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Research

Bigger genomes provide environment-dependent growth benefits in grasses

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Summary

- Increasing genome size (GS) has been associated with slower rates of DNA replication and greater cellular nitrogen (N) and phosphorus demands. Despite most plant species having small genomes, the existence of larger GS species suggests that such costs may be negligible or represent benefits under certain conditions.
- Focussing on the widespread and diverse grass family (Poaceae), we used data on species' climatic niches and growth rates under different environmental conditions to test for growth costs or benefits associated with GS. The influence of photosynthetic pathway, life history and evolutionary history on grass GS was also explored.
- We found that evolutionary history, photosynthetic pathway and life history all influence the distribution of grass species' GS. Genomes were smaller in annual and C_4 species, the latter allowing for small cells necessary for C_4 leaf anatomy. We found larger GS were associated with high N availability and, for perennial species, low growth-season temperature.
- Our findings reveal that GS is a globally important predictor of grass performance dependent on environmental conditions. The benefits for species with larger GS are likely due to associated larger cell sizes, allowing rapid biomass production where soil fertility meets N demands and/or when growth occurs via temperature-independent cell expansion.

Introduction

Genome-related costs to plant growth are widely reported to scale with genome size (GS; Knight *et al.*, 2005; Simonin & Roddy, 2018). Data suggest that species with larger genomes have slower cell cycle rates (Bennett, 1972; Šímová & Herben, 2012), and higher nitrogen (N) and phosphorus (P) demands (Kang *et al.*, 2015; Anneberg & Segraves, 2023). They are also associated with larger nuclear and cell sizes (Beaulieu *et al.*, 2008; Roddy *et al.*, 2020, but see Jordan *et al.*, 2015), which may reduce gas exchange efficiency (Théroux-Rancourt *et al.*, 2021) and growth rates (Zhukovskaya *et al.*, 2019). Despite these costs, plant species vary significantly in GS (ranging > 2400-fold in 1C-value, the amount of DNA in an unreplicated gametophytic nucleus, across angiosperm species; Pellicer *et al.*, 2018). While most plant species have small genomes (modal GS = 0.6 pg per 1C; Pellicer *et al.*, 2018), the existence of larger GS species (up to 152.23 pg per 1C) suggests that such costs may be negligible or actually represent benefits under certain conditions (e.g. Grime & Mowforth, 1982; Boman & Arnqvist, 2023).

Cellular N and P costs likely increase with GS (Faizullah *et al.*, 2021; Anneberg & Segraves, 2023) due to enhanced demands of N- and P-rich nucleic acids and the costs of making and maintaining larger cells. Such costs can be overcome when these nutrients are readily available. For example, nutrient-addition experiments show that species with larger genomes are more competitive than those with smaller genomes when grown in soils fertilised with N and/or P (Šmarda *et al.*, 2013; Guignard *et al.*, 2016; Peng *et al.*, 2022). Thus, a larger cell size associated with larger GS may provide environment-dependent growth advantages over small GS when nutrients are not limiting. It is also likely that species with larger GS can increase the production of biomass more rapidly than smaller GS species when nutrients are available because of the scaling effects of GS on cell size (e.g. Pacey *et al.*, 2022).

Given the inverse relationship between stomatal size and density (Beaulieu *et al.*, 2008), it has been hypothesised that larger GS species will be more water use efficient than small GS species, due to their larger guard cells and stomatal pore sizes, which may reduce rates of stomatal conductance (Faizullah *et al.*, 2021) and enable greater water storage in the larger vacuoles of leaf cells (Leitch & Leitch, 2022). Consistent with this hypothesis, controlled growth experiments of *Solidago gigantea* showed that tetraploids had higher water use efficiency than diploids (Walczyk &

Hersch-Green, 2022) and species of *Schoenus* and *Tetraria* (Cyperaceae) with larger GS had lower stomatal conductance (van Mazijk *et al.*, 2024). However, other studies have shown different trends (e.g. Schley *et al.*, 2022; Šmarda *et al.*, 2023), meaning the role GS plays in influencing a species' response to reduced water availability is unclear.

At lower temperatures, where growth is limited by slower cell division rates, the larger cell size of species with larger GS may enable rapid growth by expansion of existing cells (which is unaffected by low temperatures) and negates the material costs associated with cell division (Haber & Luippold, 1960; Grime & Mowforth, 1982). This contrasts with tropical growth temperatures, where cell division rates are high but are slowed in large GS species by the greater time needed to replicate DNA, negatively affecting plant performance (e.g. Francis *et al.*, 2008) and/or because larger cells are more difficult to maintain at higher temperatures, resulting in selection for small cells and hence genomes (Sabath *et al.*, 2013). Therefore, plants with larger genomes may enhance plant growth performance under high soil fertility, low growth temperatures and possibly low water availability, and these effects may play a role in influencing the distribution of species across climatic and edaphic gradients (Fig. 1), as observed at the global scale (Bureš *et al.*, 2024).

The impact of GS on cell size may also interact with physiological and life-history adaptations. For example, C_4 photosynthesis is associated with a suite of leaf anatomical characters that constrain cell size and hence may also limit the evolution of C_4 photosynthesis in species with larger GS. The rapid diffusion of C_4 metabolites from the site of carbon assimilation (mesophyll cells) to that of carbon reduction (bundle sheath cells) requires that mesophyll cells are adjacent to a vein (Dengler & Nelson, 1998). C_4 leaves therefore have a higher vein density than C_3 leaves (Christin *et al.*, 2013). Such a requirement means that C_4 photosynthesis may not be possible in larger GS species, as the larger cells mean that such species are restricted to having lower leaf cell packing densities and slower rates of CO_2 diffusion (Théroux-Rancourt *et al.*, 2021). Likewise, life-history strategies may also exert strong selection on GS, as genomes may be constrained in size in annual, but not perennial, species due to selection for rapid growth and reproduction associated with the annual life history (Bennett, 1972; Enke *et al.*, 2011; Qiu *et al.*, 2019). As photosynthetic pathway and life history are key determinants of plant growth rate (Atkinson *et al.*, 2016; Wade *et al.*, 2020), any limitations imposed by GS related to these factors should be accounted for when assessing how the performance of plants relates to their GS.

To explore the costs and benefits of having a larger GS, we investigate how GS varies across grasses (Poaceae), and how it relates to their environmental niches and growth performance under different conditions. Here, we carried out two analyses: (1) examining the relationship between GS and grass species' environmental niches and (2) examining the effect of GS on the relative growth rate (RGR, the rate of growth proportional to size) of grass species under a range of controlled environmental

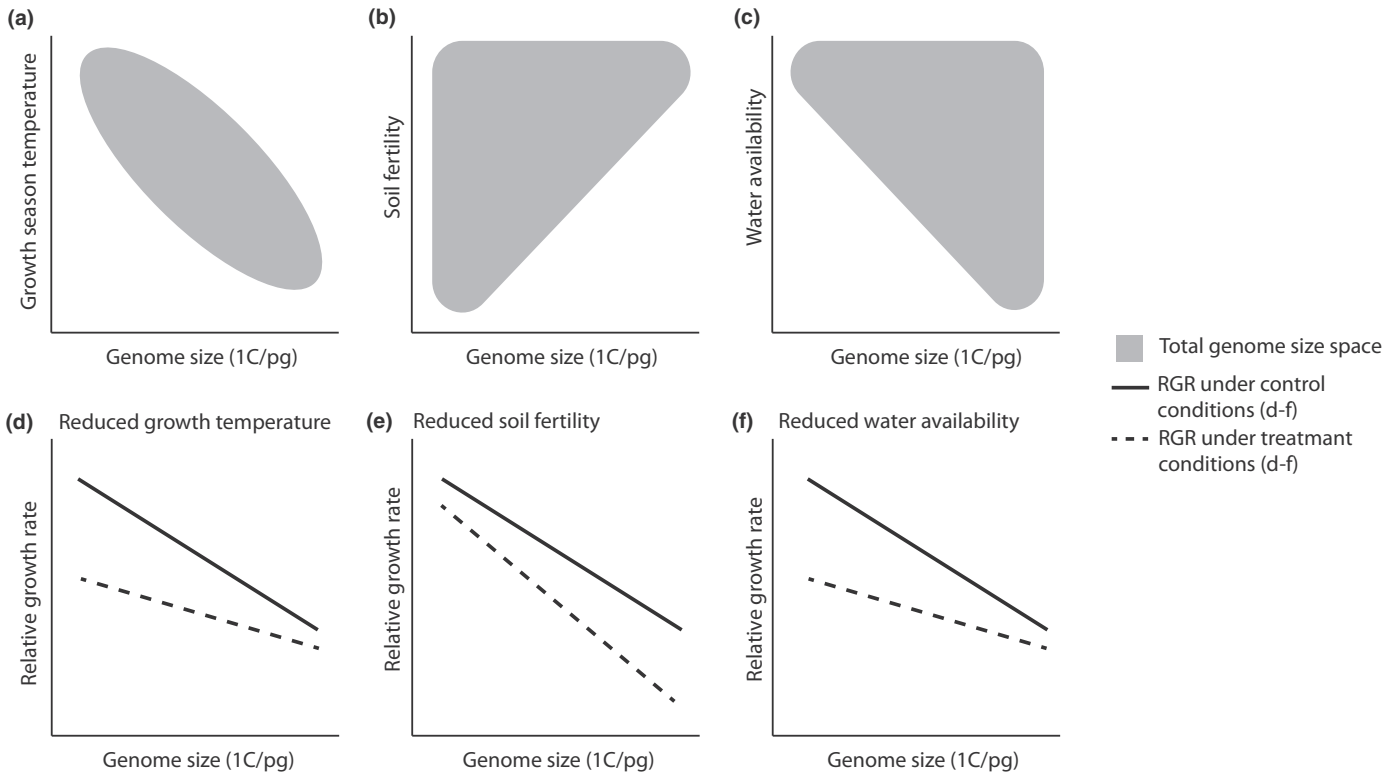

Fig. 1 Predicted relationships between grass genome size and (a–c) climatic/edaphic niche and (d–f) relative growth rate (RGR; rate of growth proportional to size) under controlled experimental treatments (reduced levels (dashed line) from controls (solid line)).

conditions (in which the control treatment was tropical (high) temperatures, high water availability and high nutrients, and these conditions were reduced or limited upon experimentation). These were used to test the following predictions (1–3 illustrated in Fig. 1):

(**1**) Relationships between GS, growing season temperature and RGR – Low growing season temperatures will be associated with species with larger GS (especially perennials; Fig. 1a). In the RGR experiment, we predict there will be a negative relationship between RGR and GS under tropical (control) conditions, but this relationship will be weaker when the same species are grown under lower temperatures (Fig. 1d).

(**2**) Relationships between GS, soil fertility and RGR – Low fertility soils will be associated with smaller GS species because of the higher nutrient costs associated with increasing GS (Fig. 1b; Anneberg & Segraves, 2023). Smaller GS species are therefore expected to tolerate a wider range of soil fertilities. In the growth rate experiment, the RGR of species with larger genomes will be more limited by low nutrient availability conditions than those with smaller GS (Fig. 1e).

(**3**) Relationships between GS, water availability and RGR – Dry conditions will be associated with species with large GS, which may be more water use efficient (Fig. 1c; e.g. Faizullah *et al.*, 2021; but see Schley *et al.*, 2022; Šmarda *et al.*, 2023). Therefore, we predict that larger GS species will be more competitive when water availability is low and hence more likely to be present on sites experiencing these conditions. Likewise, in the growth experiment, the RGR of species with larger genomes will be less limited by low water availability than those with smaller GS (Fig. 1f).

(**4**) GS interactions with evolutionary history, photosynthetic pathway and life history – GS will be strongly influenced by evolutionary history, with closely related species having a similar GS than those most distantly related. Genomes will be smaller in species that have an annual life history and use the C_4 photosynthetic pathway relative to perennial and C_3 species.

Materials and Methods

Study taxa

Grasses are an ideal study system to test hypotheses about GS – environment relationships as they show a huge variation in 1C-values (0.3–23 pg per 1C; Pellicer & Leitch, 2020) and have a global distribution, growing in a wide range of climatic conditions (Lehmann *et al.*, 2019). In addition, different life histories and photosynthetic pathways are well represented in grasses (*c.* 20/80% annual/perennial split with the Poaceae having the highest number of annual species (*c.* 1700) joint with the Asteraceae (Christin *et al.*, 2013; Hjertaas *et al.*, 2022); 60/40% C_3/C_4 split; Osborne *et al.*, 2014), allowing a robust examination of relationships with GS.

The grass family consists of *c.* 11 000 species (Clayton *et al.*, 2016) that are divided into two major clades, the BOP clade (Bambusoideae, Oryzoideae and Pooideae) and the PACMAD clade (Panicoideae, Arundinoideae, Chloridoideae, Micrairoideae, Aristidoideae and Danthonioideae). BOP grasses, which only use the C_3 photosynthetic pathway, dominate cooler climates (particularly species of the Pooideae family; Lehmann *et al.*, 2019). PACMAD grasses, which are both C_3 and C_4 (with C_4 photosynthesis evolving independently 22–24 times in this clade; Christin *et al.*, 2013), dominate grassy biomes in warmer climates (with species of the Andropogoneae and Chloridoideae families dominating high- and low-rainfall tropical and sub-tropical grasslands respectively; Lehmann *et al.*, 2019).

Genome size and functional trait data

1C-values for 785 Poaceae taxa were extracted from the Plant DNA C-values database (v.7.1; Pellicer & Leitch, 2020). The data were cleaned to remove 45 taxa not identified to the species level, which therefore could not be placed in a phylogeny used for analyses (see 'Statistical analyses' in the Materials and Methods section). We also directly measured 1C-values for an additional 111 species not listed in the database but included in the grass RGR experiment (described below) using a one-step flow cytometry procedure (Supporting Information Table S1; see Pellicer *et al.*, 2021 for a detailed description of this methodology), resulting in a final dataset of 851 grass species that we had both 1C-values and phylogenetic information for. To determine 1C-values, *c.* 1cm^2 of leaf material from the species of interest was co-chopped with the same amount of leaf material from the calibration standard species in 2 ml of the nuclei isolation buffer and then filtered through a 30 µm nylon filter. The nuclei were then stained by adding 100 µl of propidium iodide (1 mg ml^{-1}) and incubated on ice for 15 min. The sample was then run on a Sysmex CyFlow Space (Sysmex Europe GmbH, Norderstedt, Germany) flow cytometer, fitted with a 100 mW green solid-state laser. Flow histograms were analysed with the WindowsTM-based FLOWMAX software (v.2.92014; Sysmex GmbH) and the average of each sample used to estimate GS (Pellicer *et al.*, 2021). For details of the calibration standard and buffer used for each grass species, see Table S1.

Information on life history and photosynthetic pathway were extracted for each grass species from GrassBase (Clayton *et al.*, 2016) and the grass photosynthesis database of Osborne *et al.* (2014) respectively.

Grass species environmental niche

To determine the climatic niche of each grass species, we first extracted georeferenced occurrence records (c. 18.6 M records) for Poaceae taxa from the Global Biodiversity Information Facility (GBIF) (GBIF.org (5 November 2019) GBIF Occurrence Download doi: 10.15468/dl.rckugp) using the package RGBIF (Chamberlain *et al.*, 2023) in the R environment (R Core Team, 2022). To control the quality of occurrence records, species names were standardised against GrassBase (Clayton *et al.*, 2016) using the software package TAXONOME (Kluyver & Osborne, 2013). Longitude and latitude data were checked to ensure values were sensible using the COORDINATECLEANER package (Zizka *et al.*, 2019) and were accurate to at least three decimal

places. Finally, to ensure that records represented individuals within the native range of a species, all records were checked against Kew GrassBase distributions (Clayton *et al.*, 2016). After these cleaning steps, species were excluded if they had < 10 unique occurrence points remaining. The data cleaning process and focus on species for which we have GS and phylogenetic information resulted in a dataset containing 584 species (occurrence points per species range = 10–418 365; median = 433).

Two climatic variables appropriate for testing the predictions outlined in Fig. 1 were selected from the WorldClim database (WORLDCLIM v.2; Fick & Hijmans, 2017). These were mean temperature of warmest quarter (Bio10; we consider that this variable best reflected the temperature of the growing season) and precipitation seasonality (Bio15; to reflect seasonal limitations in water availability). Values were extracted for each occurrence point for the two Worldclim products (accessed at a 30-s spatial resolution) and summarised as a mean for each species across the geographical area represented by the occurrence data. Species-mean values of soil N density (g m^{-2}) were calculated from values extracted from the International Satellite Land-Surface Climatology Project (ISLSCP Initiative II) Global Gridded Soil Characteristic dataset (1 × 1 degree spatial resolution; Scholes & Brown de Colstoun, 2011). While both soil N and P availability are tightly linked to the costs of a larger GS (e.g. Anneberg & Segraves, 2023), we focus on soil N due to the availability of a global dataset and the fit between this variable and the low N treatment in the RGR experiment described below.

Grass relative growth rate under different environmental conditions

Relative growth rate estimates were obtained from a series of experiments performed at the University of Sheffield (UK), which focused on differences in growth patterns between C_3 and C_4 grasses grown under different environmental conditions. In brief, seedlings of up to 382 grass species, representing a broad sample across the BOP and PACMAD clades, and including species belonging to 13 of the 22–24 evolutionary origins of C_4 photosynthesis in grasses (Grass Phylogeny Working Group, 2012), were grown in 1-l pots (90 : 10 mix of vermiculite and sand) in a controlled environment chamber (MTPS 120; Conviron, Winnipeg, Canada). Detailed methodology on experimental design, plant growth conditions and growth rate modelling for the 'control' treatment can be found in Atkinson *et al.* (2016).

Plants were grown under four treatments that differed in growth temperature, water- and N availability. The 'control' treatment, which contained the most species (382 species), mimicked growth season temperatures of some tropical savannas (e.g. northern Australia or Campo Grande, Brazil; Global Climate Normals 1961–1990) with 30°C day-time and 25°C night-time temperatures, and with nonlimiting water- and nutrient availability (watered twice daily and fed twice weekly with 50% nitrate-type Long Ashton solution containing 1 mM ammonium nitrate; Hewitt, 1966). The three other treatments were carried out with a reduced sample of species from the 'control' treatment (between 130 and 140 species) due to space constraints.

The 'reduced temperature' treatment differed from the control conditions only in having a lower day-time (20°C) and night-time (15°C) temperature (comparable to growing season temperatures in parts of Northern Europe albeit with a smaller daily temperature range; Global Climate Normals 1961–1990). The 'reduced watering' treatment differed from the control conditions only in the watering frequency (twice weekly watering), and the 'reduced nitrogen' treatment differed only in the amount of N supplied (fed twice weekly with 50% nitrate-type Long Ashton solution but with the nitrate component diluted 10-fold). All four treatments had the same light intensity (maximum photosynthetic photon flux density of 1600 μmol m^{-2} s^{-1} at canopy height), day length (14 h), and relative humidity (70%).

To determine species growth rates, two individuals of each species were harvested weekly for 5 wk. After harvesting, above- and belowground biomass was oven-dried at 70°C and weighed to calculate total dry plant biomass. Relative growth rate values were calculated as a linear regression of log(mass) against time for each species.

Of the grass species with RGRs estimated, 1C-values for 67 species were available from the Plant DNA C-values database (Pellicer & Leitch, 2020) and 1C-values for an additional 111 species (i.e. 178 species in total) were determined as described above (based on the availability and successful germination of seeds, and therefore the ability to generate leaf material for estimating 1C-values). Of these 178 species, 80 were grown in all four experimental conditions (control, reduced temperature, reduced N and reduced watering), with the remainder being grown in two (13 species) or three (15 species) experimental conditions, or just the control conditions (70 species).

Statistical analyses

In order to test for associations between GS and the climatic/edaphic niche of hundreds of grass species, the effects of phylogeny must be accounted for Felsenstein (1985). To do this we used a completely sampled, dated Bayesian phylogeny of over 11 000 grass taxa (Forrestel, 2015) and the 'MCMCglmm' function in R (MCMCGLMM package; Hadfield, 2010). This method implements Markov chain Monte Carlo (MCMC) routines for fitting generalised linear mixed models (GLMM), and accounts for nonindependence and correlated random effects due to the phylogenetic relationships between species. This approach uses the inverse of a phylogenetic correlation matrix of the study species (see Hadfield & Nakagawa (2010) for details). We created a MCMCglmm model with either the climatic or soil variable of interest as the response variable. GS (log-transformed 1C-values), photosynthetic pathway (C_3 or C_4) and life history (annual or perennial) were fitted as explanatory variables in addition to any significant interaction between GS and life history/photosynthetic pathway (as established during preliminary model fitting; an interaction between GS and life history for the temperature of the warmest quarter model was fitted regardless because of the hypothesis regarding differential relationships for annual and perennial species (see prediction 1)). 'Species' was fitted as a random effect. The model was run for 100 000

iterations with a burn-in of 1000 iterations and using parameter-expanded priors. From the model, we calculated the posterior probability of Pagel's lambda and the mean and 95% credible interval (Hadfield & Nakagawa, 2010).

The influence of GS on RGR under the different experimental conditions was analysed using MCMCglmm and using a phylogeny created specifically for the species studied in the RGR experiment (see Atkinson *et al.*, 2016 for details). Photosynthetic pathway and life history, key determinants of RGR in grasses (Atkinson *et al.*, 2016; Simpson *et al.*, 2020), were also included as explanatory variables in addition to GS, as well as any significant interaction terms between these and GS (as established during preliminary model fitting). Models were fitted for each of the four experimental conditions following the same routine as above. To account for species differences in each of the conditions (e.g. the larger number of species in the control conditions relative to the three treatments), these models were also fit to the 80 species that occurred in all four conditions. In these 80 common species, both photosynthetic pathways (37 C_3 species/43 C_4 species) and life histories (38 annual species/42 perennial species) were well represented, as well as a wide range of GSs (1C-value range: 0.4–23 pg per 1C).

To determine how GS is distributed across grass species, in relation to evolutionary history, photosynthetic pathway and life history, similar MCMCglmm modelling approaches were used. We created a grand mean MCMCglmm model with GS (log-transformed 1C-values) as the response variable and 'species' as the random effect, in order to estimate Pagel's lambda (a measure of phylogenetic signal in GS). We did this both across all species, and within each of the two major clades to see whether a phylogenetic signal is primarily driven by the BOP/PACMAD split. To test for life history and photosynthetic pathway influences on GS, a MCMCglmm model was run with GS as the response variable and life history or photosynthetic pathway as the explanatory variable (for the latter analysis C_3 species were grouped by clade to see whether there were differences between C_3 BOP and PACMAD species).

Marginal R^2 values (i.e. those associated with fixed effects only) and conditional R^2 values (i.e. associated with both fixed and random effects) for all MCMCglmm models were estimated following Nakagawa & Schielzeth (2013). We acknowledge that variation apportioned to phylogeny (i.e. the difference between marginal and condition R^2 values) in the models fit here likely reflects both the influence of evolution history and geographical location, since major clades of grasses have quite strongly structured geographic patterns (Lehmann *et al.*, 2019).

Results

Grass genome size variation

Using GS data for 851 grasses (i.e. *c.* 7% of Poaceae species), we find most grasses have small genomes with a modal GS of 1.8 pg per 1C (although this is three times larger than the modal GS for all angiosperms, that is 0.6 pg per 1C based on data for 10 770 species; Pellicer *et al.*, 2018). Overall, grass GS ranges 77-fold, from 0.30 pg per 1C in *Oropetium thomaeum* and *Chloris gayana* (both Chloridoideae subfamily; PACMAD clade) to 23.00 pg per 1C- in octoploid *Leymus karellinii* (Pooideae subfamily; BOP clade), with 10% of species possessing larger genomes, that is > 10 pg per 1C.

Relationships between GS, growing season temperature and RGR

In the temperature niche analysis, there was a significant but weak negative relationship between GS and mean temperature of the warmest quarter in perennial species ($P = 0.04$; $n = 593$; Fig. 2a,d; Table 1), whereas in annual species, there was no association ($P = 0.87$; Table 1). This negative relationship is common for C_3 and C_4 perennial species, with C_4 species being restricted to the smaller GS/warmer end of the relationship (Fig. 2a). A large proportion of variation in temperature niche was explained by evolutionary history, and a much smaller proportion accounted for by GS, photosynthetic pathway and life history (conditional R^2 (cR^2) = 0.70; marginal R^2 (mR^2) = 0.08).

In the growth rate experiment, RGR was unrelated to GS under high temperature 'tropical' conditions (= 'control' treatment; $P > 0.05$; $n = 174$; Fig. 3a; Table S2), but moderately positively related to GS under low temperature 'temperate' growth conditions (= 'reduced temperature' treatment; $P = 0.09$; $n = 93$; Fig. 3b). Independently of GS, the lower growth temperature removed the C_4 growth advantage, as RGR was significantly higher in C_4 species than C_3 species in 'control' ($P = 0.006$) but not in the 'reduced temperature' treatment ($P > 0.05$; contrast Fig. 3a with b). For a given GS, perennials grew slower than annuals under temperate and tropical growth temperatures ($P < 0.001$ for both; contrast Fig. 3e with f). The differences in the species present in each experimental condition did not influence the relationships between RGR, GS and plant traits (i.e. models run for the 80 species common to all experimental conditions showed consistent results to the full model; see Table S3 for the output for the reduced 80-species dataset).

Relationships between GS, soil fertility and RGR

We predicted that the nutrient costs of larger GS would be alleviated under fertile soils (Fig. 1b). In keeping with this prediction, there was a strong positive relationship between GS and the soil N density niche ($P = 0.009$; $n = 572$; Fig. 2b; Table 1). However, rather than occurring across the range of soil N availabilities as predicted, smaller GS species are generally excluded from high N soils (i.e. fewer points in the top left of Fig. 2b). This positive relationship was common to both C_3 and C_4 species, with C_4 species falling at the low GS/low soil N density end of the relationship (Fig. 2b). For a given GS, perennials live on soils with higher N availability than annuals ($P < 0.001$). A larger proportion of variation in soil fertility niche was explained by evolutionary history than by grass traits (conditional R^2 (cR^2) = 0.75; marginal R^2 (mR^2) = 0.04).

When grown under reduced N availability in the growth experiment, species with larger genomes had lower RGR than

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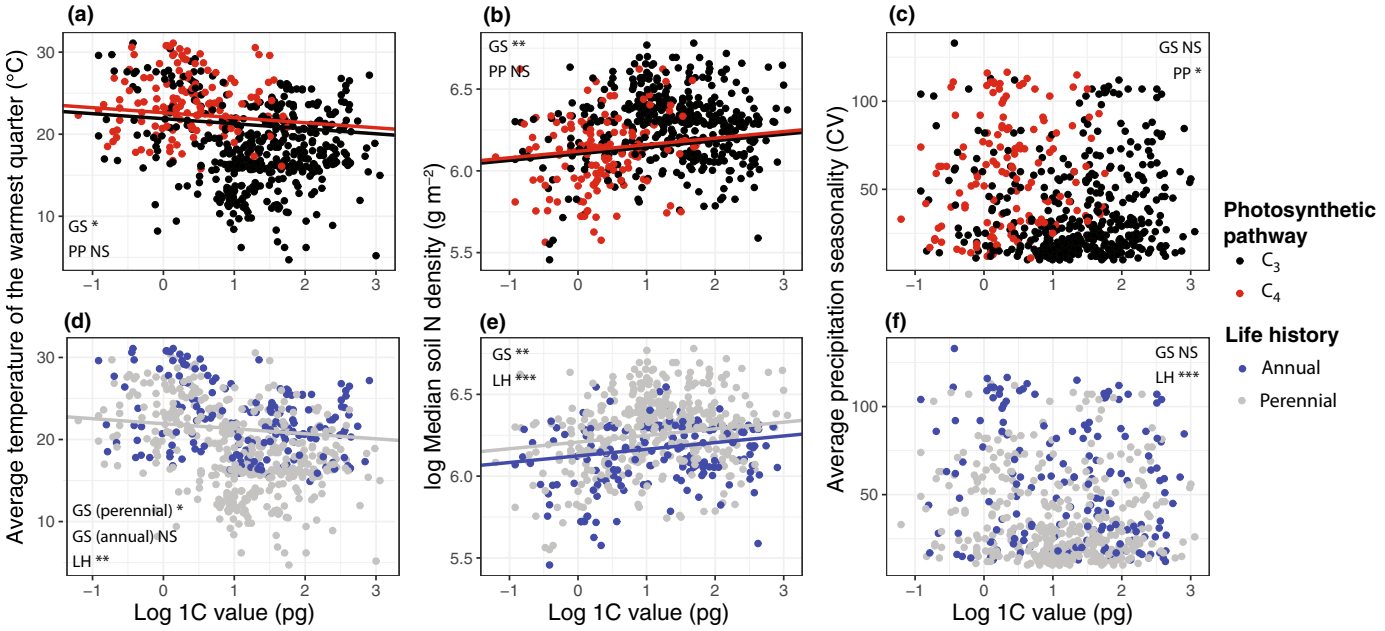

Fig. 2 Genome size relationships with the climatic and edaphic niche of grass species. Climate/soil characteristics are means of values extracted across each species range. Figures for the same niche variable (i.e., a, d; b, e; c and f) show results from the same model but are separated out to show photosynthetic pathway and life history effects. Fitted lines indicate significant relationships when phylogeny is accounted for using generalised linear mixed models. GS, genome size (expressed as 1C-values); LH, life history; ns, not significant; PP, photosynthetic pathway. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

Table 1 Contributions of genome size, photosynthetic pathway and life history to the climatic and edaphic niche of grass species.

	Temperature of the warmest quarter (n = 593; mR^2 = 0.08; cR^2 = 0.70)		Soil Nitrogen density (n = 572; mR^2 = 0.04; cR^2 = 0.75)		Precipitation seasonality (n = 593; mR^2 = 0.09; cR^2 = 0.62)	
	Estimate (95% CI)	*P*-value	Estimate (95% CI)	*P*-value	Estimate (95% CI)	*P*-value
Intercept	21.89 (18.24–25.43)	**<0.001*****	6.10 (5.87–6.31)	**<0.001*****	50.96 (30.50–71.35)	**<0.001*****
Genome size	Annual: −0.07 (−0.78 –0.68) Perennial: −0.59 (−1.21 to −0.04)	Annual: 0.87 Perennial: **0.04***	0.04 (0.01–0.07)	**0.009****	−1.45 (−4.83–1.83)	0.39
Photosynthetic pathway (C_3→C_4)	0.89 (−1.10–3.03)	0.39	0.02 (−0.11–0.15)	0.76	14.05 (1.41–26.30)	**0.025***
Life history (Annual→ Perennial)	−1.99 (−3.07 to −0.94)	**0.001****	0.08 (0.04–0.13)	**<0.001*****	−10.70 (−15.18 to −6.31)	**<0.001*****
Genome size: Life history	−0.525 (−1.250–0.211)	0.14				

Note: Values represent posterior mean estimates of the slopes as determined by MCMC phylogenetic GLMMs. The explanatory variables together with evolutionary history (i.e. the fixed and random effects) could explain a significant proportion of variation in growth season temperature, soil nitrogen density variation and precipitation seasonality (conditional R^2 (cR^2) = 0.62–0.75). The explanatory variables alone explained a much lower proportion of niche variation (marginal R^2 (mR^2) = 0.04–0.09), indicating a substantial role of phylogeny in determining grass species climatic and edaphic niches. Significant model terms ($P < 0.05$) are in bold. CI, confidence interval; $P < 0.01$; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

smaller genomes, but only in annual species ($P = 0.005$; $n = 102$; Fig. 3g; Table S2) and regardless of photosynthetic pathway (Fig. 3c). Perennial species had lower RGR under N limitation ($P < 0.001$), but this was unrelated to GS.

Relationships between GS, water availability and RGR

Grass GS was unrelated to their precipitation seasonality niche (Fig. 2c,f; $n = 584$; Table 1). For a given GS, C_4 and annual species were found in places with greater precipitation seasonality than C_3 ($P = 0.026$; Table 1; Fig. 2c) and perennial ($P < 0.001$; Table 1; Fig. 2f) species, respectively. A larger proportion of variation in precipitation seasonality niche was explained by evolutionary history than by grass traits (conditional R^2 (cR^2) = 0.62; marginal R^2 (mR^2) = 0.09).

The lack of relationship between GS and water availability is supported in the growth rate experiment, with grass RGR being unrelated to GS in the 'reduced watering' treatment ($P > 0.05$;

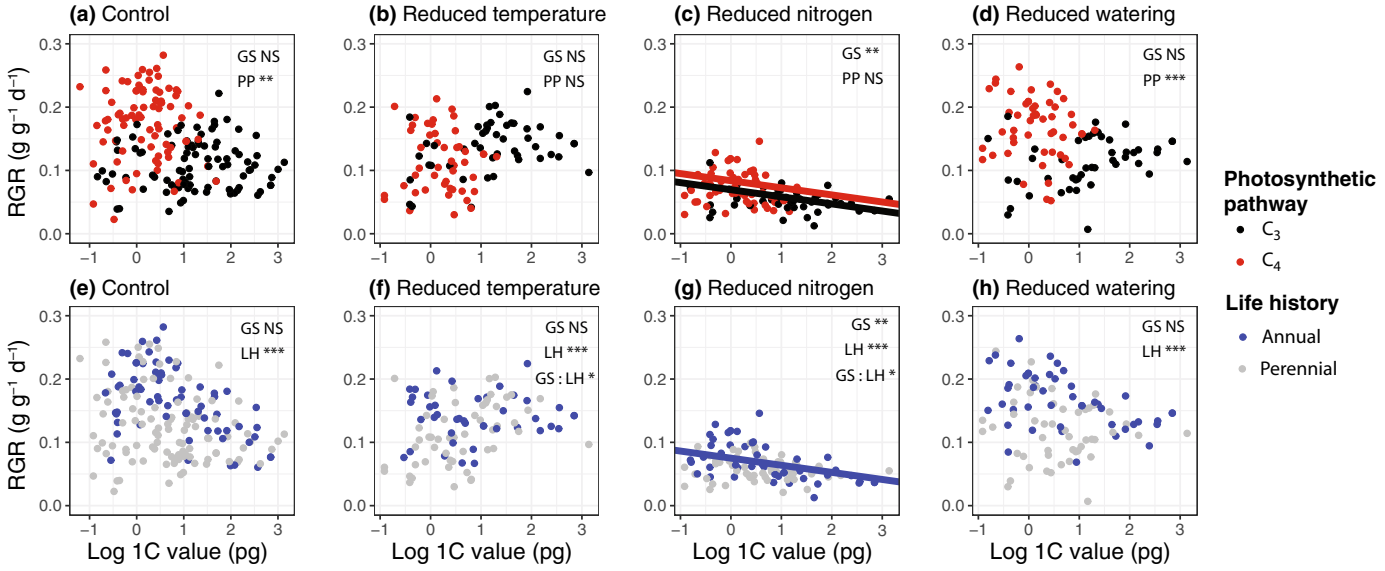

Fig. 3 Genome size relationships with relative growth rate (RGR) under different environmental conditions in grasses. Data are from a comparative growth experiment on grass seedlings grown in a controlled environment chamber. 'Control' treatment conditions are tropical temperatures with nonlimiting water and nutrient supply. Other treatments have reduced temperature, soil nitrogen, or watering. Figures for the same treatment (i.e., a, e; b, f; c, g; d and h) show results from the same model but are separated out to show photosynthetic pathway and life history effects. Fitted lines indicate significant relationships when phylogeny is accounted for using generalised linear mixed models. GS, genome size (expressed as 1C-values); LH, life history; ns, not significant; PP, photosynthetic pathway. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

$n = 95$; Fig. 3d,h; Table S2). Irrespective of GS, the C_4 photosynthetic pathway ($P = 0.001$) and annual life history ($P < 0.001$) were associated with higher RGR under water limitation.

GS interactions with evolutionary history, photosynthetic pathway and life history

GS is not randomly distributed across grass taxa but instead shows strong phylogenetic signal (Pagel's lambda = 0.80, 95% confidence interval (CI) = 0.74, 0.86), with larger GS predominantly found in the major BOP clade (Fig. 4). The strong phylogenetic signal in GS holds within the BOP (Pagel's lambda = 0.81, 95% CI = 0.74, 0.87) and PACMAD (Pagel's lambda = 0.75, 95% CI = 0.59, 0.88) clades, suggesting that the significant signal seen across grass taxa is not a product of the major BOP/PACMAD clade split.

As predicted, C_4 grasses have a smaller mean and narrower range of GS (mean = 1.7 pg per 1C; range 19.3-fold, 0.3–5.8 pg per 1C; $n = 196$) compared with C_3 species (mean = 5.3 pg per 1C; range 57.5-fold, 0.4–23.0 pg per 1C; $n = 655$; Fig. 5a). Within the PACMAD clade, where all instances of C_4 evolution occur, C_3 species have a larger mean and range in GS (mean = 2.5 pg per 1C; range 15.5-fold, 0.7–10.8 pg per 1C) than the C_4 species, but the differences in mean values are not significant ($P > 0.05$). Relative to PACMAD species, the C_3-only BOP clade has a greater range of GS (mean = 5.9 pg per 1C; range 57.5-fold, 0.4–23.0 pg per 1C), and a mean GS that is significantly higher than the C_4 PACMAD species ($P = 0.025$) but not the C_3 PACMAD species ($P > 0.05$; Fig. 3a; Table S4).

Perennial species have a significantly larger GS than annuals when evolutionary history is accounted for ($P = 0.003$; Table S5), with fewer species with small GS and more species with larger GS than annual species (Fig. 5b). However, the occurrence of larger genomes is not limited to only perennial species, as predicted, with annual and perennial species having similar ranges in GS (annual = 0.3–18.8 pg per 1C; perennial = 0.3–23 pg per 1C; Fig. 5b).

Discussion

GS diversity in grasses

Our analyses have shown that the diversity of GS encountered in grasses is influenced by evolutionary history, photosynthetic pathway and life history. GS is strongly shaped by evolutionary history, such that species with larger genomes are clustered particularly in the Pooideae of the BOP clade. Larger GS are absent in C_4 species which could be because C_4 photosynthesis has only evolved in taxa where GS is smaller. Phylogenetic patterns in GS support this explanation as GS is smaller and less variable (smaller mean and range in GS) in the PACMAD clade, where all origins of C_4 photosynthesis in grasses occur. Nevertheless, C_4 photosynthesis may also constrain GS indirectly through cell size. As C_4 photosynthesis requires high vein densities in leaves (to minimise diffusion distances from mesophyll cells to veins), it may only be possible in species with smaller GS and therefore smaller cell sizes (Théroux-Rancourt *et al.*, 2021). This may contribute to explaining the high prevalence of C_4 origins in the PACMAD clade relative to its

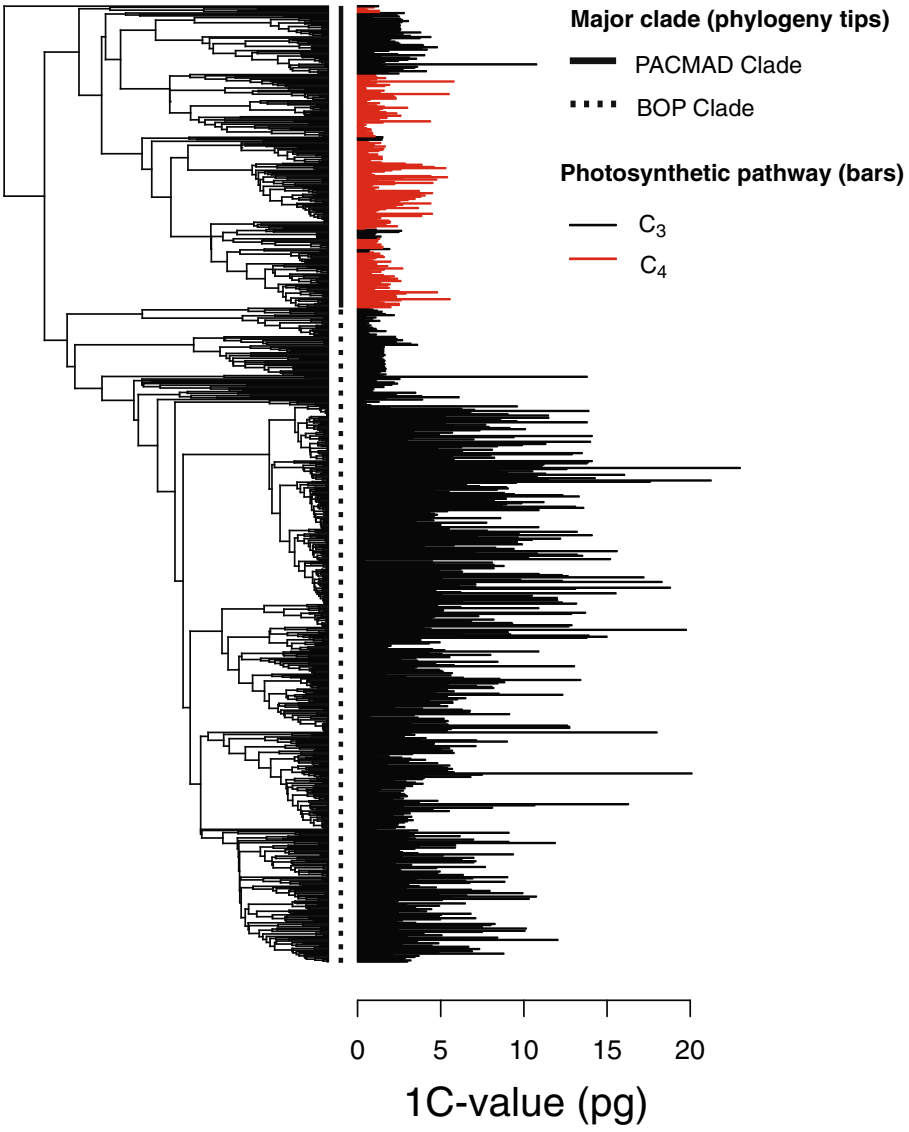

Fig. 4 Distribution of genome size across 851 grass species. Genome size (expressed as 1C-values, i.e. the amount of DNA contained within a unreplicated gametophytic nucleus given in picograms (pg)) shows a strong phylogenetic signal in grasses (Pagel's lambda = 0.80 (95% confidence interval = 0.74, 0.86)), with larger values seen in the BOP (Bambusoideae, Oryzoideae, and Pooideae) clade but not in the other major clade, PACMAD (Panicoideae, Arundinoideae, Chloridoideae, Micraioideae, Aristidoideae and Danthonioideae). Species included are those with available phylogenetic, genome size and photosynthetic pathway information.

C_3-only, sister BOP clade where species with larger GS are found (i.e. largest GS encountered in PACMAD clade was just 10.8 pg per 1C compared with 23.0 pg per 1C in the BOP clade). Macroevolutionary models may provide more insights into the correlated evolution of GS, cell size and photosynthetic pathway.

There was some evidence that life history may influence GS, with annual species having a smaller mean GS compared with perennials, but a similar range. Contrary to the prediction that annual species will only have small genomes due to selection for accelerated growth and rapid reproduction, GS effects on replication time may not be important to grass species' performance over the length of a growing season. Additionally, the maximum GS for an annual species is reported to be *c.* 25 pg per 1C (Bennett, 1972; Leitch & Bennett, 2007). Given that the largest GS so far reported for any grass is 23 pg per 1C, an annual life history may be feasible across the range in GS in this family.

GS-temperature interactions influence grass growth and niches

Our findings suggest larger genomes may provide a competitive advantage in cooler climates. Grass species with larger genomes were predominantly found in a cooler growing season niche (Fig. 2a), and grew marginally faster under temperate, but not tropical, growth temperatures, than those with smaller genomes (Fig. 3a,b). The precise mechanisms driving these results are likely due to the larger minimum cell sizes of larger GS species, or potentially related to heterosis associated with polyploidy (Chen, 2010). Studies in the grass species *Zea mays* (maize) show that cold temperatures cause a significant decline in cell production rates but have no effect on mature cell sizes (Ben-Haj-Salah & Tardieu, 1995; Rymen *et al.*, 2007). Therefore, while low temperatures slow growth through reductions in cell division rate, this may be overcome in larger GS perennial species by expansion of their large cells formed during the preceding warm, dry season

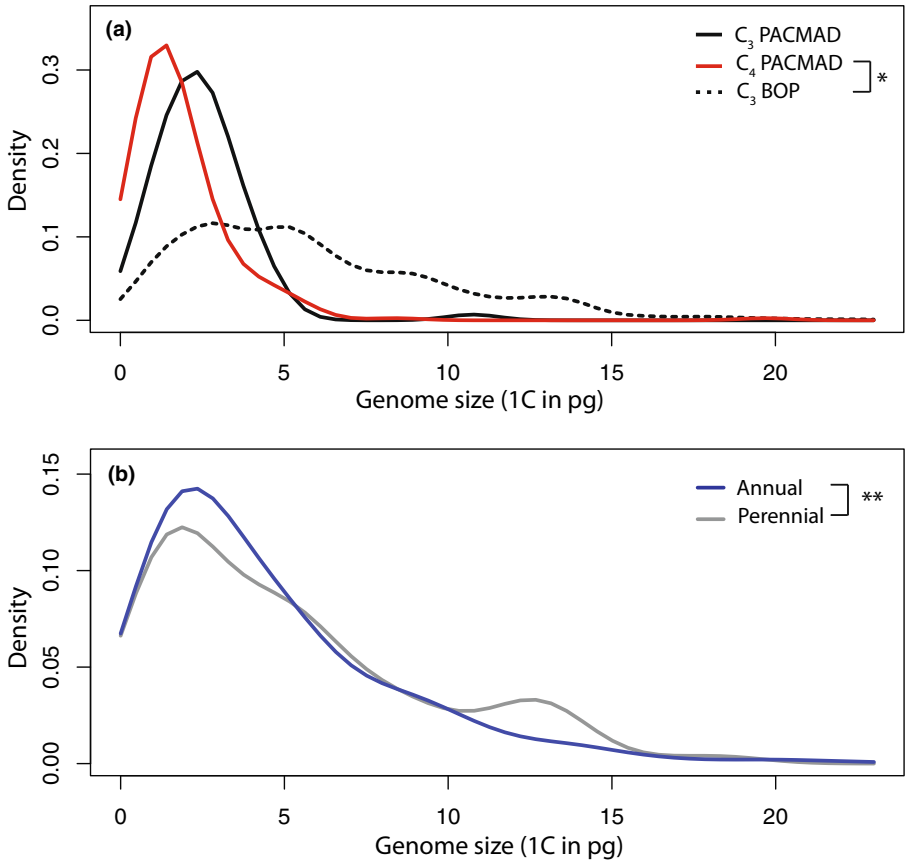

Fig. 5 Constraints on genome size in grasses. Genome size (GS; expressed as 1C-values in pg) is restricted to being smaller in both C_3 and C_4 species in the PACMAD (Panicoideae, Arundinoideae, Chloridoideae, Micraioideae, Aristidoideae and Danthonioideae) clade, but not in the C_3-only BOP (Bambusoideae, Oryzoideae, and Pooideae) clade (a). GS is significantly larger in perennial compared with annual grass species (b); however, GS is not limited by life history (i.e. the range of GS in annual and perennial species is similar). Figures are kernel density plots which display the distribution of values using one continuous curve, and therefore density is a proxy of species richness. Legends indicate line colours and where significant differences exist in mean GS between named groups (once phylogeny is accounted for using generalised linear mixed models). *, $P < 0.05$; **, $P < 0.01$.

when temperatures are low (Grime & Mowforth, 1982). Fitting with this hypothesis, the association between colder growth season temperatures and large genomes was stronger in perennial grass species (Table 1), which have the capacity to undergo cell division in the previous year unlike annual species. A recent analysis exploring GS in angiosperms also revealed the importance of temperature in influencing plant distributions, with larger GS species dominating temperate latitudes compared with tropical regions (Bureš *et al.*, 2024).

Nutrient costs of grass GS

Given the elevated nutrient costs of synthesising nucleic acids (Sterner & Elser, 2002) and data from pot experiments (Walczyk & Hersch-Green, 2019; Anneberg & Segraves, 2020), we expected the performance of grass species with larger genomes to be more impacted when N was limiting compared with smaller genome species, as has been found elsewhere (Šmarda *et al.*, 2013; Guignard *et al.*, 2016). We found that larger GS grasses grew slower than smaller GS species under N limitation (Fig. 3c,g), and that their global distribution was limited to high N soils (Fig. 2b,e). In the growth rate experiment, species with larger genomes grew slower in the reduced N treatment but not in the two other treatments or control, which had high N availability. This suggests that when soil fertility is high, larger genomes are not detrimental to growth, and may provide a competitive growth advantage to species over those with smaller GS (e.g. through more vigorous growth and shading; Peng *et al.*, 2022). This mechanism may explain our finding of smaller GS grasses typically occurring on low nutrient soils (Fig. 2b). The N costs associated with GS might be expected to vary depending on the photosynthetic pathway, as C_3 plants have a lower photosynthetic N use efficiency than C_4 ones (Brown, 1978; Ghannoum *et al.*, 2011). However, we found there was no effect of photosynthetic pathway on GS–soil N density relationships (Fig. 2b). In addition, C_3 grasses fared no worse than C_4 grasses with the same GS under the reduced N treatment in our growth experiment (Fig. 3c). Thus these data do not support Kelly (2018) who proposed that C_3 plant genomes experienced stronger selection for less N-costly DNA codons and amino acids than C_4 plant genomes.

While we only studied the influence of N availability on grass growth performance and species distribution here, P availability may also be highly influential. Co-limitation of N and P has been shown to restrict larger GS species occurrence, with experiments showing that only when angiosperms are released from both N and P limitation is there an associated increase in biomass of large genome species (Šmarda *et al.*, 2013; Guignard *et al.*, 2016). Here, our global analysis of soil fertility considered only N but still detected a significant positive relationship with GS. We were not able to extend our analysis to soil P as although the understanding of the spatial patterns of bioavailable soil P is improving, a global map does not yet exist (He *et al.*, 2021). Examining how the co-availability of soil N and P influences the global

distribution of GS in grasses would be a natural next step to this analysis. We predict that where soil N and P are both highly limiting (e.g. Australia), grass genomes will be restricted to smaller sizes, and where both are highly available (e.g. Europe), larger GS grasses will dominate (Scholes & Brown de Colstoun, 2011).

No relationships between GS and grass water use efficiency

We find no support for a water use efficiency advantage of large or small genomes in grasses. Species with larger genomes were not associated with more seasonally dry environments (Fig. 2c), nor did they have higher RGR under experimental water limitation (Fig. 3d). Such findings add to the mixed results in the growing number of studies exploring the relationship between GS and water. For example, palms with small GS are associated with arid conditions, while those species growing in wet conditions have a wide range in GS (Schley *et al.*, 2022). This is consistent with data from multiple diploid-polyploid comparisons of pot-grown plants, where polyploids with larger GS had reduced water use efficiency compared with diploids (Šmarda *et al.*, 2023). In contrast, in the studies presented here, no association between GS and water availability was found, as also reported from experiments conducted on diploid and tetraploid cytotypes of *Solidago gigantea* (Asteraceae; Walczyk & Hersch-Green, 2022). Indeed, we found that grass species living in seasonally dry environments display a wide range of GS (Fig. 2c), suggesting that different strategies may be employed to cope with low water availability that relate to cell size and GS. For example, while one might expect stomatal conductance to be low and water use efficiency high under a low density of large stomata (associated with larger GS; Bertolino *et al.*, 2019), an alternative strategy for high water use efficiency is to possess small stomata that are highly responsive (i.e. close quicker) to changing environmental conditions, which may be only possible in species with small cell sizes and genomes (Drake *et al.*, 2013; Lawson & Vialet-Chabrand, 2019; but see Elliott-Kingston *et al.*, 2016). In addition, relationships between GS and grass water use efficiency will be impacted by photosynthetic pathway, as C_4 plants have an intrinsically higher water use efficiency than C_3 plants (Rawson *et al.*, 1977; Monson, 1989; Osborne & Sack, 2012), although as shown here, C_4 grasses only possess small genomes.

The water availability niche of grass species, as well as the growing season temperature and soil N niches, were partially explained by the combination of GS, photosynthetic pathway and life history (marginal $R^2 < 0.10$), but with a much larger proportion of variation was accounted for by evolutionary history (conditional $R^2 > 0.59$). Here, we considered environmental factors individually (in line with the specific predictions 1–3), while plants are experiencing these different conditions simultaneously as well as others that have not been considered here. For example, grass-dominated systems tend to experience high-frequency disturbance, such as recurrent fire or herbivory. The two main strategies for persistence through disturbance, resprouting from surviving meristems or recruiting from stored seeds, may be related to GS to some extent. For example, the ability to resprout after fire in Restionaceae species was associated with lower GS (Linder *et al.*, 2017), and GS may influence seed mass (Beaulieu *et al.*, 2008) and therefore the ability of seeds to survive disturbance *in situ* or disperse into disturbed areas (Chen *et al.*, 2020). Therefore, GS may play a role in influencing the ability of grass species to persist through frequent disturbance, but associations between GS and disturbance regimes or plant performance through disturbance have yet to be tested.

Conclusion

Grass-dominated biomes occupy 40% of the land surface and almost every part of Earth's vegetated climate space (Lehmann *et al.*, 2019). Here, we explored how GS relates to grass performance under different environmental conditions. We found evidence that both small and large genomes provide environment-dependent growth benefits (e.g. large GS associated with faster growth under lower temperatures and higher N availability, and small GS generally associated with faster growth elsewhere) that likely relates to minimum cell sizes, and this diversity in GS likely contributes to the global success of grasses (Linder *et al.*, 2018). Our findings suggest that GS is an important predictor of a grass species growth performance, under contrasting temperature and soil nutrient conditions, filtering for species based on their GS and related cell size. This study adds to a growing body of work (Guignard *et al.*, 2016; Faizullah *et al.*, 2021; Peng *et al.*, 2022) that proposes GS plays a role in influencing plant community composition and ecosystem responses to environmental challenges, such as climate change and N deposition.

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Competing interests

None declared.

Author contributions

KJS, IJL and ARL conceived the study, in discussion with JAM and JH. KJS and SM collected the data. EJF provided the large grass phylogeny. KJS wrote the manuscript with all authors contributing critically to drafts.

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Data availability

Growth rate: Growth rate data under the various experimental treatments is available in the Dryad entry: 10.5061/dryad.d7wm37q7n. Environmental niche: Species-level climatic and edaphic niche data is available in the Dryad entry 10.5061/dryad.d7wm37q7n. Genome size: 1C-values were extracted from the Plant DNA C-values database which is freely available at https://cvalues.science.kew.org (Pellicer & Leitch, 2020; https://doi.org/10.1111/nph.16261). New 1C-values determined for this work are available in the Supplementary Information (alongside methodological details) and also in the Dryad entry 10.5061/dryad.d7wm37q7n. Phylogeny: The maximum clade credibility tree of the study species used for the environmental niche analyses is available in the Dryad entry. The phylogeny used in the growth rate analysis is published as supplementary data in the study by Atkinson *et al.*, 2016 (https://doi.org/10.1038/nplants.2016.38). Code: R script demonstrating the fitting and running of a Markov chain Monte Carlo generalised linear mixed effects model (MCMCglmm), plotting the results of the model and extracting R^2 values for it is available in the Dryad entry 10.5061/dryad.d7wm37q7n.

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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 Genome sizes (GS) for 111 of the species present in the grass relative growth rate experiment estimated using a one-step flow cytometry procedure.

Table S2 The contributions of genome size and traits to the relative growth rate (RGR) of grass species under different experimental environmental conditions.

Table S3 The contributions of genome size and traits to the relative growth rate (RGR) of 80 grass species found under all environmental treatments in a controlled growth experiment.

Table S4 Differences in 1C-values between the two major grass clades.

Table S5 Differences in 1C-values between annual and perennial grass species.

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