root tips. Mean shoot and root phytomer sizes were calculated by dividing total shoot mass or root mass by the numbers of leaves or root forks.

To investigate the structural constraints on creating larger leaves, we quantified investment in tissues with functions in mechanical

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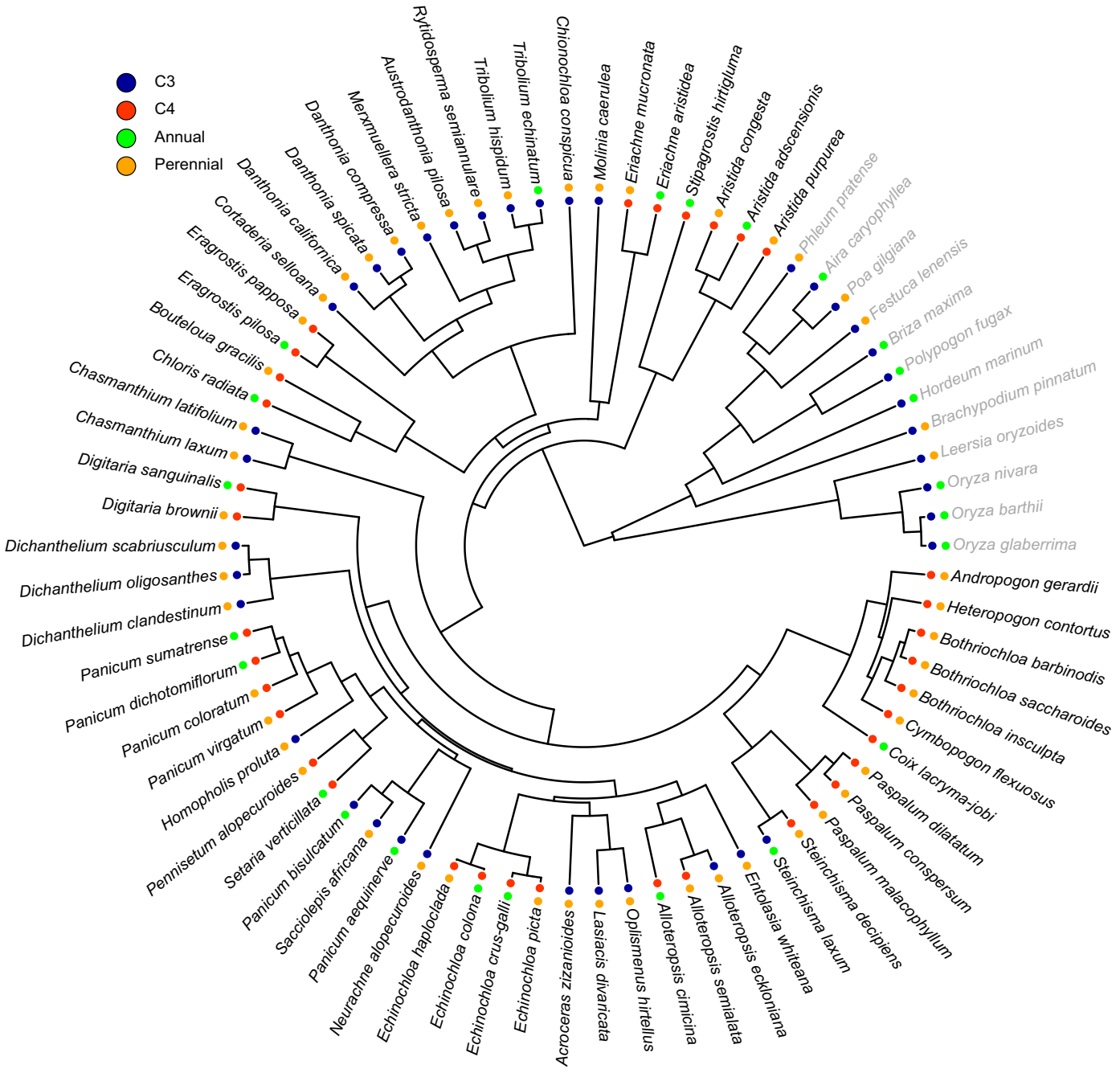

Fig. 1 Phylogeny of the species used in the experiments. Species names in the Bambusoideae–Oryzoideae–Pooideae (BOP) clade are coloured in grey, while those in the Panicoideae–Arundinoideae–Chloridoideae–Micrairoideae–Aristidoideae–Danthonioideae (PACMAD) lineage that contains all C_4 taxa are black. The photosynthetic pathway and life history of each species are indicated by coloured tips on the phylogenetic tree.

support (especially veins and fibres). We measured investment in tissues associated with veins, and which therefore had a potential function in mechanical support, using image analysis of transverse sections. Leaf transverse sections from 86 species of grasses representing all subfamilies as well as numerous C_4 lineages and their C_3 relatives were retrieved from a published dataset (Christin *et al.*, 2013). The original study measured the total cross-sectional area of veins, outer bundle sheath, inner bundle sheath, and mesophyll for each section, in a segment covering several veins (Christin *et al.*, 2013). For the same segments, we added measures of the total cross-sectional area of epidermis, bundle sheath extension (extra bundle sheath cells), and fibre tissues, so that all tissue types were included. Each area was normalised by dividing by the number of veins within the measured area. To scale for a whole leaf, we then multiplied these per vein values by the total number of veins per leaf. The total number of veins per leaf was counted and leaf width measured from leaves that had been cleared and stained following Scoffoni & Sack (2011).

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Phylogenetic analysis

A phylogeny for the species involved in the growth analysis was reconstructed using a set of sequences from four regions of the chloroplast genome that have been widely used in grass phylogenetics: *trnKmatK*, *rbcL*, *ndhF* and *trnLtrnF*. These markers were retrieved from NCBI databases when available for the species used here, and were amplified and Sanger sequenced using published protocols (Grass Phylogeny Working Group II, 2012) for species that had never been analysed. Each marker was then aligned using MUSCLE v.3.8.31 (Edgar, 2004), and the alignment was manually verified. The four markers were then concatenated, and a time-calibrated phylogenetic tree was inferred using BEAST v1.8.4 (Drummond & Rambaut, 2007). The GTR + G+I substitution model was used, and the speciation prior was set to a Yule process. A relaxed molecular clock with a log-normal distribution was used. The monophyly of each of the BOP and PACMAD clades was enforced to root the tree, and the split of the two clades was constrained by a normal distribution with a mean of 51.2 (age in million years estimated by Christin *et al.*, 2014) and a standard deviation of 0.0001. Two analyses were run for 10 000 000 generations, with sampling frequency of 1000 generations. Convergence of the runs and effective sampling sizes were monitored with TRACER v.1.6 (Rambaut & Drummond, 2015), and the burn-in period was set to 5000 000. Posterior trees from the two analyses were combined, and median ages were mapped on the highest credibility tree, which was used for comparative analyses. For the cross-section dataset, the phylogeny from the original publication was used (Christin *et al.*, 2013).

Statistical information

Bayesian mixed effects models (MCMCglmm (Hadfield, 2010)) were fitted using R STUDIO v.1.0.153 to account for nonindependence due to phylogeny, species, experiment and block and, if necessary, repeated measures. All models included random effects accounting for phylogenetic relatedness, between-species difference in means unrelated to phylogeny, experiment and block. For traits that could be measured nondestructively (e.g. number of tillers and leaves, and main stem length) an additional individual-specific random effect was fitted. All models were fitted using parameter-expanded priors (Hadfield, 2019). Continuous data were analysed assuming a Gaussian error distribution, whereas count data were analysed using a zero-truncated Poisson distribution. We determined the number of iterations, burn-in and thinning by visual assessment. We let the MCMC algorithm run for 5000 000 iterations with a sampling interval of 100. All models fitted were of the form:

$$\log_e(y) = \beta_0 + \beta_t t.$$

where $\log(y)$ indicates a log transformation or link function. This means the time slope, β_t, can be interpreted as average RGR over the growth period. Models were fitted with the following two-way interactions: life history (i.e. perennial vs annual) * time, photosynthetic pathway (i.e. C_3 vs C_4) * time and life history * photosynthetic pathway. We tested the effects of fitting three-way interactions between these factors, but none was statistically significant. We removed the species *A. cimicina* from the analysis for number of tillers, as it was an outlier, but there was no effect of removing it (Table S1).

To provide a more intuitive way of interpreting changes in RGR, we also calculated doubling times from the fitted models using a simple transformation. If M_0 is initial size, then a plant will be $2M_0$ some time later. We can calculate this time as:

$$2M_0 = M_0 \exp(r t_D)$$

where r is RGR, and so

$$t_D = \log_e(2)/r.$$

When comparing the results of multiple significance tests within each data table, we applied a sequential Bonferroni correction, which sequentially adjusts the threshold value for significance to account for multiple testing and to avoid type I errors (Rice, 1989).

Results

RGR was faster in annuals than perennials, and greater in C_4 than C_3 grasses (Table S2), as expected under the hot, high-light conditions of our experiment. In all fitted models the interactions (life history × photosynthetic pathway) and (life history × photosynthetic pathway × time) were not significant. As a consequence, we concentrate from here on the interactions of (photosynthetic pathway × time) and (life history × time), as these were the primary focus of the experiment.

The initial size and number of phytomers (intercept at time zero, the day of germination; Table 1) were similar for plants with each photosynthetic pathway. However, C_4 species initiated leaves at a slower rate compared with the C_3 type, such that the number of leaves on the main stem, and the average number of leaves per tiller (i.e. leaves per branch), increased more slowly in C_4 than C_3 grasses (Table 1). The rate of increase in the number of tillers (i.e. the production of new shoot branches) did not differ between C_4 and C_3 species (Table 1).

Faster growth in C_4 grasses could therefore not be attributed to either leaf or branch initiation rates. However, the rate at which shoot phytomers enlarged during the experiment differed substantially between C_4 and C_3 grasses. Shoot phytomers (Fig. 2a,b), including leaves, internodes and tillers, increased in size faster in C_4 than C_3 plants (Table 1), corresponding to an *c.* 50% reduction in doubling time for the size of C_4 shoot phytomers compared to the C_3 type.

Below ground, a marginally slower rate of primary root initiation, combined with a marginally faster rate of secondary root initiation, meant that secondary root branching on each primary root was faster in C_4 than C_3 species (Table 1; Fig. 3c,d). The outcome of this faster branching was that mass accumulation occurred more quickly for each primary root in C_4 than C_3

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Table 1 Effects of transition from $C_3 \rightarrow C_4$ or perennial → annual on the initial size or number of phytomers and their relative growth rate (RGR).

			Transition $C_3 \rightarrow C_4$		Transition perennial → annual	
			ΔIntercept	Δ*RGR*	ΔIntercept	Δ*RGR*
Number of modules	Above ground	No. of leaves	ns	−0.005	ns	**0.010****
		No. of tillers	ns	ns	−0.977	**0.037****
		No. of leaves per tiller	ns	**−0.007****	ns	ns
		No. of leaves per main tiller	ns	**−0.014****	ns	ns
	Below ground	No. of root forks	ns	ns	ns	0.024
		No. of primary roots	ns	−0.009	ns	0.010
		No. of secondary roots	ns	0.022	ns	0.026
		No. of secondary roots per primary root	ns	**0.031***	ns	ns
Size of modules	Above ground	Shoot phytomer mass	ns	**0.034****	−1.330	**0.032****
		Internodal mass	ns	**0.034****	−1.203	**0.031****
		Tiller-phytomer mass	ns	**0.027****	−1.239	**0.031****
	Below ground	Root phytomer mass	ns	0.021	ns	ns
		Root diameter	ns	**0.017****	ns	ns
		Primary root phytomer mass	ns	**0.045****	−1.021	**0.027***

ΔIntercept is the change in the intercept (initial size or number at time, $t = 0$), and Δ*RGR* is the change in time slope (RGR, d^{-1}).
Results in plain type were individually significant at $P < 0.05$, but became nonsignificant after the sequential Bonferroni correction, which adjusts the significance threshold to account for multiple testing within the table. The results in bold type remained significant after this correction had been applied.
ns, nonsignificant ($P > 0.05$); *, $P < 0.05$; **, $P < 0.01$.

plants (Table 1). Root phytomers increased in diameter faster in C_4 than C_3 plants (Fig. 2c,d), but there was only weak evidence that this was accompanied by greater root phytomer mass (Table 1).

Overall, faster above-ground growth in C_4 than C_3 grasses was therefore achieved through gigantism in shoot structures. The leaves and tillers of C_4 species enlarged more rapidly, despite a slower leaf initiation rate and no change in the rate of tiller branching. Below ground, the roots of C_4 species increased in diameter faster and produced secondary branches more quickly than the roots of C_3 grasses.

The effects of annual and perennial life histories on all measured growth parameters were the same for C_3 and C_4 grasses, that is there were no interactions between annual vs perennial and C_3 vs C_4. There was some evidence that annual grass seedlings had smaller initial shoot phytomer size than the perennials (Table 1). However, the relative increase in the numbers of shoot phytomers was much faster in annuals than perennials (Table 1). Tiller branching was especially rapid, with a *c.* 30% decrease in the tiller doubling time in annuals compared with perennials (Fig. 3a,b; Table 1). The higher growth rate in leaf number for annuals compared with perennials arose entirely because of faster tiller branching, as the rate of leaf initiation per tiller remained unchanged (Table 1). This difference in branching rate between annuals and perennials contrasts markedly with the situation for C_3 and C_4 plants (Table 1).

Similar to the situation for C_4 compared with C_3 grasses, the enlargement of above-ground phytomers was faster in annual than perennial species (Fig. 2a,b). The average mass of leaves, internodes and tillers increased more quickly in annuals than perennials, leading to a *c.* 50% reduction in the time taken for each to double in size (Table 1). Conversely, the growth rate of root phytomer mass and thickness (Fig. 2c,d) did not differ between annual and perennial plants.

Although root phytomer size was the same in annuals and perennials, the mass growth per primary root was greater in annuals than perennials (Table 1). There was some evidence of faster root initiation in annual than perennial species, with the number of primary roots and root branching increasing more rapidly (Table 1). Although none of these effects was statistically significant after the correction for multiple testing had been applied, the data suggest that quicker branching is a more likely explanation than greater phytomer size for the faster root growth in annuals (Table 1; Fig. 3c,d).

Overall, the higher growth rates in annual than perennial grasses were achieved through the faster branching of tillers, and more rapid enlargement of tillers and leaves. There was some evidence of faster root branching. However, as with the C_3 vs C_4 contrast, the leaves on each tiller (i.e. leaves per tiller and leaves per main tiller) were not initiated more quickly in annuals than perennials.

Differences in morphogenesis between C_4 and C_3 plants, and between annuals and perennials, gave rise to significant structural changes at the whole-plant scale. Height growth, measured by the rate of main stem elongation, was faster in C_4 and annual plants compared with C_3 and perennials, leading to reductions in the time taken for height to double of *c.* 20% for C_4 and *c.* 30% for annual plants (Table 2). The whole-plant leaf area also enlarged faster in C_4 than C_3 grasses despite the slower leaf initiation of C_4 species (Table 2), leading to a *c.* 30% reduction in doubling time for the total plant leaf area. Similarly, there was a significant difference in total leaf area growth between annuals and perennials (Table 2). Underpinning these differences, individual leaves grew larger in both C_4 and annual grasses than in

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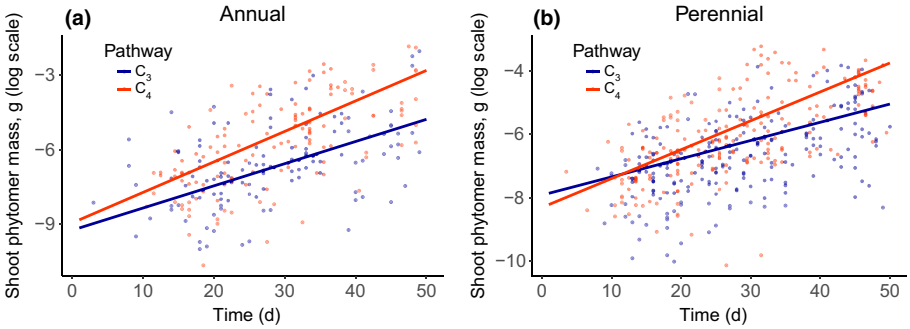


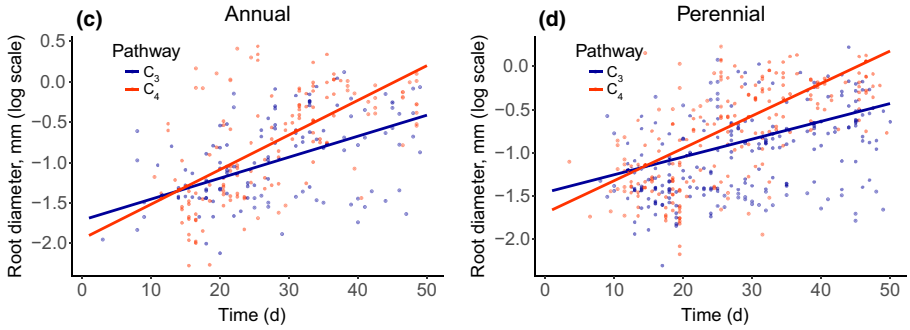
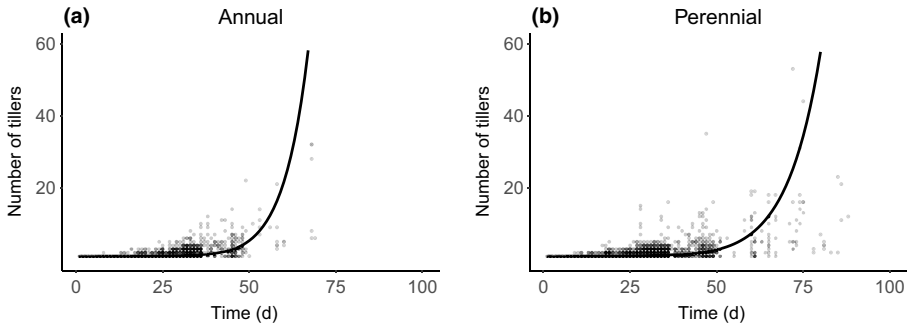


Fig. 2 Growth in the size of shoot and root phytomers. Growth over time in the mass of shoot phytomers, which each consist of one leaf, sheath and internode (g DM) (a, b), in C_3 and C_4, annual and perennial grasses. Mean root diameter (mm) over time (c, d) in C_3, C_4, annual and perennial grasses. The density of colour in each case indicates the number of stacked points corresponding to each value.



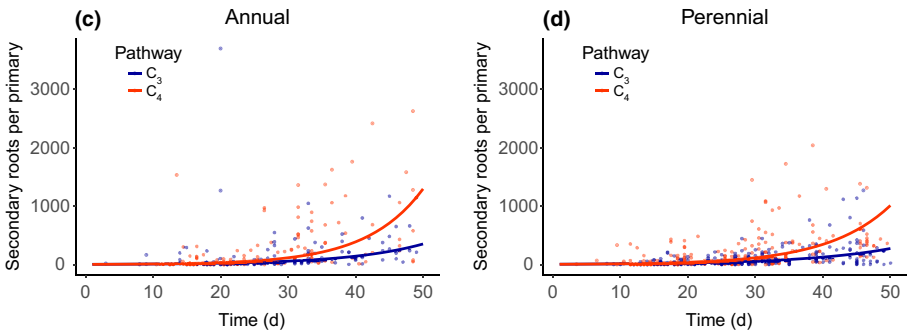


Fig. 3 Branch development in shoots and roots. The upper panels (a, b) shows the numbers of tillers through time in annuals and perennials, while the lower panels (c, d) show the numbers of secondary roots per primary root in C_3, C_4, annual and perennial grasses. The density of colour in each case indicates the number of stacked points corresponding to each value.

C_3 and perennial species (Table 2). The root systems of both C_4 and annual plants also enlarged significantly faster than C_3 and perennial species, respectively. The surface area and total length of roots enlarged faster in C_4 than C_3 species, causing a *c.* 20% reduction in doubling time, and in annuals than perennials, resulting in a *c.* 30% reduction in doubling time (Table 2).

After the sequential Bonferroni correction, there were no significant differences in the RGRs of SLA and specific root length (SRL) between C_4 and C_3 species, or between annual and perennial species (Table 2). These results did not change when plants were compared at a common size (Table S2). After the Bonferroni correction, we found no differences in the RGR of root allocation based on photosynthetic pathway or life history (Table S2). There was also no evidence of differences in dry weight relative to fresh weight, when compared over time (Table S2).

The relationship between the cross-sectional area of support tissues and leaf width was not significantly different from isometric, (slope = 0.94, 95% CI = 0.55–1.37; Fig. 4). There was some evidence that C_4 species have higher investment than C_3

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Table 2 Effects of transition from $C_3 \rightarrow C_4$ or perennial → annual on the initial size of shoot and root systems and their relative growth rate (RGR).

		Transition $C_3 \rightarrow C_4$		Transition perennial → annual	
Growth parameter		ΔIntercept	Δ*RGR*	ΔIntercept	Δ*RGR*
Above ground	SLA	0.358	−0.010	ns	ns
	Total plant leaf area	ns	**0.033*** **	ns	**0.037*** **
	Lamina area of individual leaves	ns	**0.027*** **	ns	**0.027*** **
	Main stem length	ns	**0.019*** **	ns	**0.024*** **
Below ground	SRL	ns	−0.013	ns	ns
	Total surface area of the root system	ns	**0.027***	ns	**0.034****
	Total length of the root system	ns	**0.021***	ns	**0.032*** **

ΔIntercept is the change in the intercept (initial size or number at time, $t = 0$), and Δ*RGR* is the change in time slope (RGR). SLA, specific leaf area; SRL, specific root length.
Results in plain type were individually significant at $P < 0.05$, but became nonsignificant after the sequential Bonferroni correction, which adjusts the significance threshold to account for multiple testing within the table. The results in bold type remained significant after this correction had been applied.
ns, nonsignificant ($P > 0.05$); *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

plants in support structures ($P < 0.05$; using a phylogenetic regression fitted in MCMCglmm). However, the cost of making leaves did not differ among species with different life histories (i.e. annuals vs perennials; $P > 0.3$).

Discussion

This study characterises the association between plant morphogenesis and increased rates of carbon fixation and biomass accumulation. Fast growth is achieved through contrasting patterns of morphogenesis in C_4 and annual grasses (Fig. 5), but these differences are additive and there is no evidence of an interaction between photosynthetic pathway and life history. However, our analysis is unable to infer causality. Higher rates of photosynthesis in C_4 and annual plants may have caused morphogenesis to adapt by developing larger sinks for the extra carbon fixed. Alternatively, C_4 photosynthesis and annual life history may have evolved more easily in lineages that already had faster phytomer growth, as this provided the sinks needed to utilise fixed carbon. Recent work has clarified the situation for annuals from the BOP grass lineage (Fig. 1), showing that fast RGR is not a prerequisite for the evolution of annual life history. Instead, annuals evolve at a faster rate in lineages with a larger investment in shoot relative to root mass (Lindberg *et al.*, 2020).

The faster growth of C_4 than C_3 grasses is manifested as gigantism in the size of shoot phytomers, but with no change in shoot branching. This result implies important ecological benefits within C_4 grass communities for plants with larger leaves and taller shoots, rather than more branches. In highly modular herbaceous plants like grasses, larger leaf modules lead to a taller shoot stature (Niinemets, 2010), which increases the ability of plants to compete for light with neighbours (Violle *et al.*, 2009). However, although structural investment is greater in C_4 than C_3 leaves, we found that larger leaves are no more efficient to deploy than smaller ones (Fig. 4).

Faster growth below ground in C_4 than C_3 grasses is used to produce more secondary roots, leading to more densely branched root systems. Greater secondary root branching in crops is associated with a more efficient exploration of the soil volume and better scavenging of immobile nutrients such as phosphorus (Lynch, 2019). More generally, a higher total length of roots within a particular soil volume increases the ability of a species to pre-empt the supply of nutrients (Craine & Dybzinski, 2013). These observations imply that nutrient capture may be an

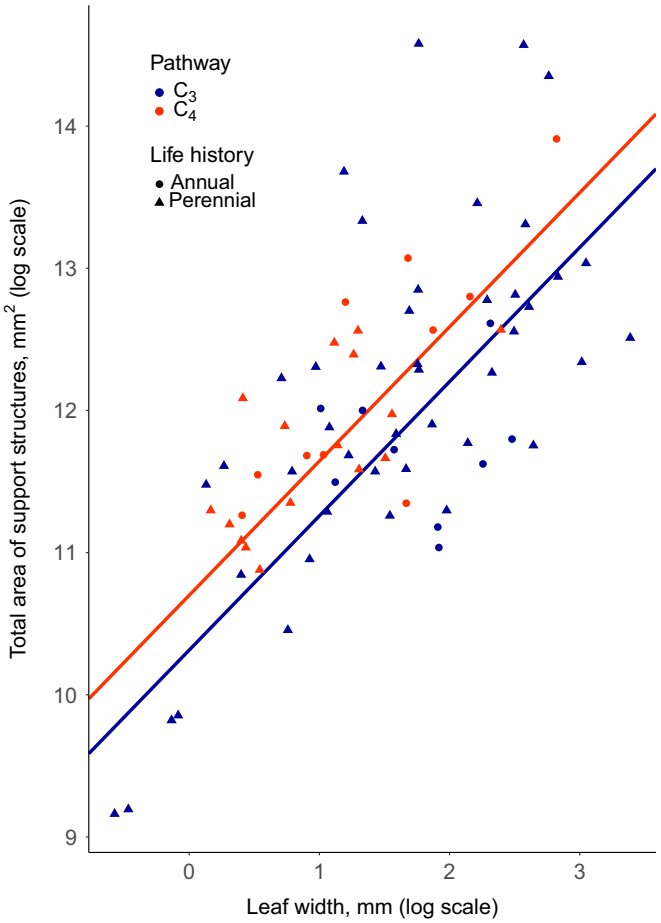

Fig. 4 Investment in leaf support structures. The relationship between the total area of leaf support structure in cross-section (mm^2) and the size of leaf (leaf width, mm). The effect of C_4 photosynthesis is marginally significant ($P < 0.05$), the slope of the lines is 0.94 (95% CI = 0.55–1.37).

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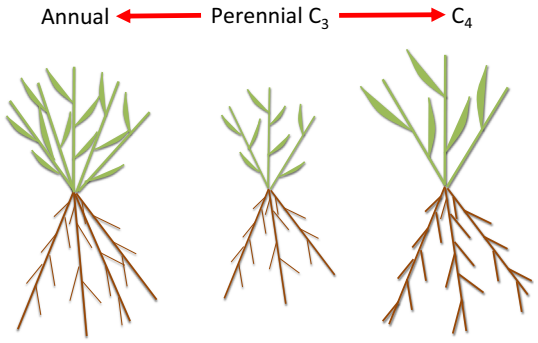

Fig. 5 Morphogenesis of growth in C_3 perennial, C_4 and annual grasses. Diagrammatic representations of the morphogenesis that underlies the growth rate differences between C_3 and C_4, and annual and perennial plants. Supporting values and statistical evidence are given in Table 1.

important selection pressure for the morphogenesis of fast root growth in C_4 grasses. Faster root elongation, and increased root surface area and diameter in C_4 than C_3 plants, has the potential to increase nutrient uptake, giving better access to water, greater resilience to root herbivores (Johnson *et al.*, 2016), a larger tissue volume for storage and a larger surface for interactions with soil mutualists.

Based on our results, C_4 plants should therefore capture light more effectively and pre-empt resource capture from a larger soil volume than their C_3 counterparts, making them stronger competitors during seedling establishment and vegetative growth.

By contrast with the effects of C_4 photosynthesis, the transition to an annual life history is associated with a developmental pattern of faster shoot branching in addition to shoot gigantism. Although annuals produce smaller seeds and have a smaller initial size (Rees, 1996), they grew faster than the perennials in our experiment (Table 1). The fast growth of annuals is usually interpreted in terms of changes to resource allocation and photosynthetic nitrogen-use efficiency (Garnier, 1992; Garnier & Vancaeyzeele, 1994). Our finding of faster branching in annuals than perennials brings two potential ecological benefits in the disturbed habitats occupied by these species. First, in disturbed, open habitats with a low density of competitors, more rapid branching enables faster lateral spread, reducing the aggregation of foliage and self-shading, facilitating more efficient light capture (Niinemets, 2010). Such a strategy would be disadvantageous in a densely occupied sward, where vertical growth is more important. Secondly, as each tiller in grasses has the potential both to terminate in a seed-bearing inflorescence and to generate further branches, faster branching in annuals rapidly enhances their reproductive potential.

Although phytomer size and branch initiation rate differed systematically among functional groups, the initiation of new leaves by each tiller was not consistently used as a mechanism to grow faster. Instead, the rate of leaf initiation remained unchanged in annuals compared with perennials, and consistently slowed in C_4 compared with C_3 species. This occurred despite published evidence that the leaf emergence rate accelerates within a particular genotype in response to the carbon supply relative to demand (Baumont *et al.*, 2019). One interpretation of our results is that having more leaves does not bring the same ecological benefits as having larger leaves or more branches (i.e. ecological selection). An alternative is that leaf initiation rate may trade off against leaf size (Huang *et al.*, 2016), such that producing larger leaves inevitably slows the rate of leaf initiation. Finally, the developmental process of leaf initiation may be constrained, such that it is unable to go faster. Crop research shows genetic variation in leaf emergence rate (e.g. Morita *et al.*, 2005) and differences among species (e.g. Frank & Bauer, 1995), but to our knowledge the hypothesis of an upper limit to leaf initiation rate remains untested.

Fast growth is inversely related to storage, maintenance and defence (Atkinson *et al.*, 2012; Huot *et al.*, 2014), such that it is most beneficial in resource-rich environments and trades off against survival in resource-poor and disturbed environments (Grime, 1977; Rose *et al.*, 2009). Our work illuminates a previously unrecognised facet of fast growth, revealing that the relationships of rapid biomass accumulation to morphogenesis depend on ecological context and may be constrained by development. Sampling multiple independent lineages of C_4 and annual plants has enabled us to infer that fast growth is consistently linked to differing strategies of morphogenesis in each case. We find that fast growth enables resource acquisition and allocation to be coordinated with morphogenic changes that enhance either competitive ability or reproductive potential.

Acknowledgements

We thank University of Sheffield summer placement students (Carrie Alderley, Joseph Booth, Will Burn, Robert Hardy, Anthony Hodder, Alana Jones, Caroline Milson, Harriet Myers, Barbara Ojei Agwaziam, Charles Philpot, Anthony Putzfeld, James Rafferty, David Rapley, Lucy Vaughan, and Christopher Weston) for their technical assistance. The work was funded by NERC via a standard grant (NE/N003152/1) awarded to CO, PAC, KT and MR. PAC is supported by a Royal Society Research Fellowship (grant no. URF/R/180022).

Author contributions

RNW, CPO, MR and KT designed the study. RNW, PS and EM carried out the comparative growth experiment, and data on leaf structural support were collected by PAC and HEW. PAC produced the phylogeny. RNW, MR and CPO analysed the data and interpreted the results. RNW, CPO and MR wrote the paper. All the authors provided critical comments on drafts of the manuscript.

Data availability

Plant growth data and phylogenies were deposited at the NERC Environmental Information Data Centre (EIDC), https://doi.org/10.5285/cb0d7a37-45c5-4645-b5ef-ba097d92fc20. DNA sequences have been deposited with NCBI, with the accession numbers MT587581 for *Entolasia whiteana* matK and MT586483 for *Entolasia whiteana* ITS.

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Supporting Information

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

Table S1 Effects of transition from $C_3 \rightarrow C_4$ or perennial → annual on the number of shoot branches (i.e. tillers) and their relative growth rate.

Table S2 Effects of transition from $C_3 \rightarrow C_4$ or perennial → annual on plant mass or area and its relative growth rate.

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