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THE GLOBAL DISTRIBUTION OF GRASS FUNCTIONAL TRAITS WITHIN GRASSY BIOMES

Running Title: Distribution of traits within grasslands

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We confirm that all authors have no conflict of interest

Data Accessibility Statement

Data will be uploaded to Dryad upon acceptance of submission

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ABSTRACT

Aim

The sorting of functional traits along environmental gradients is an important driver of community and landscape scale patterns of functional diversity. However, the significance of environmental factors in driving functional gradients within biomes and across continents remains poorly understood. Here, we evaluate the relationship of soil nutrients and climate to leaf traits in grasses (Poaceae) that are hypothesised to reflect different strategies of resource-use along gradients of resource availability.

Location

Global

Taxon

Poaceae

Methods

We made direct measurements on herbarium specimens to compile a global dataset of functional traits and realised environmental niche for 279 grass species that are common in grassland and savanna biomes. We examined the strength and direction of correlations between pairwise trait combinations and measured the distribution of traits in relation to gradients of soil properties and climate, while accounting for phylogenetic relatedness.

Results

Leaf trait variation among species follows two orthogonal axes. One axis represents leaf size and plant height, and we showed positive scaling relationships between these size-related traits. The other axis corresponds to economic traits associated with resource acquisition and allocation, including leaf tensile strength (LTS), specific leaf area (SLA) and leaf nitrogen content (LNC). Global-scale variation in LNC was primarily correlated with soil nutrients, whilst LTS, SLA and size related traits showed weak relationships to environment. However, most of the trait variation occurred within different vegetation types, independent of large-scale environmental gradients.

Main conclusions

Our work provides evidence among grasses for relationships at the global scale between leaf economic traits and soil fertility, and for an influence of aridity on traits related to plant size. However, large unexplained variance and strong phylogenetic signal in the model residuals imply that at this scale the evolution of functional traits is driven by factors beyond contemporary environmental or climatic conditions.

Keywords: functional traits, soil fertility, climate, phylogenetic conservatism, biomes, grasses

INTRODUCTION

Functional traits govern competitive interactions and differences in growth and survival, which are responsible for variation in abundance and distributions across environmental gradients. Plant functional types (for example evergreen, deciduous, C_3 and C_4) have been widely used to group plants from geographically separate communities which are thought to share response to environmental variation (Woodward, Lomas, & Kelly, 2004). However, there can be significant variation in functional traits within these plant functional groups (Liu, Edwards, Freckleton, & Osborne, 2012). Crucially, the environmental responses and biotic interactions of a plant functional type may not apply across all ecological settings in which it is found (Keith, Holman, Rodoreda, Lemmon, & Bedward, 2007), and there is growing interest in how traits vary within plant functional types, and how they differ among and within biomes and continents (Lehmann et al., 2014). To address these issues, we investigate the relationships between functional traits that reflect different strategies of resource capture and allocation, and investigate whether these correlate with environmental gradients across the globe, focusing on the grasses that characterise global grassy biomes (grasslands and savannas).

Functional traits of species contribute to ecosystem function according to their relative abundance / biomass in the community, so that dominant species contribute the most, and a number of studies have supported this view (Garnier, Navas, & Grigulis, 2015; Grime, 1998). Globally there are ~1,000 species of grass that are dominant within grassy vegetation types in at least part of their range, and their dominance may reflect the evolution of particular sets of functional traits that give each species advantages in terms of competition and survival (Edwards, Osborne, Strömberg, Smith, & Consortium, 2010). Physiological and morphological constraints mean there are limits to the trait combinations that a species can deploy, resulting in economic trade-offs between the investment of resources (i.e. water, light, nutrients and CO_2) in fast, but cheaply constructed leaves, versus the conservation of these resources in slow growing, yet long lived tissues (Diaz et al., 2004; Grime et al., 1997; Ian J. Wright et al., 2004). It has been proposed that trade-offs reflecting differences in the way plants acquire and allocate resources to growth or conservation of tissues provide mechanisms that can determine distribution patterns across resource gradients (Fine et al., 2006; Herms & Mattson, 1992).

In environments where resources are limited, species are predicted to have a slow growth rate, high investment in carbon-based compounds, low leaf nitrogen content (LNC), long leaf lifespan and low specific leaf area (SLA) (Craine et al., 2002; Grime et al., 1997; Reich, Walters, & Ellsworth, 1997; Westoby, Falster, Moles, Vesk, & Wright, 2002). These traits reflect the high cost of tissue loss to for example herbivory, for individuals where growth is resource limited (Coley, Bryant, & Chapin, 1985; Herms & Mattson, 1992). Conversely in resource-rich environments (where water, light or nutrients are not limiting), community assembly is determined by the ability to rapidly acquire and allocate resources to growth and thereby out-compete neighbouring individuals (Grime, 1977). Traits including low investment in secondary metabolites, high SLA, high maximum photosynthetic rate, short leaf lifespan, high relative growth rate and high LNC are predicted to promote dominance in environments where resource availability does not limit growth (Craine et al., 2002; Grime et al., 1997; Reich et al., 1997; Westoby et al., 2002) .

Functional traits that reflect different strategies of resource acquisition and allocation correlate strongly with resource availability at the community scale (Katabuchi, Kurokawa, Davies, Tan, & Nakashizuka, 2012; Kraft & Ackerly, 2010). Recent studies spanning environmental gradients have shown that similar sorting processes also drive economic trait distribution in predictable ways at a landscape scale (Asner et al., 2014; Fortunel, Paine, Fine, Kraft, & Baraloto, 2014). However, environmental trait relationships may not vary predictably across biomes and continents, and can be specific to areas of unique evolutionary history when considered at larger scales (Knapp et al., 2004; Lehmann et al., 2014). The relationships between traits and environment at these large scales may, in fact, be in large part explained by evolutionary history. For example, in tropical forests, trait variation is phylogenetically partitioned independently of variation in contemporary environmental conditions (Asner et al., 2014), and yet global-scale analyses rarely consider the role of evolutionary history when examining the relationships of traits to environment.

Current estimates of the global extent of tropical savannas and temperate grasslands suggest that ~40% of the Earth's terrestrial surface is covered in grassy ecosystems (White, Murray, & Rohweder, 2000). These store large amounts of carbon, and support livelihoods and food security globally (Parr, Lehmann, Bond, Hoffmann, & Andersen, 2014). Grassy biomes are an ideal system for studying the global-scale relationships of functional traits with environment, as they occur on every continent and most climates. Since these systems are each typically dominated by relatively few species, these traits are also crucial determinants of ecosystem function. Here, we investigate the global distribution of functional traits linked to resource economics in grassy systems, and their relationship to soil fertility and climate, whilst accounting for the role of evolutionary history in trait distribution. We first measure the strength and direction of pairwise correlations between traits to test whether co-variation is consistent with the hypothesis of trade-offs. Secondly, we investigate the distribution of traits in relation to the environment, testing whether ecological theory explaining the sorting of species among communities at the landscape scale can be applied to explain the equivalent sorting along global environmental gradients. Specifically, we test the hypothesis that dominant species in resource rich grassy environments are characterised by traits associated with fast acquisition and the maximum allocation of resources to growth. Conversely in environments where soil nutrients or climate limit plant growth we expect dominant species to exhibit traits that reflect the conservation of long-lived tissues.

MATERIALS AND METHODS

Species sampling

A global database of the species that characterise grassy biomes was compiled from regional maps of potential vegetation (Lehmann et al., 2019) using the taxonomy of Grassbase (Clayton, 2006 onwards). Within the map of Lehmann et al grassy vegetation was defined as grasslands, savannas or woodland with a continuous grassy underlayer. Grassy vegetation types and also their characteristic grass species were identified from the description and metadata associated within original vegetation maps. These species were therefore based on the expert opinion of vegetation mappers, however, they showed good correspondence to lists of dominant species generated for each vegetation units from plot survey datasets (Lehmann et al., 2019). The maps documented 1635 grassy vegetation types, characterised by, 1154 species of which 841 were identified to species level. This map was used as the basis for our species sampling (Appendix S1 in Supporting Information).

We first generated a randomly ordered list, without replacement, of the 841 globally dominant grass species. Random draws were weighted by the area over which each species is common, to ensure that globally important species were represented higher up the list. We searched the herbarium collection of the Royal Botanic Gardens, Kew, for as many species as time would feasibly allow, starting from the top of the list and working down. We were able to search for herbarium specimens for the first 300 species on the list, of which 279 were present within the herbarium and could therefore be included in our sample. These 279 species represent around one quarter of the world's dominant grass species and can be found within 1012 of the vegetation types (Appendix S2 in Supporting Information). Vegetation types were characterised by between 1 to 19 dominant species and on average 4. A map showing the global distribution of our species sample is in Appendix S3.

Traits measured and their significance

The following traits were selected for study because they reflect different strategies of resource use along gradients of resource availability and can be measured from herbarium specimens. SLA is a good predictor of growth rate (Rees et al., 2010) and reflects the return on previously acquired resources, since there is a trade-off between quickly growing large, light-capturing, yet vulnerable leaves and producing strong, long-lived leaves (Westoby et al., 2002; Westoby & Wright, 2006). Maximum leaf size is associated with light capture (Poorter & Rozendaal, 2008). Plant height declines along gradients of decreasing moisture and/or nutrient availability (I. J. Wright, Reich, & Westoby, 2001), and is also thought to reflect different ecological strategies among species in relation to disturbance (Weiher et al., 1999; Westoby, 1998). Taller plants compete more effectively for light (Cavender-Bares, Ackerly, Baum, & Bazzaz, 2004; Tilman, 1988), however, smaller plants may be selected for in highly disturbed environments since there is a trade-off between fast reproduction and competitive ability (Westoby, 1998). Foliar nitrogen is positively correlated with maximum photosynthetic rates (Field, Merino, & Mooney, 1983). Leaf tensile strength is an important form of defence against herbivory (Choong et al., 1992) and is strongly correlated with leaf life span (Onoda et al., 2011).

Trait measurements from herbarium specimens

Protocols for measuring functional traits usually prescribe the use of fresh leaf material. However, access to a global range of species was not possible from fresh material, and so we developed methods for taking measurements from herbarium specimens. Measurements taken from herbarium specimens have the additional advantage of being from plants grown in their native range under natural soil and climatic conditions. Prior to gathering our data, we conducted preliminary tests (Appendix S4 in Supporting Information), showing that measurements taken from fresh grass leaves correlate strongly with those from rehydrated herbarium material for SLA ($r^2 = 0.90$) and LTS ($r^2 = 0.84$) (Appendix S4 in Supporting Information). LNC is typically measured using dried leaf material and can be estimated directly from dried herbarium samples.

Trait measurements

Herbarium specimens were only selected for sampling from areas where the species formed a dominant part of the vegetation. Herbarium sheets were also selected, where possible, to be distributed along the extent of the range where each species was dominant.

SLA: A full leaf where possible or, if not, a section of leaf was removed from the herbarium sheet, weighed using a five-point balance, and rehydrated for 24 hours in distilled water. The rehydrated leaf was photographed and the one-sided surface area calculated using image analysis software (WINDIAS, Delta-T Devices Ltd, Cambridge, UK). The SLA (expressed in cm^2 g^{-1} of dry mass) was calculated by dividing the value of the leaf area by the dry leaf mass. *LNC:* Leaf material was ground to a fine powder for 15 minutes at 25 beats per second using a tissue lyser (Tissuelyser II, Qiagen, Netherlands). Between 10-20mg was weighed into tin capsules and analysed using an elemental analyser (Vario EL Cube, Elementar, Germany). *LTS:* Leaves collected from herbarium specimens were rehydrated in distilled water for 24 hours. A section cut away from the midrib was clamped using a texture analyser (Lloyds TA500, AMETEK Test & Calibration Instruments), and the force measured at point of tearing (expressed in MPa). Three replicates per species were measured.

Values for maximum culm height, leaf length and leaf width were established from GrassBase, the Kew taxonomic database (Clayton, 2006 onwards). The former was used as a measure of plant height, while maximum leaf length and width were used to estimate leaf size, assuming an elliptical shape.

Environmental variables

All mapping of environmental variables was implemented in R (Core Development Team R, 2016). Global maps of the total topsoil exchangeable bases, soil pH, topsoil and sand content (an indicator of drainage), were obtained from the Harmonized World Soils Database (IIASA, 2008). These were used to calculate the mean soil pH, percentage topsoil sand content and total topsoil exchangeable bases (a measure of fertility, and hereafter referred to as "soil nutrients"), across the geographical area in which each species dominated grassy vegetation. The total topsoil exchangeable bases is defined as the sum of exchangeable cations, including sodium (Na$^+$), calcium (Ca^{2+}), magnesium (Mg^{2+}) and potassium (K$^+$).

Global data for nineteen climatic variables was obtained from the Worldclim database (Hijmans, Cameron, Parra, Jones, & Jarvis, 2005) and summarised as a mean for each species across the geographical area in which it dominated vegetation. Climatic variables can be highly correlated with one another. We therefore used principal components analysis (PCA) to reduce the dimensionality of the nineteen climate variables to axes that describe general patterns. The full results of the PCA are in Table 1. In summary, the first six principal component (PC) axes accounted for 95% of the total climatic variation and were used in multiple regression models of trait ~ soil + climate. PC1 was a gradient relating to temperature, PC2 was an axis of dryness and diurnal temperature range. PC3 relates to precipitation of the warmest and wettest months. PC4 is an axis of temperature and isothermality, PC5 is a gradient of temperature in combination with precipitation, and PC6 is a gradient of temperature, moisture and temperature range (Table 1).

Table 1. Climate variables with the highest loadings following principal components analysis (PCA). Mean diurnal range is the mean of monthly (max temp - min temp) and isothermality is the mean diurnal range/ temperature annual range (*100).

	Highest Loading Climate Variables	
	Negatively Loading	Positively Loading
PC1	Mean annual temperature, Min. temperature of coldest Month, Mean temperature of coldest quarter	Temperature seasonality
PC2	Precipitation of Driest Quarter, Precipitation of driest month	Mean Diurnal Range
PC3	Precipitation of Warmest Quarter, Precipitation of Wettest Month	Precipitation of Driest Month
PC4	Mean Temperature of Warmest Quarter	Isothermality
PC5	Precipitation of Warmest Quarter	Precipitation of coldest quarter
PC6	Mean temperature of wettest quarter	Temperature annual range

Phylogenetic hypothesis

A Bayesian distribution of one hundred phylogenetic trees was constructed of hypothesised relationships between all 279 dominant species in this study, including 94 C$_3$

and 185 C_4 species. Molecular data from 39 genes for all Poaceae species present in Genbank was downloaded using PHLAWD (Smith & Dunn, 2008) in April of 2014 to build an initial phylogeny including all grass species with sufficient genetic coverage (Forrestel et al. unpublished). There was no genetic data available for 66 of the species included in the study, and these species were therefore included using a set of taxonomic constraints based on existing expert knowledge of grasses. The phylogeny of Christin et al. (2014) was utilized as a dated backbone, and the methods of (Jetz, Thomas, Joy, Hartmann, & Mooers, 2012) were employed to insert taxa for which there was no genetic data available using the "pastis" package in R (Thomas et al., 2013) . One hundred trees from the final Bayesian distribution of phylogenies were subsequently pruned down to the 279 species included in our study. The trees are deposited in the Dryad database.

Statistical analyses

We first investigated the relationships among traits using a PCA to identify the main axes of variation between SLA, LTS, LNC, maximum culm height, maximum leaf area and maximum leaf width. To verify whether trade-offs operate at a global scale in this plant group, as Reich et al. (1997), Ian J. Wright et al. (2004), and Díaz et al. (2015) have all shown across all plant groups, we used a phylogenetic generalised least squares (PGLS) model to determine the strength and direction of correlations between all combinations of pairwise plant traits. PGLS accounts for phylogenetic autocorrelation in model residuals that is expected due to common ancestry.

We also used a PGLS model to investigate associations between the traits and environment. We fitted two models; trait ~ nutrients + sand + pH + PC1 + PC2 + PC3 + PC4 + PC5 + PC6 and its results are shown in Tables 3 and 4. Secondly, to evaluate whether traits differed systematically between continents, we fitted continent as a factor in the PGLS models trait ~ soil nutrients*continent + soil pH *continent + soil % sand*continent + PC1*continent + PC2*continent + PC3*continent + PC4*continent + PC5*continent + PC6*continent (results in Appendix S8). All model residuals were checked for normality and logarithmically transformed where necessary. PGLS analyses were performed using the R package "Caper" (Orme et al., 2012)

We measured phylogenetic signal in both the residuals of the models and the individual traits using Pagel's Lambda (λ), which estimates how much trait variation depends on phylogeny according to a Brownian model of evolution. A λ value of 0 implies no phylogenetic signal, while a value of 1 indicates phylogenetic dependence consistent with a Brownian motion model.

For all phylogenetic analyses, the tree used was randomly selected from the 100 Bayesian distribution of phylogenies. The analyses were repeated on another five randomly selected trees to assess sensitivity of our statistical models to phylogenetic uncertainty. We found no difference in any of the results based on using the different trees and so present results from a single phylogeny.

To assess how much of the trait variation occurred within versus between each of the grassy vegetation types defined by Lehmann et al (2019), we performed variance partitioning using the lme function ((Trait ~ 1, random = ~ 1| vegetation type) and the varcomp function in the R package "nlme" in R (Pinheiro J, 2017).

RESULTS

Geographical and phylogenetic distributions

Global patterns in the distribution of traits are shown in Figure 1. Mapping the mean trait values for dominants in each of the vegetation types revealed clear geographic patterns in trait values. The lowest values of LNC occurred across areas of the tropics (Fig. 1), areas characterised by very low soil nutrients, low pH, high rainfall and consistently high temperatures. The highest LNC occurred across the Eurasian Steppe (Fig. 1), a dry region with high soil fertility and seasonally low temperatures. Interestingly, regions with notably high SLA included both parts of the North American Great Plains, where there is a continental climate and high soil fertility, as well as the Brazilian Cerrado where the climate is tropical and soil nutrients very low. Despite being characterised by high SLA, areas of the Cerrado also exhibited very low values of LNC (Fig. 1). The toughest-leaved plants were in areas of Australia and the Eurasian Steppe (Fig. 1), where SLA was also the lowest (Fig. 1). The tallest and largest-leaved plants were in areas of the tropics, but particularly tropical Africa (Fig.1).

The phylogenetic distributions of trait values are shown in Figure 2 and reveal important differences in traits values between lineages. Individual traits including LNC, LTS, leaf width and height all showed strong and significant phylogenetic signals ($P<0.001$ for $\lambda = 0$), with λ values ranging from 0.57 to 0.96 (see Table 2). SLA displayed weaker, but statistically significant phylogenetic dependence ($\lambda = 0.14$). However, maximum leaf area showed no evidence of a phylogenetic signal ($\lambda = 0.24$).

Differences in clade mean height resulted from the divergence between dominant grasses in the Chloridoideae and Panicoideae lineages with Chloridoideae species being shortest. Panicoideae and Pooideae lineages were also significantly different in height with Panicoide being taller ($P<0.001$) (Fig. 2; Appendix S5 in Supporting Information). Differences in clade mean trait values for LTS were most distinct for Danthonioideae species, which are characterised by the toughest leaves (Fig. 2; Appendix S5). Significant differences in LNC were also observed between grasses from Pooideae and Panicoideae clades, with Pooideae grasses having the highest LNC (Fig. 2; Appendix S5). Smaller lineages also contributed to the phylogenetic signal in all traits (Fig. 2; Appendix S5).

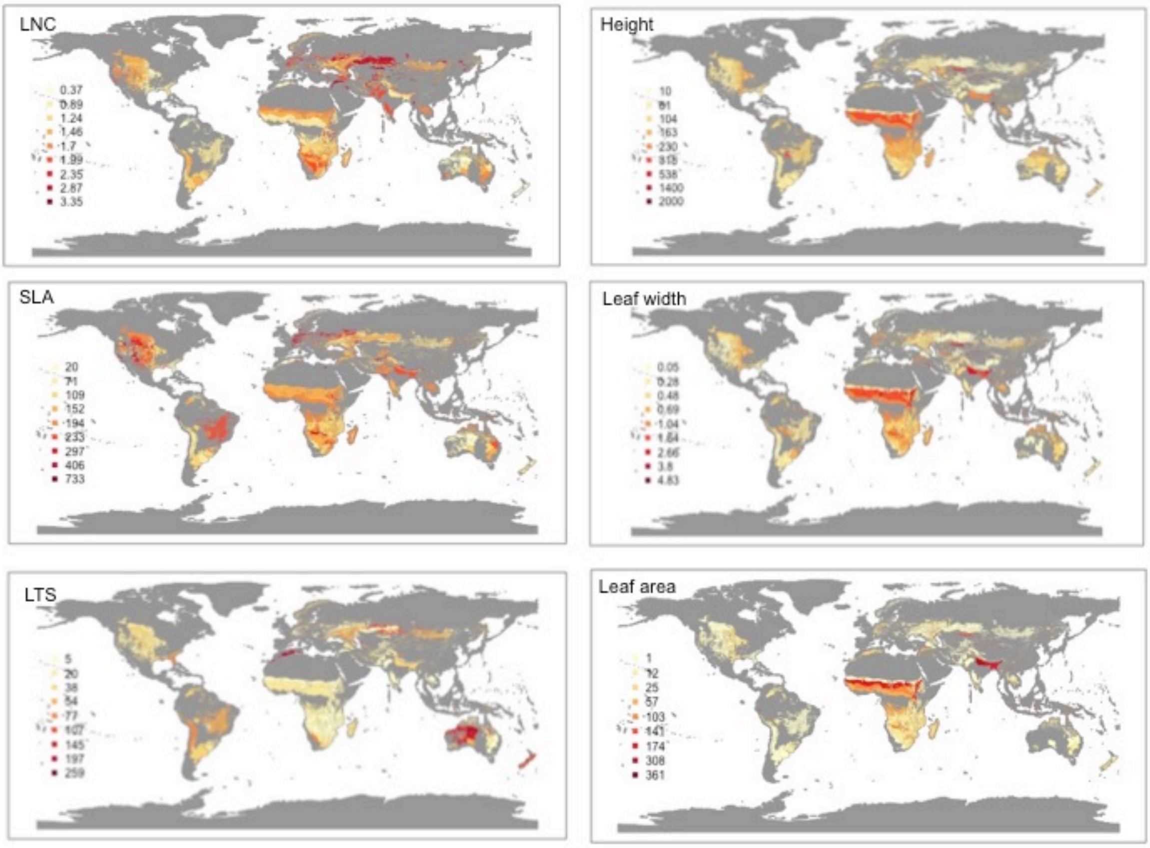

Figure 1. Global distributions of functional traits. Traits are: (LNC) leaf nitrogen content (%), (SLA) specific leaf area (cm^2/g), (LTS) leaf tensile strength (MPa), (Height) maximum culm height (cm), (Leaf width) maximum leaf width (cm), and (Leaf area) maximum leaf area (cm^2). Mapping is based on the mean trait values for dominant species in each of the grassy vegetation types determined by Lehmann et al. (2019).

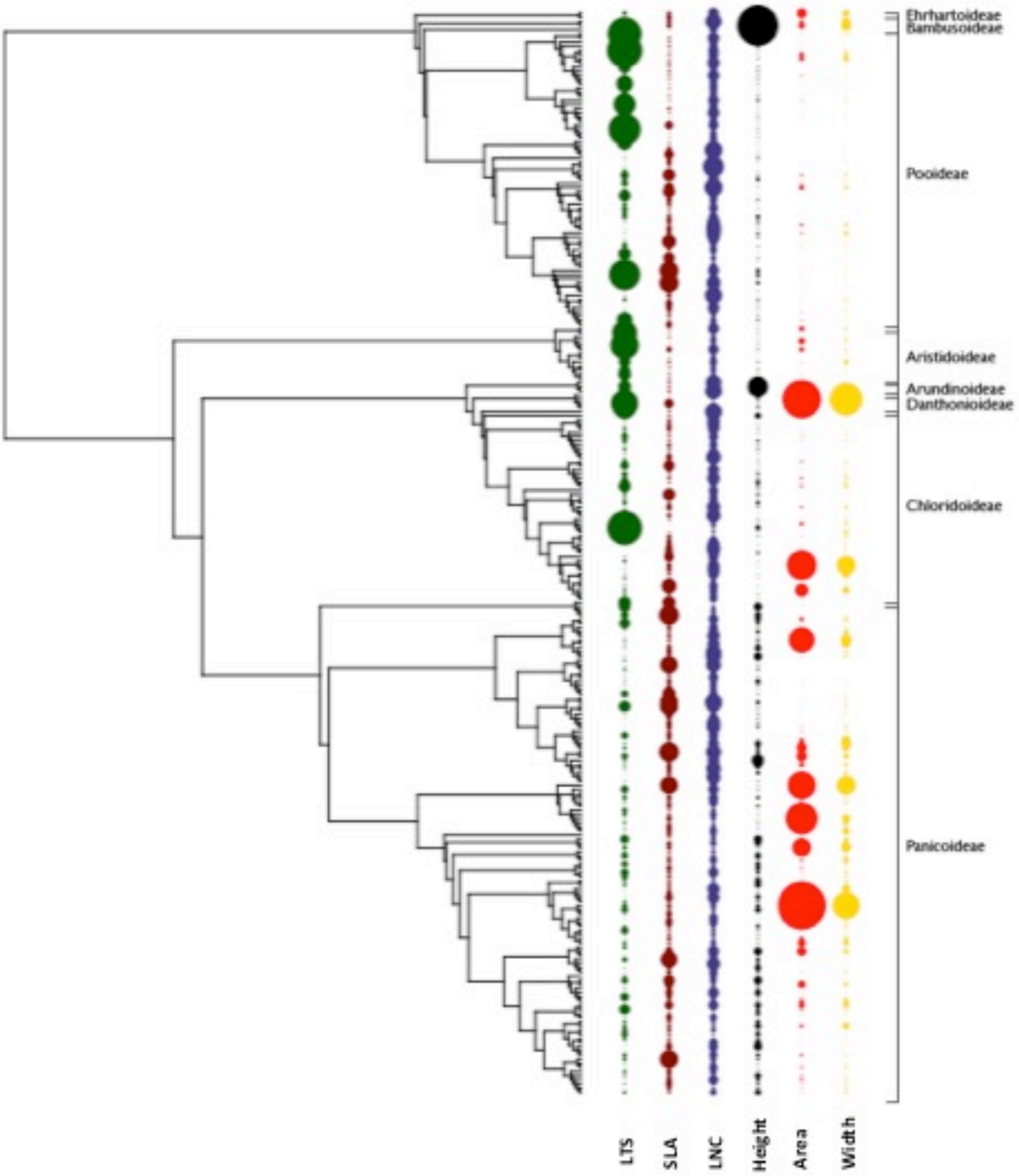

Figure 2. Functional trait values mapped across the phylogenetic tree. From left to right, traits are: leaf tensile strength, LTS (green), SLA (burgundy), % leaf nitrogen content, LNC (blue), maximum culm height (black), maximum leaf area (orange) and maximum leaf width (yellow) mapped across the phylogenetic tree. A full list of species in the tree is in Appendix S1 in supporting information. Dot sizes represent trait values and are scaled to fit the figure, which means scaling differs between traits.

Table 2. Pagel's λ for the individual traits. All traits except maximum leaf area showed strong and significant phylogenetic signal based on a likelihood ratio test against λ=0 with 1 degree of freedom. *$P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; *ns* not significant

Trait	λ	P (λ = 1)
LTS	0.77	***
N	0.59	***
SLA	0.14	*
Height	0.96	***
Leaf Area	0.24	N.S
Leaf Width	0.80	***

Trait coordination

Traits were separated on two orthogonal axes of variation (Fig. 3). One was identified as an axis corresponding to size-related traits including maximum culm height, maximum leaf width and maximum leaf area (Fig. 3). Orthogonal to this axis was an axis of resource capture and usage, and ranged from low to high SLA and LNC and high to low LTS, all traits corresponding to the leaf economic spectrum (Fig. 3). PC1 accounted for 35% of the total variance, PC2 accounted for 29%, PC3 14%, PC 4 10% and PC5 6% (Appendix 6a in Supporting Information). The loadings of traits on each axis are reported in Appendix 6b (Supporting Information).

Leaf economic traits all showed a statistically significant association with each other in the PGLS analysis. SLA and LNC were positively correlated (Fig. 4; Appendix S7 Supplementary Information). In contrast, SLA and LNC were negatively correlated with LTS (Fig. 4; Appendix S7). There was also a strong association among size-related traits, which all showed positive relationships (Fig. 5; Appendix S7). A weaker relationship was observed between leaf width and SLA, LNC and LTS, and between LTS and maximum culm height (Appendix S7).

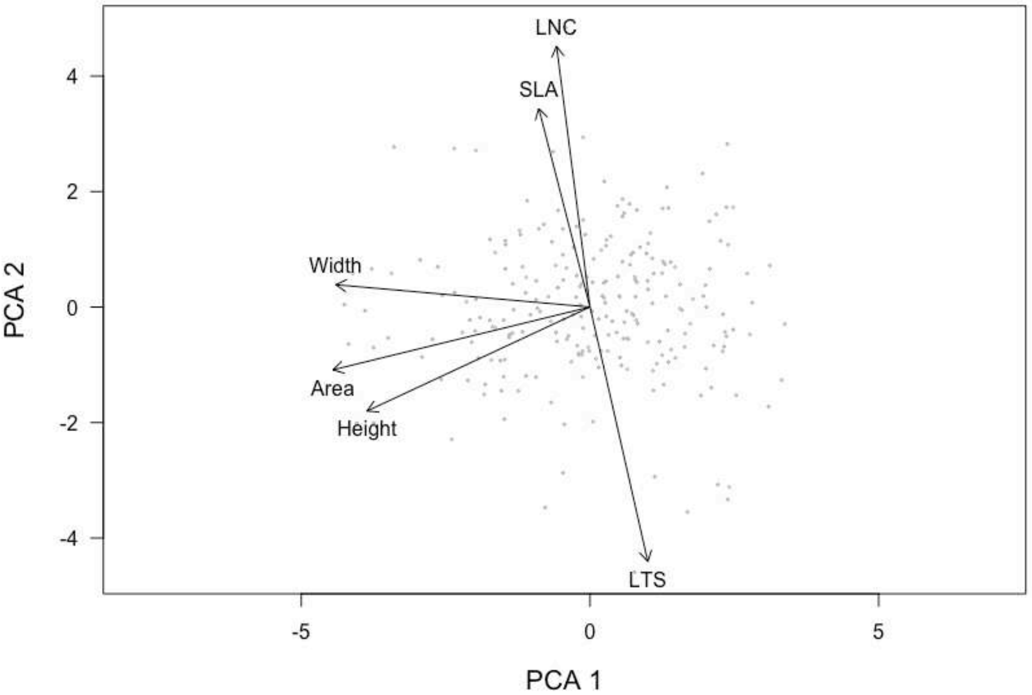

Figure 3 Principal components analysis (PCA) of traits, including specific leaf area (SLA), leaf tensile strength (LTS), leaf nitrogen content (LNC), maximum culm height (Height), maximum leaf area (Area) and maximum leaf width (Width). Orthogonal axes of trait variation are identified involving leaf economic traits and traits relating to size. Arrows represent the direction of increase of the trait values.

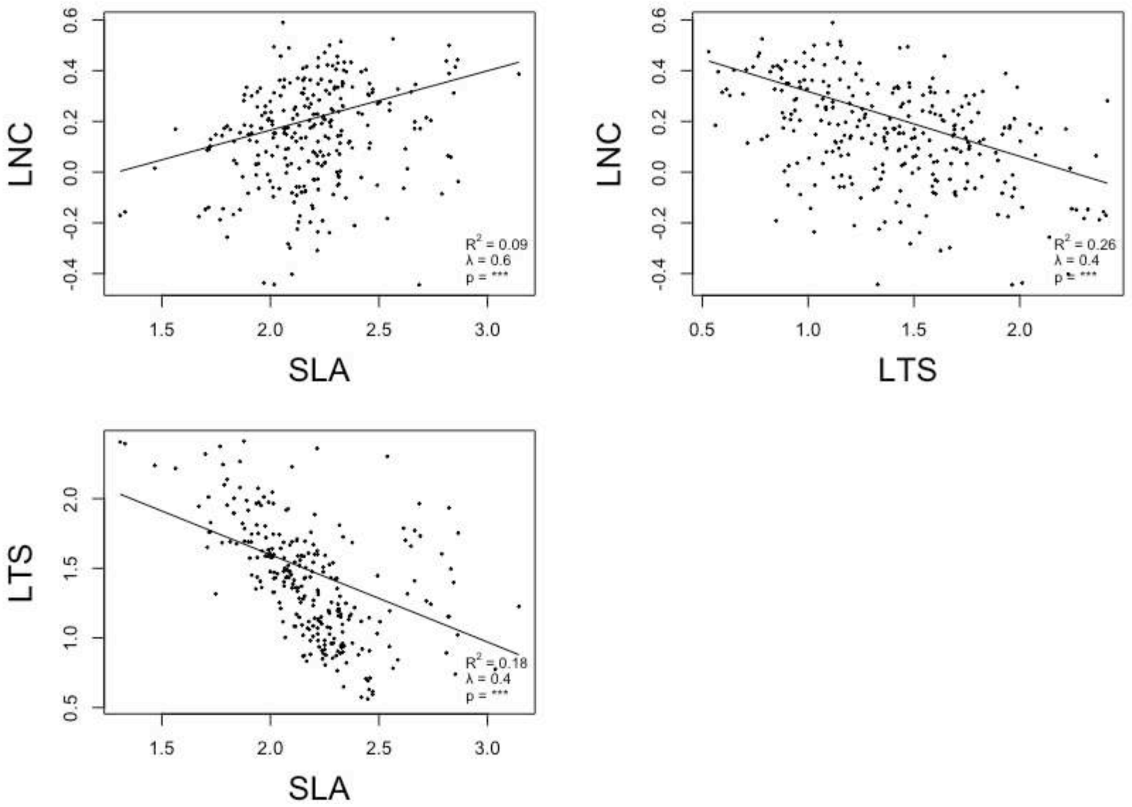

Figure 4 Relationship between pairwise combinations of the leaf economic traits, specific leaf area (SLA), leaf tensile strength (LTS) and leaf nitrogen content (LNC). Regression lines result from PGLS models of pairwise traits. All trait values are logarithmically transformed. Lambda values range between 0 and 1. Values closer to 1 indicate higher phylogenetic signal in the residuals of the models.

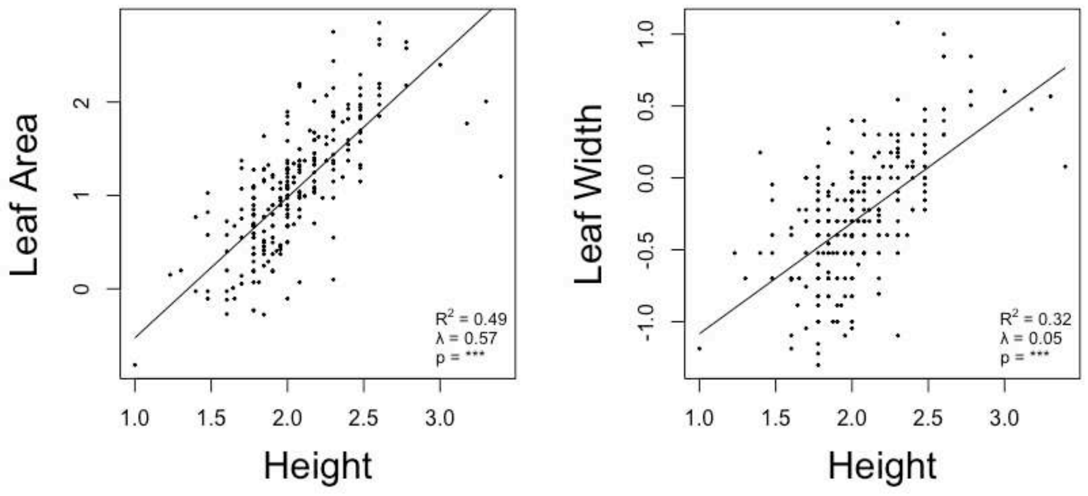

Figure 5 Relationship between pairwise combinations of the size traits: maximum leaf area, maximum culm height and maximum leaf width. Regression lines result from PGLS models of pairwise traits. All trait values are logarithmically transformed. Lambda values range between 0 and 1. Values closer to 1 indicate higher phylogenetic signal in the residuals of the models.

Traits and environment

ANOVA revealed that, with the exception of the relationship between precipitation and LNC, there was no significant difference in the slopes of the linear model fits for the different continents (Appendix 8 in Supporting Information). The interaction was therefore dropped from all subsequent analyses. The only leaf economic trait that showed moderately strong and significant associations with climate and soil was LNC ($r^2 = 0.20$) (Table 3). Soil nutrients made a significant contribution to the explanatory power of the model for LNC ($P<0.001$) as did PC axes 2 (dryness and diurnal temperature), PC5 (temperature in combination with precipitation) and PC 4 (temperature and isothermality) ($P<0.05$) (Table. 3). The remaining traits show hardly any relationship to environment, with very little of the variation in LTS and SLA explained by environmental gradients ($r^2 = 0.05$ and 0.03 respectively). Only soil nutrients significantly contributing to the explanatory power of the model for LTS. Soil pH and % sand were significant predictors of SLA although this association was again very weak (Table 3). Size-related traits were barely associated with environment, maximum culm height ($r^2 = 0.05$), maximum leaf width ($r^2 = 0.08$) and maximum leaf area ($r^2 = 0.06$), PC2 (dryness and diurnal temperature) was a significant predictor of the variation in both height and leaf width (Table 3). Leaf area and leaf width were significantly influenced by PC 1 (temperature) and PC 3 (precipitation and temperature) (Table 4). There were strong phylogenetic signals in the residuals of the model for all of the leaf economic spectrum traits, including LNC ($\lambda = 0.64$), LTS ($\lambda = 0.60$), SLA ($\lambda = 0.42$) (Table 3), as well as height ($\lambda = 0.69$), maximum leaf area ($\lambda = 0.55$) and maximum leaf width ($\lambda = 0.59$) (Table 4).

Variance partitioning was used to compare how much of the trait variation occurred within and between each of the grassy vegetation types defined by Lehmann et al. (2019). This showed that 60% of variation in LTS occurred within rather than between vegetation types, and a large amount of variation within the vegetation types was also evident for SLA (95%), LNC (64%), maximum culm height (55%), maximum leaf area (83%) and maximum leaf width (81%), suggesting that global- and regional-scale changes in environment are not key drivers of variation in grass traits.

Table 3. Relationship between species means of leaf economic traits relating to resource capture and release and environmental predictors of geographical trait variation. The full model is defined as trait ~ soil nutrients +soil pH + soil % sand + PC1+PC2+PC3+PC4+PC5+PC6. Data were logarithmically transformed before tests. *$P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; *ns* not significant

	N			SLA			LTS		
	Slope	S.E	P	Slope	S.E	P	Slope	S.E	P
Soil TEB	0.02	0.01	***	0.02	0.01	**	-0.03	0.01	*
Soil pH	-0.07	0.03	*	-0.13	0.05	**	0.04	0.07	ns
Soil %Sand	0.00	0.00		0.01	0.00	**	-0.01	0.00	ns
PC1	0.00	0.01	ns	0.00	0.01	ns	0.01	0.01	ns
PC2	0.02	0.01	**	0.00	0.01	ns	-0.02	0.02	ns
PC3	-0.01	0.01	ns	-0.01	0.01	ns	0.00	0.02	ns
PC4	0.02	0.01	*	0.01	0.02	ns	-0.04	0.02	ns
PC5	-0.03	0.01	**	0.02	0.02	ns	-0.02	0.03	ns
PC6	0.00	0.01	ns	0.00	0.02	ns	0.03	0.03	ns
Lambda	0.64			0.42			0.60		
r2	0.20			0.03			0.05		

Table 4 Relationship between variation in species means of traits relating to size and environmental predictors of traits variation from the model trait ~ soil fertility +soil pH + soil % sand + PC1+PC2+PC3+PC4+PC5+PC6. Data were logarithmically transformed before tests. *$P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; *ns* not significant

		Height			Leaf Width			Leaf Area	
	Slope	S.E	P	Slope	S.E	P	Slope	S.E	P
Soil nutrients	-0.01	0.01	ns	0.00	0.01	ns	-0.01	0.02	ns
Soil pH	-0.08	0.05	ns	-0.04	0.07	ns	-0.10	0.12	ns
Soil %Sand	0.00	0.00	ns	0.00	0.00	ns	0.00	0.01	ns
PC1	-0.02	0.01	ns	-0.03	0.01	**	-0.05	0.02	**
PC2	0.04	0.01	***	0.04	0.02	*	0.05	0.03	ns
PC3	-0.02	0.01	ns	-0.05	0.02	**	-0.07	0.03	*
PC4	0.00	0.02	ns	-0.01	0.02	ns	-0.04	0.04	ns
PC5	0.00	0.02	ns	0.01	0.02	ns	0.02	0.04	ns
PC6	0.01	0.02	ns	0.03	0.03	ns	0.04	0.06	ns
λ	0.69			0.59			0.55		
r2	0.05			0.08			0.06		

DISCUSSION

Using a global comparative analysis of traits from around one quarter of the globally dominant grass species, we demonstrate that leaf nitrogen content is significantly correlated with soil nutrients and to a lesser extent climate within the world's grassy biomes. However, overall, global gradients in the abiotic environment explain a relatively small amount of variation in commonly measured traits that are thought to reflect trade-offs in the acquisition and allocation of resources across grassy biomes. Instead, our analyses reveal that large amounts of trait variation occurs within as opposed to between vegetation types, and we show strong phylogenetic patterns in the distribution of traits. We were only able to sample a subset of the dominant grass species and vegetation types across the globe. However, our sample included multiple species from every continent, which represent a global latitudinal, climatic and soil gradient including species from all of the major and most minor grass phylogenetic lineages.

Trait relationships

We wanted to establish how leaf economic and size traits are coordinated among species. The traits of species dominating grassy biomes vary at the global scale along orthogonal axes of variation previously predicted by theory (Díaz et al., 2015; Grime, 1977; Sandel, Monnet, & Vorontsova, 2016) providing further evidence for trade-offs being a fundamental mechanism underlying plant functional strategies at a global scale. One axis revealed trade-offs between traits associated with the rapid acquisition of resources and allocation to growth, and traits linked to the conservation of resources in well-defended tissues, a relationship which is concurrent with other work (Coley et al., 1985; Diaz et al., 2004; Herms & Mattson, 1992; Reich et al., 1997; Westoby et al., 2002). An orthogonal axis of variation was identified relating to size and this is also consistent with previous studies that were conducted across broad taxa (Diaz et al., 2004; Sandel et al., 2016; Westoby, 1998). Interestingly, our study reveals that traits that are tightly correlated do not necessarily share the same relationships to environment.

Leaf economic traits and environment

We wanted to determine whether the trade-offs underlying plant strategies sort according to abiotic gradients of soil nutrients and climate at the global scale, and if this depends upon continent, since the magnitude of trait-environment relationships may vary between regions with different evolutionary histories (Lehmann et al., 2014). There was a marginally significant difference between continents in the responses of LNC to precipitation. However, for all other traits and environmental variables this was non-significant, showing that relationships between traits and environment are at the global scale independent of geographical location.

Although we found little evidence that continents differed in their trait relationships with environment, we did find strong evidence that evolutionary history shapes the distribution of traits. All leaf economic traits corresponding to trade-offs associated with a long leaf life span (SLA, LNC and LTS) exhibited strong phylogenetic signals in both the individual traits and the residuals of the models. This finding is consistent with previous work showing phylogenetic signals in both the traits and habitat associations of grasses (Liu et al., 2012; Visser, Woodward, Freckleton, & Osborne, 2012).

LNC was associated with soil fertility and also climate, with high values of LNC found in dry climate regions with high diurnal temperature range – i.e. semi-arid or desert regions. Ordoñez et al. (2009), also showed using published data consisting of mostly trees and shrubs, that globally an increase in LNC corresponds with increasing soil nutrients, and that soil influences traits more than climate. Species that are distributed across drier sites are known to have higher LNC, which may be a mechanism for improving leaf water-use efficiency by increasing investment in photosynthetic proteins and raising CO_2-fixation for a given stomatal conductance (Schulze et al., 1998; I. J. Wright et al., 2001). Arid regions also often coincide with areas of high soil fertility, which exerted a stronger effect on the variation in LNC in our analysis than climate. The observed increase in LNC with increasing soil nutrients may therefore be a plastic response to resource availability in the environment, as opposed to an adaptive strategy. However, we note that, in general, LNC varies more between species than within them (Kichenin, Wardle, Peltzer, Morse, & Freschet, 2013). Furthermore, our results show that LNC is highly conserved across the phylogeny, indicating that this pattern is driven by evolutionary adaptations of species that reflect historical processes rather than by the contemporary environment.

Variation in LTS and SLA were barely associated with soil properties and not at all with climate. A previous global scale analysis of leaf mechanical properties, which included forest as well as grassland species, showed the influence of mean annual precipitation on mechanical properties of leaves to be minimal but did not consider properties of soil (Onoda et al., 2011). Soil fertility has previously been linked to toughness in leaves at smaller scales (J Read, Sanson, & Lamont, 2005), which is consistent with theory that predicts better defended leaves in resource-limited habitats (Coley et al., 1985). Toughening of the leaves caused by lignin production is commonly observed in plants from arid habitats (Jennifer Read, Sanson, de Garine-Wichatitsky, & Jaffre, 2006), and it was therefore surprising that the PC axis describing precipitation did not have a significant effect upon LTS at a global scale and that the relationship with soil fertility were not stronger.

SLA showed the weakest relationship of all the leaf economic traits to environment and was barely explained by a combination of all measured soil properties. Although we did sample individuals that represented as much of the geographical range of each species as possible, we were unable to incorporate intraspecific trait variation into our study. This may account for some of the weak relationships observed. Traits, and in particular SLA can be highly plastic in response to environment, and this may explain some of the weakness of our correlations. However, since interspecific variation contributes less to trait variation than interspecific variation and in the case of SLA mirrors interspecific differences across environmental gradients (Carlucci, Debastiani, Pillar, & Duarte, 2015) we believe our results to be robust. Soil pH and sand content were significant predictors of SLA but did not explain variation in any other traits, and the relationship of LNC to soil nutrients was much stronger than that of SLA. This shows, importantly, that highly correlated traits do not necessarily share the same responses to environmental predictors.

SLA had the weakest relationship to environment but also had the highest amount of variation within vegetation types. Variance partitioning showed that 95% of variance in SLA, 64% of variance in LNC and 40% of variance in LTS, occurred within-vegetation type. A large part of the variance in these traits therefore occurs at finer scales (i.e. landscape and habitat patch) than can be explained by climate, a pattern also observed in other studies which included a broader range of taxa and biomes (Freschet, Cornelissen, van Logtestijn, & Aerts, 2010; Ian J. Wright et al., 2004). Unexplained variation within vegetation types may result from phylogenetically correlated environmental factors, as indicated by the strong phylogenetic signal, that vary at the landscape or community scales and could result from changes in woody plant cover, fire, herbivory or microsite variation in soil properties and moisture. Soil nutrients and hydrological properties can vary over small spatial scales that would not be captured by the resolution of our gridded soil data e.g. (Fridley, Grime, Askew, Moser, & Stevens, 2011). Furthermore, our measure of soil nutrients does not necessarily reflect plant nutrient uptake, which can be influenced by other factors including soil structure and compaction. It is possible that our data may capture broad scale patterns but underestimate fine scale relationships between traits and soils. However, He et al. (2010) provide evidence that in grasslands soil does not explain trait variation that is unexplained by climate.

Size related traits and environment

There was strong allometric scaling between maximum culm height, leaf area and leaf width, and all size-related traits were weakly correlated with climate, but not soil. Leaves perform several functions including light capture, water transport and defence, and optimal leaf size and shape therefore depends on environmental factors such as irradiance, energy balance, water availability and water loss, as well as biotic interactions such as competition and herbivory. Smaller leaves have higher major vein density which contributes to drought tolerance by directing water around blockages caused by drought-induced xylem embolism, and helping to protect the hydraulic system from damage (Sack et al., 2012). We found smaller and narrower leaves in drier habitats and larger, wider leaves in warm, humid regions. Aridity and diurnal temperature range were weakly associated with both maximum culm height and leaf width. Taller plants were found in the wettest regions, which are also the most productive areas of the world. Height is an important component of competition as taller plants are better competitors for light and cast shade on neighbouring individuals. Increased stature can therefore confer dominance in wet, productive areas where competition is likely to be most intense. Shorter plants and narrower leaves were found in the driest areas with a high temperature diurnal range, indicating semi-arid or desert climates It was therefore surprising that relationships between size and climate were not stronger over global scales. As with the leaf economic traits, we found a large amount of small-scale variation in size-related traits (between 55 and 83% of variation in size-related traits occurred within rather than between vegetation types). It is likely that unexplained variation in size related traits is driven by predictors which vary over smaller scales than climate, for example herbivory or fire.

Conclusions

Our results demonstrate that leaf traits of the dominant species of grassy biomes vary along orthogonal axes relating to size, and to resource capture and allocation. Trait correlations along these axes provide further evidence for trade-offs being a fundamental mechanism that underlie plant functional strategies at a global scale, however, correlated traits do not necessarily share the same response to environment. With the exception of LNC, traits linked to resource economics are barely correlated with global gradients in soil nutrients. Size-related traits are weakly correlated with climate. After accounting for global environmental gradients, there remain robust phylogenetic patterns in leaf and size traits, demonstrating that the trait combinations of dominant grass species depend strongly on their evolutionary history. There is considerable trait variation among the dominant species within grassy biomes, such that most trait variation occurs within rather than between different vegetation types. In combination, these patterns suggest that mechanisms of co-existence and phylogenetically linked environmental correlates varying over small spatial scales are important determinates of species occurrence.

BIOSKETCH

Emma Jardine has worked at Oxford University and at the German Centre for Integrative Biodiversity Research (iDiv)/FSU. Her research interests focus on understanding variation in plant functional traits, how plant strategies are associated with different environments and the processes that determine their distribution. This work formed a component of her PhD in the Animal and Plant Sciences department at the University of Sheffield.

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SUPPORTING INFORMATION

Appendix S1 Supplementary Methods
Appendix S2 Grass species incuded in this study
Appendix S3 Map showing the distirbution of species sampling
Appendix S4 Correlation between traits measured from fresh leaves and rehydrated herbarium specimens
Appendix S5 Clades with significantly different mean trait values
Appendix S6 Results of principal components analysis of traits
Appendix S7 Table showing all pairwise trait correlations
Appendix S8 ANOVA table comparing slopes of the full model with continent as an interaction.

SUPPORTING INFORMATION

Appendix S1 Supplementary methods

The map that was the basis for our species sampling was produced by Lehmann et al (2019), which integrated and reclassified 20 national and regional vegetation maps. These original maps were the products of a combination of botanical surveys, geographic analysis and expert opinion. References for these maps are listed in Lehmann et al (2019). From these data Lehmann et al identified global grassy vegetation types. In total 1635 grassy vegetation types were identified. Grassy vegetation was defined as having > 50% of the relative ground cover or biomass composed of grasses so that the classification of deserts and areas with sparse vegetation cover was not problematic. Vegetation units were considered grassy deserts where the total above-ground biomass was either <50 g m^2, or where total ground cover was <25%, throughout the year. Any region where grasses were the dominant component of the ground layer, irrespective of tree cover were also included in the map so as to include tropical savannas and woodlands which behave functionally as savanna due to a continuous grassy layer. Areas identified as mosaics of open and closed canopy but with a continuous grassy layer e.g. across the Steppe region of Russia were also classified as grassy. For areas of the world where no other maps were available, the WWF Ecoregions map was used and assessed by the above criteria to re-define units as grassy or otherwise. Artificial vegetation units (ie agricultural units or those planted by humans) were excluded from the map.

The species which characterise each vegetation type were also identified from the same maps and species that were invasive in vegetation types were excluded for the purposes of our study.

Appendix S2 List of all species included in the study following the taxonomy of GrassBase.

Species	Author
Acroceras macrum	Stapf
Aeluropus lagopoides	(L.) Thw.
Aeluropus littoralis	(Gouan) Parl.
Agropyron cristatum	(L.) J. Gaertn.
Agrostis capillaris	L.
Agrostis leptotricha	E. Desv.
Alloteropsis semialata	(R. Br.) Hitchcock
Andropogon bicornis	L.
Andropogon brazzae	Franch.
Andropogon gayanus	Kunth
Andropogon lateralis	Nees
Andropogon lima	(Hack.) Stapf
Andropogon schirensis	Hochst.
Andropogon selloanus	(Hack.) Hack.
Andropogon tectorum	Schum. & Thonn.
Anthephora argentea	Goossens
Anthephora pubescens	Nees
Anthoxanthum odoratum	L.
Apluda mutica	L.

Aristida adscensionis	L.
Aristida contorta	F. Muell.
Aristida diffusa	Trin.
Aristida jubata	(Arech.) Herter
Aristida junciformis	Trin. & Rupr.
Aristida murina	Cav.
Aristida pallens	Cav.
Aristida purpurea	Nutt.
Aristida rhiniochloa	Hochst.
Aristida rufescens	Steud.
Aristida similis	Steud.
Aristida stricta	Michx.
Arundinella mesophylla	Nees ex Steud.
Arundo donax	L.
Astrebla lappacea	(Lindl.) Domin
Axonopus canescens	(Nees) Pilger
Axonopus compressus	(Sw.) Beauv.
Axonopus fissifolius	(Raddi) Kuhlm.
Axonopus purpusii	(Mez) Chase
Bambusa polymorpha	Munro
Bambusa tulda	Roxb.
Blepharoneuron tricholepis	(Torr.) Nash
Bothriochloa ischaemum	(L.) Keng
Bothriochloa saccharoides	(Sw.) Rydb.
Bouteloua curtipendula	(Michx.) Torr.
Bouteloua megapotamica	(Spreng) Kuntze
Brachiaria deflexa	(Schum.) C. E. Hubb. ex Robyns
Brachiaria nigropedata	(Fic. & Hiern.) Stapf
Brachiaria serrata	(Thunb.) Stapf
Brachypodium pinnatum	(L.) Beauv.
Briza brizoides	(Lam.) Kuntze
Briza subaristata	Lam.
Bromus auleticus	Trin. ex Nees
Bromus sclerophyllus	Boiss.
Bromus speciosus	Nees
Bromus tectorum	L.
Calamagrostis arundinacea	(L.) Roth
Calamagrostis epigeios	(L.) Roth
Calamagrostis rubescens	Buckl.
Calamagrostis varia	(Schrad.) Host
Calamovilfa longifolia	(Hook.) Scribn.
Cenchrus biflorus	Roxb.
Cenchrus ciliaris	L.
Centropodia glauca	(Nees) T. A. Cope
Chionochloa flavescens	Zotov
Chionochloa pallens	Zotov

Chionochloa rubra	Zotov
Chloris virgata	Sw.
Chondrosum eriopodum	Torr.
Chondrosum gracile	H. B. & K.
Chondrosum hirsutum	(Lag.) Sweet
Chrysopogon aciculatus	(Retz.) Trin.
Chrysopogon fulvus	(Spreng.) Chiov.
Chrysopogon nigritanus	(Benth.) Veldkamp
Cleistogenes squarrosa	(Trin.) Keng
Cortaderia jubata	(Lemoine) Stapf
Ctenium newtonii	Hack.
Cymbopogon caesius	(Hook. & Arn.) Stapf
Cymbopogon distans	(Nees) W. Watson
Cymbopogon flexuosus	(Nees) W. Watson
Cymbopogon giganteus	Chiov.
Cymbopogon nardus	(L.) Rendle
Cymbopogon nervatus	(Hochst.) Chiov.
Cymbopogon pospischilii	(K. Schum.) C. E. Hubb.
Cynodon dactylon	(L.) Pers.
Cynodon incompletus	Nees
Dactylis glomerata	L.
Dactyloctenium aegyptium	(L.) Willd.
Dactyloctenium giganteum	B. S. Fisher & Schweickerdt
Dactyloctenium radulans	(R. Br.) Beauv.
Danthonia californica	Boland.
Dendrocalamus strictus	(Roxb.) Nees
Deschampsia cespitosa	(L.) Beauv.
Deschampsia flexuosa	(L.) Trin.
Desmostachya bipinnata	(L.) Stapf
Dichanthium fecundum	S. T. Blake
Dichanthium foveolatum	(Delile) Roberty
Dichanthium sericeum	(R. Br.) A. Camus
Digitaria abyssinica	(A. Rich.) Stapf
Digitaria brazzae	(Franch.) Stapf
Digitaria californica	(Benth.) Henrard
Digitaria debilis	(Desf.) Willd.
Digitaria eriantha	Steud.
Digitaria macroblephara	(Hack.) Paoli
Digitaria milanjiana	(Rendle) Stapf
Diheteropogon amplectens	(Nees) Clayton
Echinochloa colona	(L.) Link
Echinochloa haploclada	(Stapf) Stapf
Echinochloa pyramidalis	(Lam.) Hitchc. & Chase
Echinolaena inflexa	(Poir.) Chase
Eleusine coracana	(L.) Gaertn.
Elionurus muticus	(Spreng.) Kuntze

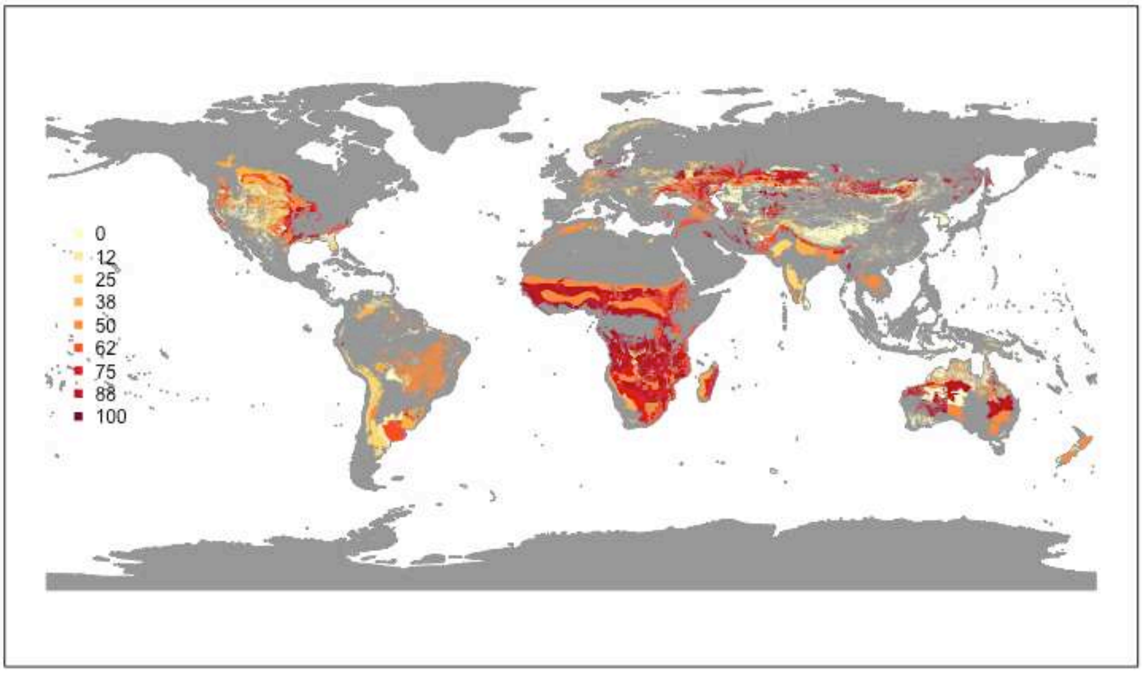
Enneapogon desvauxii	Beauv.
Entolasia imbricata	Stapf
Eragrostis biflora	Hack. ex Schinz
Eragrostis ciliaris	(L.) R. Br.
Eragrostis curvula	(Schrad.) Nees
Eragrostis cylindriflora	Hochst.
Eragrostis lugens	Nees
Eragrostis neesii	Trin.
Eragrostis obtusa	Munro ex Ficalho & Hiern
Eragrostis superba	Peyr.
Eriochloa fatmensis	(Hochst. & Steud.) Clayton
Exotheca abyssinica	(Hochst.) Anderss.
Festuca caprina	Nees
Festuca idahoensis	Elmer
Festuca lenensis	Drobov
Festuca novae-zealandiae	(Hack.) Cockayne
Festuca ovina	L.
Festuca pratensis	Huds.
Festuca quadriflora	Honck.
Festuca valesiaca	Schleich. ex Gaud.
Fingerhuthia africana	Lehm.
Helictotrichon desertorum	(Less.) Pilger
Heteropogon contortus	(L.) Beauv. ex Roem. & Schult.
Heteropogon melanocarpus	(Ell.) Benth.
Hyparrhenia anthistirioides	(Hochst.) Anderss. ex Stapf
Hyparrhenia cymbaria	(L.) Stapf
Hyparrhenia dichroa	(Steud.) Stapf
Hyparrhenia diplandra	(Hack.) Stapf
Hyparrhenia familiaris	(Steud) Stapf
Hyparrhenia filipendula	(Hochst.) Stapf
Hyparrhenia hirta	(L.) Stapf
Hyparrhenia newtonii	(Hack.) Stapf
Hyparrhenia nyassae	(Rendle) Stapf
Hyparrhenia schimperi	(Hochst.) Anderss. ex Stapf
Hyparrhenia smithiana	(Hook.) Stapf
Hyparrhenia subplumosa	Stapf
Hyperthelia dissoluta	(Nees) Clayton
Imperata cylindrica	(L.) Raeusch.
Ischaemum afrum	(J. F. Gmel.) Dandy
Koeleria glauca	(Spreng.) DC.
Koeleria macrantha	(Ledeb.) Schult.
Leersia hexandra	Sw.
Leptochloa fusca	(L.) Kunth
Leptocoryphium lanatum	(HBK) Nees
Leymus cinereus	(Scribn. & Merr.)
Leymus racemosus	(Lam.) Tsvelev

Leymus triticoides	(Buckl.) Pilger
Loudetia arundinacea	(A. Rich) Hochst. ex Steud.
Loudetia phragmitoides	(Peter) C. E. Hubb.
Loudetia simplex	(Nees) C. E. Hubb.
Melica brasiliana	Ard.
Melica minuta	L.
Melica nutans	L.
Melica picta	C. Koch
Melinis amethystea	(Franchet) G. Zizka
Melinis minutiflora	P. Beauv.
Mesosetum loliiforme	(Steud.) Hitchcock
Mesosetum penicillatum	Mez
Microchloa caffra	Nees
Milium effusum	L.
Monocymbium ceresiiforme	(Nees) Stapf
Muhlenbergia richardsonis	(Trin.) Rydb.
Nardus stricta	L.
Nassella charruana	(Arech.) M. E. Barkworth
Nassella neesiana	(Trinius & Ruprecht) M. E. Barkworth
Nassella pulchra	(A. Hitchc.) M. E. Barkworth
Nassella viridula	(Trin.) M. E. Barkworth
Neyraudia reynaudiana	(Kunth) Keng ex Hitchcock
Oryza longistaminata	A. Chevalier & Roehrich
Panicum kalaharense	Mez
Panicum lanipes	Mez
Panicum maximum	Jacq.
Panicum phragmitoides	Stapf
Panicum repens	L.
Panicum virgatum	L.
Paspalum dilatatum	Poir.
Paspalum notatum	Fluegge
Paspalum scrobiculatum	L.
Paspalum vaginatum	Sw.
Pennisetum massaicum	Stapf
Pennisetum mezianum	Leeke
Pennisetum orientale	Rich.
Pennisetum polystachion	(L.) Schult.
Pennisetum purpureum	Schum.
Pennisetum sphacelatum	(Nees) T. Dur. & Schinz
Pennisetum stramineum	Peter
Pennisetum unisetum	(Nees) Benth.
Phleum alpinum	L.
Phleum phleoides	(L.) Karst.
Phragmites australis	(Cav.) Trin. ex Steud.
Phragmites vallatorius	(Pluk.) J. F. Veldkamp
Piptatherum microcarpum	(Pilg.) Tsvelev

Poa bonariensis	(Lam.) Kunth
Poa bulbosa	L.
Poa cita	E. Edgar
Poa hiemata	Vickery
Poa labillardieri	Steud.
Poa lanuginosa	Poir.
Poa nemoralis	L.
Poa pratensis	L.
Poa secunda	J. & C. Presl
Pogonarthria squarrosa	(Roem. & Schult.) Pilger
Pseudoraphis spinescens	(R. Br.) Vickery
Puccinellia gigantea	(Grossh.) Grossheim
Rytidosperma oreoboloides	(F. Muell.) H. P. Linder
Saccharum bengalense	Retz.
Saccharum spontaneum	L.
Schizachyrium sanguineum	(Retz.) Alston
Schizachyrium scoparium	(Michx.) Nash
Schizachyrium spicatum	(Spreng.) Herter
Schizachyrium tenerum	Nees
Schmidtia kalahariensis	Stent
Schmidtia pappophoroides	Steud.
Sehima ischaemoides	Forsk.
Sehima nervosum	(Rottler) Stapf
Setaria incrassata	(Hochst.) Hack.
Setaria sphacelata	(Schumach.) Stapf & C. E. Hubb. ex Moss
Sorghastrum nutans	(L.) Nash
Sorghum arundinaceum	(Desv.) Stapf
Sorghum purpureosericeum	(A. Rich.) Schweinf. & Aschers.
Spartina patens	(Ait.) Muhl.
Sporobolus airoides	(Torr.) Torr.
Sporobolus compositus	(Poir.) Merrill
Sporobolus contractus	Hitchcock
Sporobolus cubensis	Hitchcock
Sporobolus indicus	(L.) R. Br.
Sporobolus ioclados	(Trin) Nees
Stenotaphrum secundatum	(Walt.) Kuntze
Stipa arabica	Trin. & Rupr.
Stipa barbata	Desf.
Stipa capillata	L.
Stipa caucasica	Schmalh.
Stipa comata	Trin. & Rupr.
Stipa dasyphylla	(Lindem.) Czern. ex Trautv.
Stipa eremophila	Reader
Stipa hohenackeriana	Trin. & Rupr.
Stipa ichu	(Ruiz & Pav) Kunth

Stipa krylovii	Roshev.
Stipa lessingiana	Trin. & Rupr.
Stipa neaei	Nees ex Steud.
Stipa pulcherrima	C. Koch
Stipa richteriana	Kar. & Kir.
Stipa sareptana	Beck.
Stipa speciosa	Trin. & Rupr.
Stipa tenacissima	L.
Stipa thurberiana	Piper
Stipa tirsa	Stev.
Stipa trichophylla	Benth.
Stipa turkestanica	Hack.
Stipa zalesskii	Wilensky
Stipagrostis ciliata	(Desf.) de Winter
Stipagrostis uniplumis	(Licht.) de Winter
Themeda anathera	(Nees) Hack.
Themeda arundinacea	(Roxb.) A. Camus
Themeda tremula	(Nees) Hack.
Themeda triandra	Forsk.
Themeda villosa	(Lam.) A. Camus
Trachypogon spicatus	(L.) Kuntze
Tragus berteronianus	Schult.
Tragus koelerioides	Aschers.
Tragus racemosus	(L.) All.
Triodia basedowii	E. Pritzel
Triodia longiceps	J. M. Black
Triodia pungens	R. Br.
Triodia wiseana	C. A. Gardner
Tristachya leiostachya	Nees
Urochloa mosambicensis	(Hack.) Dandy
Vossia cuspidata	(Roxb.) Griff.

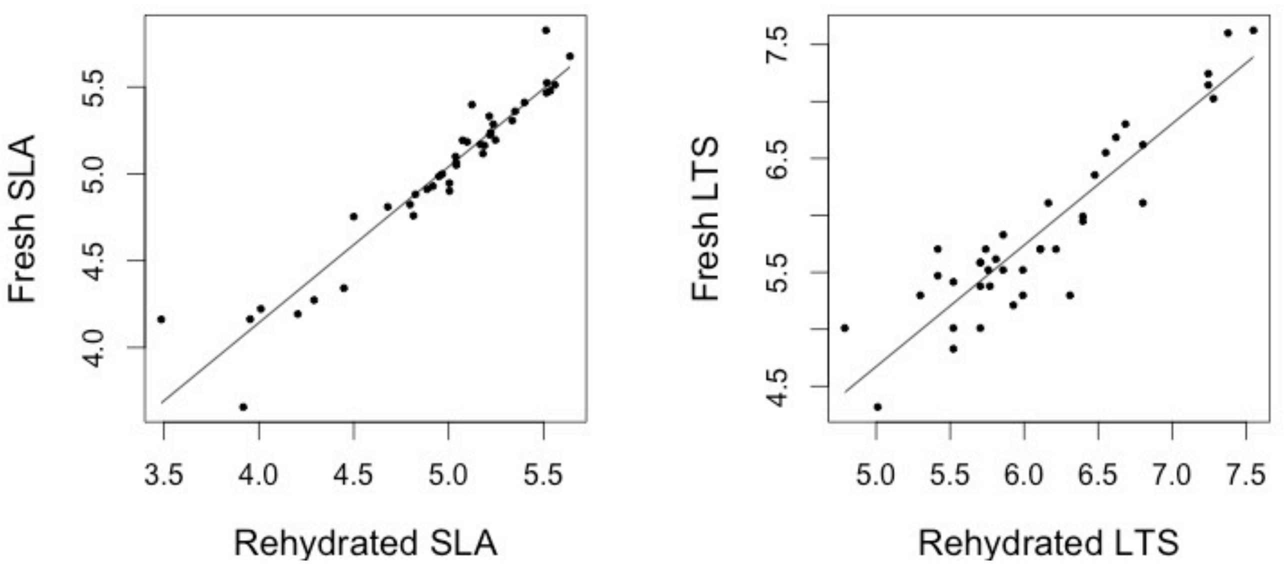
Appendix S3 The global extent of grassy vegetation used as the basis for sampling in this study. Polygons, which make up the map represent different vegetation types. Polygons/vegetation types are coloured by the percentage of the total species per polygon/vegetation that were sampled in this study. The numbers of dominant species for vegetation types ranged from 1 to 19, with a mean of 4.



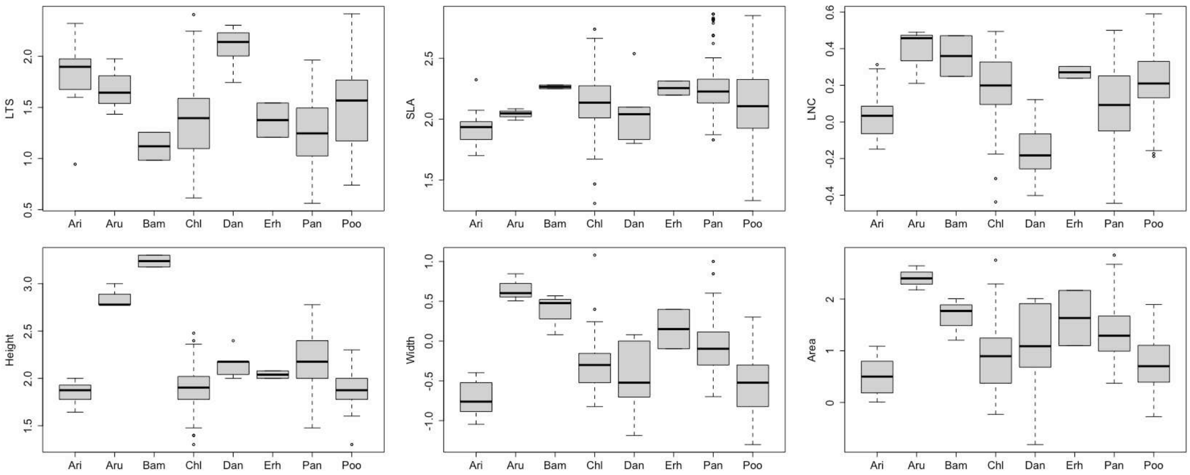
Measuring traits from herbarium specimens

We measured the SLA and LTS on the fresh leaves of 39 grass species using standard protocols (Cornelissen, Lavorel et al. 2003). Leaf area was determined using image analysis software (WINDIAS, Delta-T Devices Ltd, Cambridge, UK) and dry leaf weight using a five-point balance. Leaves were then dried in herbarium presses for 10 days, weighed, rehydrated in de-ionised water for 24 hours before being scanned and then measured again. Using linear regression we showed strong correlations between the fresh and rehydrated trait measurements for SLA ($r^2 = 0.90$, $P<0.001$) (Figure S1) and LTS ($r^2 = 0.84$, $P<0.001$).

Appendix S4 The relationship between trait values measured on fresh leaves and the same leaves that had been subjected to drying in herbarium presses then rehydrated for (a) specific leaf area (SLA) ($r^2 = 0.90$, $P<0.001$) and (b) leaf tensile strength (LTS) ($r^2 = 0.84$, $P < 0.001$). All data were logarithmically transformed.



Appendix S5 Comparison of trait values for Aristoideae (Ari), Arundoideae (Aru), Bambusoideae (Bam), Chloridoideae (Chl), Danthonioideae (Dan), Erhartoideae (Erh), Panicoideae (Pan) and Pooideae (Poo). Solid lines show the median and solid circles the mean for each clade. Ouliers are unfilled circles. All traits were logarithmically transformed.



Appendix 6a The proportion of variance explained by each axis of a principal components analysis of the traits specific leaf area (SLA), leaf tensile strength (LTS), leaf nitrogen content (LNC), leaf carbon content (LCC), maximum culm height, maximum leaf area and maximum leaf width.

	PC1	PC2	PC3	PC4	PC5	PC6
Standard deviation	1.60	1.23	0.91	0.83	0.59	0.24
Proportion of Variance	0.43	0.25	0.14	0.11	0.58	0.01
Cumulative Proportion	0.43	0.68	0.82	0.93	0.99	1.00

Appendix 6b Loadings of each traits on each of the PC axis identified following principal components analysis of the traits specific leaf area (SLA), leaf tensile strength (LTS), leaf nitrogen content (LNC), maximum culm height, maximum leaf area and maximum leaf width.

	PC1	PC2	PC3	PC4	PC5	PC6
Height	-0.52	-0.24	0.04	-0.70	-0.81	-0.14
Leaf Area	-0.59	-0.14	0.01	-0.03	0.29	0.73
Leaf Width	-0.59	0.05	-0.12	0.02	0.47	-0.65
LNC	-0.08	0.60	-0.29	-0.73	-0.10	0.07
SLA	-0.11	0.46	0.88	0.04	-0.01	-0.01
LTS	0.13	-0.59	0.36	-0.68	0.19	-0.11

Appendix S7 Pairwise relationships between all combinations of species mean traits. λ values are for the residuals in the PGLS model. *P* < 0.05; ** *P* < 0.01; *** *P* < 0.001; *ns* not significant. Trait data were logarithmically transformed before tests

	Slope	S.E	λ	r^2	*P*
N~LTS	-0.256	0.027	0.40	0.26	***
N~SLA	-0.233	0.044	0.60	0.09	***
N~Height	-0.014	0.043	0.54	0	ns
N~Leaf Width	0.096	0.031	0.48	0.03	**
LTS~SLA	-0.640	0.085	0.40	0.18	***
SLA~Height	-0.036	0.056	0.32	0	ns
SLA~Leaf Width	0.107	0.042	0.30	0.02	*
SLA~Leaf Area	0.019	0.027	0.32	0	ns
Height~LTS	0.120	0.047	0.50	0.02	*
LTS~Leaf Area	0.052	0.040	0.50	0	ns
LTS~Leaf Width	-0.149	0.062	0.49	0.02	*
Leaf Width~Height	0.425	0.039	0.05	0.32	***
Leaf Area~Height	0.330	0.021	0.57	0.49	***

Appendix S8 ANOVA comparing the slopes of the full model with the continent that each species was dominant in fitted as an interaction. The full model is defined as trait ~ soil nutrients*continent +soil pH *continent + soil % sand*continent + PC1*continent +PC2*continent +PC3*continent +PC4*continent +PC5*continent +PC6*continent. $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; *ns* not significant. Trait data were logarithmically transformed before tests

		LC			LTS			SLA			Height			Leaf Area			Leaf Width	
	DF	F value	P	DF	F value	P	DF	F value	P	DF	F value	P	DF	F value	P	DF	F value	P
Soil TEB	4	1.83	ns	4	0.52	ns	4	2.30	ns	4	0.47	ns	4	1.31	ns	4	1.48	ns
Soil pH	4	0.46	ns	4	0.96	ns	4	0.93	ns	4	1.22	ns	4	2.27	ns	4	0.89	ns
Soil % Sand	4	1.14	ns	4	0.42	ns	4	3.08	ns	4	0.59	ns	4	2.03	ns	4	0.33	ns
PC1	4	0.85	ns	4	0.84	ns	4	0.74	ns	4	0.43	ns	4	1.14	ns	4	1.89	ns
PC2	4	0.78	ns	4	0.69	ns	4	0.76	ns	4	0.63	ns	4	1.53	ns	4	1.46	ns
PC3	4	0.86	ns	4	1.10	ns	4	0.47	ns	4	0.21	ns	4	1.15	ns	4	1.83	ns
PC4	4	2.28	ns	4	2.26	ns	4	0.86	ns	4	2.37	ns	4	2.13	ns	4	1.26	ns
PC5	4	4.57	**	4	0.86	ns	4	2.20	ns	4	0.60	ns	4	1.22	ns	4	0.37	ns
PC6	4	1.74	ns	4	1.58	ns	4	0.80	ns	4	0.43	ns	4	2.04	ns	4	2.02	ns