GLOBAL RELATIONSHIPS BETWEEN PLANT FUNCTIONAL TRAITS AND ENVIRONMENT IN GRASSLANDS

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A thesis submitted in partial fulfilment of the requirements for the degree of
Doctor of Philosophy

The University of Sheffield
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Submission Date

July 2017

ACKNOWLEDGMENTS

First of all I am enormously thankful to Colin Osborne and Gavin Thomas for giving me the opportunity to undertake the research presented in this thesis. I really appreciate all their invaluable support, guidance and advice. They have helped me to grow in knowledge, skills and confidence and for this I am extremely grateful.

I would like to thank the students and post docs in both the Osborne and Christin lab groups for their help, presentations and cake baking. In particular Marjorie Lundgren for teaching me to use the Licor, for insightful discussions and general support. Also Kimberly Simpson for all her firey contributions and Ruth Wade for her moral support and employment.

Thanks goes to Dave Simpson, Maria Varontsova and Martin Xanthos for allowing me to work in the herbarium at the Royal Botanic Gardens Kew, for letting me destructively harvest from the specimens and taking me on a worldwide tour of grasses. I would also like to thank Caroline Lehman for her map, her useful comments and advice and also Elisabeth Forrestel and Gareth Hempson for their contributions. I would like to thank Brad Ripley for all of his help and time whilst I was in South Africa. Karmi Du Plessis and her family and Lavinia Perumal for their South African friendliness, warmth and generosity and also Sean Devonport for sharing all the much needed teas and dub. I hope to return the hospitality one day in the UK.

I would like to give a special mention to Bethan Hindle, Shaun Coutts and Tamora James for allowing me to infiltrate their row of the office and answering all of my questions about R. A special thanks to Ronan Head whose friendship helped me greatly on my journey to beginning a PhD. To Glen Smith for putting everything into perspective in times of need and for always being able to make me laugh, usually at myself. Thanks to Gita Patel my northern sister, whose enthusiasm, positivity and ability to manifest whatever she puts her mind to is a source of inspiration. Finally to the Incredibles Jason Griffiths, Bianca Santini and Jose Moreno who have helped me immeasurably throughout my studies by being such fun, supportive and talented scientists and friends.

This research was funded by Project Sunshine.

SUMMARY

In order to understand how ecosystems are likely to respond to global anthropogenic change it is first necessary to identify general patterns and processes that can explain how they are created and maintained. Plant traits potentially provide a mechanistic explanation for the differences in growth and survival that explain a species niche that can scale up from individual to biome. In this thesis I investigate the relationships between grass functional traits and the biotic and abiotic environment, and test whether the predictions made by community ecological theory are more broadly applicable at continental and global scales.

I provide evidence that at the global scale soil nutrients is more important than climate in explaining the distribution of traits that reflect different strategies of resource use but that evolutionary history provides a stronger explanation for global trait distribution than contemporary environment. I then show the functional traits that are associated with gradients of grazing and fire and identify functional groups that have diverging responses to grazing across Sub-Saharan Africa. Finally I investigate species response to drought and identify traits which can explain a species hydrological niche.

The findings of this work provide evidence that trade-offs between carbon and nitrogen acquisition and use (leaf nitrogen content and C/N ratio) could provide a foundation for predicting plant responses to changes in climate, soil nutrients and disturbance at global scales. However, I also show that traits often used to reflect differences in leaf growth and longevity (ie. specific leaf area and leaf tensile strength) are not able to strongly predict response to either resource availability or disturbance at macro-ecological scales. This highlights the need to identify other axis of variation and organs beyond the leaf economic spectrum, for example root architecture, that are potentially important in explaining the differing aspects of a species niche and how vegetation may respond to global change.

CHAPTER ONE

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INTRODUCTION

STATEMENT OF CONTRIBUTIONS

The introduction was written by the candidate with editorial advice from Prof. Colin Osborne and Dr Gavin Thomas.

INTRODUCTION

Trait based approaches to ecology

A central goal of ecology is to find rules to describe patterns in nature and to explain how these patterns arise. There is an increasing need to understand how ecosystems are likely to respond to global anthropogenic change. The identification of general rules, patterns and processes that determine vegetation dynamics at the global scale has the potential to improve global models predicting how ecosystems will respond to global climate and land use change. Traditional methods in community ecology have relied upon grouping organisms by taxonomy and consider patterns of species diversity rather than functional diversity to understand community structure. Species have often been assigned to functional groups united by fundamental differences in traits that influence growth and survival (eg. tree/ forbs, C_3/C_4). However this approach ignores functional variation within these groups and, importantly, functional diversity is often more strongly correlated with ecosystem function than species diversity (Diaz and Cabido 2001, Lavorel and Garnier 2002, Cadotte, Carscadden et al. 2011).

There has, over the past 10 – 15 years, been a growing interest in using functional trait based approaches to describe ecological patterns and processes in plant communities (Lavorel and Garnier 2002, Wright, Reich et al. 2005, McGill, Enquist et al. 2006, Westoby and Wright 2006, Cornwell and Ackerly 2009). Trait based approaches make generalizations based upon functional traits, regardless of species identity, to predict plant responses to biotic and abiotic variation and disturbance. A functional trait can be defined as something measureable on an individual that influences the performance of an organism via trait effects on growth rate, survival and reproduction. Traits may be morphological, biochemical, physiological, phenological or anatomical and may reflect a function (eg SLA) or be a function (eg. photosynthesis) (McGill, Enquist et al. 2006, Violle, Navas et al. 2007). There is some ambiguity surrounding the definition of functional traits and the way this term is used can vary widely. Functional traits are always measured on one individual. However predictions based on trait response to environment have been scaled up to explain the processes that are responsible for community assembly, ecosystem function and global vegetation dynamics. Traits which describe how well an organism is able to survive in a given environment have been called “response traits”. Those that describe the effect of organisms on ecosystem processes have been called “effect traits” (Diaz and Cabido 2001). Some traits may be described as both response and effect traits due to having associations with certain environments but also having feedbacks to ecosystem processes. For example, specific leaf area has been associated with resource availability, but also affects ecosystem processes including decomposition.

Niche based processes

The relevance of linking variation in functional traits to biotic and abiotic gradients to make ecological predictions is linked to the importance of the niche in determining competitive interactions, assembly rules (Diamond 1975) and ecological filters (Keddy 1992). The niche can be described as the total range of conditions under which an individual organism lives (Hutchinson 1957). Species do not always occupy the full range of abiotic conditions that they are capable of tolerating and a species niche can be described as fundamental or realized. The fundamental niche of a species is defined as the total range of abiotic conditions out of n dimensional environmental space in which a species can survive in the absence of interspecific interactions (Grinnell 1917). The realized niche is the environmental space where a species is actually present and is a subset of the fundamental niche. The reduction in realized niche space is attributable to biotic interactions (competition, pathogens and symbionts) and resource-consumer dynamics which are influential at mostly local scales (Hutchinson 1957). A species niche, or its position within a population, community or ecosystem, may result from how its functional attributes are filtered by the both the biotic and abiotic environment (Chase and Leibold 2003, Cadotte, Carscadden et al. 2011). Central to this statement is the assumption that trait variation can explain differences in fitness along environmental gradients.

The importance of a species niche (determined by its functional attributes) in structuring a plant community is open to debate and theories of community assembly can be divided into those that emphasize the importance of the niche of co-occurring species (Hutchinson 1957, Wright 2002, Chase and Leibold 2003, Silvertown 2004) and those that rely on stochastic demographic processes (MacArthur and Wilson 1967, Hubbell 2001). Hubbell's Neutral Theory (2001) assumes that all species are of equal fitness and that the role of dispersal and stochastic demographic processes are more important than ecological trait differences in determining community dynamics. There is little doubt that stochastic processes influence community structure. However several studies have shown that stochastic processes cannot fully explain diversity. At community scales, neutral theory alone cannot explain many observed species distributions and functional traits are important in community assembly (McGill, Maurer et al. 2006, Kraft, Valencia et al. 2008), via their effects on recruitment, growth, reproduction and survival (Lavorel and Garnier 2002, Violle, Navas et al. 2007, Adler, Salguero-Gomez et al. 2014). What is less clear is whether the traits used to predict performance at community scales have ubiquitous relationships to environment over larger scales, with a recent global study indicating that this may not be the case (Paine, Amissah et al. 2015).

Ecology and Scale

Different patterns can emerge at different scales of study of an ecological system and patterns observed may be the result of multiple ecological processes operating at different scales (Levin 1992). Three important factors influencing a species niche are the dispersal ability of species which determines where in the world they can spread, environmental factors that are favourable to fitness which determine the broad limits of where a species can survive and also the biotic environment which can modify the limits determined by the former two (Guisan and Thuiller 2007). For example the distribution of biomes is controlled by climatic factors such as rainfall and temperature, precipitation and geology. However at smaller scales factors including soil characteristics, herbivory, fire and competition influence the structure of the vegetation and local biological interactions can decouple systems from the physical determinants of patterns. Another example occurs when at local scales most variation in litter decomposition rates is determined by physical properties of the litter and also decomposers. However at larger scales more of the variation is explained by climate (Meentemeyer 1984). Pattern can heavily depend upon scale of observation. Considerations of scale can therefore be critical in understanding the how ecological processes structure vegetation as the relative importance of these may change from community to global scale.

Ecological strategies

Central to trait-based ecology is the identification of axes of trait variation that describe ecological strategies. These are predicted to determine the response of species to environmental gradients and their effects on ecosystem processes. Much work to date has focused on trade-offs between traits that reflect differences in growth rate and resource use. Terrestrial plants are constrained in their resource capture and conservation and therefore exhibit consistent trade-offs amongst suites of correlated traits (Grime, Thompson et al. 1997, Reich, Walters et al. 1997, Diaz, Hodgson et al. 2004, Wright, Reich et al. 2004) The leaf economic spectrum describes trade-offs amongst traits that reflect differences in growth rate and longevity, arising from different strategies of resource acquisition and conservation (Wright, Reich et al. 2004). Trade-offs between these co-varying traits are expected to explain a species position along gradients of resource availability, stress and disturbance (Grime 1979). The leaf economic spectrum is just one axis of trait variation, and other ecologically important strategies have been identified. For example the leaf, height, seed spectrum reflects trade-offs in stature, structure and reproduction (Westoby 1998). Other axes of variation are less well studied, but potentially important in influencing vegetation dynamics. For example, water availability is a major factor associated with contrasting species distributions and plant species segregate along hydrological niches (Silvertown, Araya et al. 2015). In tropical forests, trade-offs have been observed between drought tolerance and light capture (Markesteijn, Poorter et al. 2011) and yet mechanisms of drought tolerance that may explain these patterns have received less attention than relationships between growth and resource availability, and have been studied at community but not larger scales.

Grass Phylogeny

The grass family consists of approximately 10,000 species globally in 645 genera (Fay 2007). Within the family there are 12 recognised sub-families. The two main early diverging clades are the BEP clade (consisting on the Bambusoideae, Erhartoideae and Pooideae), all temperate grasses with the C_3 photosynthetic type), and the PACMAD clade (consisting of the Panicoideae, Aristoideae, Chloridoideae, Micrairoideae, Arundinoideae and Danthonioideae), which include C_3 species and multiple independent origins of C_4 species (Edwards, Osborne et al. 2010). Morphological traits of grasses are known to be conserved within grass lineages (Liu, Edwards et al. 2012) and different C_4 grass lineages have different associations with gradients of fire frequency, temperature and grazing pressure in south Africa (Visser, Woodward et al. 2012). At global scales the species richness of different grass lineages separate along precipitation gradients. Species belonging to the Panicoideae (consisting of the Paniceae and Andropogoneae) are most species-rich in mesic habitats, whereas most Chloridoideae and Aristidoideae inhabit more arid regions (Hartley 1952). Taken together this indicates that both the traits and environmental niche of grass species can be phylogenetically conserved and that evolutionary history must therefore be considered when looking at trait environment relationships.

Grassy Biomes

Grasses are cosmopolitan in distribution and comprise the largest biome on Earth covering approximately 20% of terrestrial land surface and account for about 30% of global terrestrial net primary productivity (Scholes and Archer 1997). Grasses range from polar regions to the equator, from mountaintops to sea level, from deserts to wetlands, have evolved to tolerate disturbances including herbivory and fire and are represented on every continent including Antarctica. That they have evolved to tolerate such wide ranging conditions in areas if differing biogeographic history makes them ideally suited to studies of global ecological patterns. Grasslands are extremely important both economically and environmentally yet are less well studied than tropical forests. Grasslands are important carbon sinks, storing approximately 15% of the carbon on Earth (Grace, San Jose et al. 2006) and also harbor huge amounts of the Earth's biodiversity (Murphy, Andersen et al. 2016). They play an important role in global food security and provide a livelihood for many people around the world as not only are they a source of grazing for livestock but the grass family contains many important food crops including rice, millet, maize, wheat and sorghum. They are a valuable resource under threat from woody encroachment caused by CO_2 enrichment, from changes to precipitation regimes and alterations to patterns of fire and grazing.

Aims and Objectives

The aim of this thesis was to investigate the relationships between plant strategies and biotic and abiotic environments, and to test whether the predictions made by community ecological theory are more broadly applicable at continental and global scales. I use grasslands as a study system due to their wide ranging distribution in conjunction with their ease of growth to maturity (e.g. in comparison with trees), which makes them amenable to experimentation. Furthermore, grassland are in general dominated by less species than for example tropical forests making it easier to measure the number of species needed for a representative sample. In the following chapters I use comparative methods for testing the relationships between traits and environment whilst accounting for the role of evolutionary history in ecosystem assembly.

Chapter 2. THE GLOBAL DISTRIBUTION OF GRASS FUNCTIONAL TRAITS IN GRASSY BIOMES

I tested whether there is evidence of trade-offs between traits reflecting different strategies of resource acquisition in a sample of the globally dominant grass species. I then investigated whether trade-offs between leaf economic and size related traits in these species correlate with global gradients of climate and soil nutrients. Results show the relationship of some but not all traits to environmental gradients, reveal a large amount of local variation within the level of vegetation type, and emphasize the importance of evolutionary history in determining contemporary species distributions.

Chapter 3. GRASS FUNCTIONAL TRAITS ARE SORTED BY HERBIVORY AND FIRE AT THE CONTINENTAL SCALE

Disturbance is widely accepted as being an important factor in the structuring of plant communities. I tested for relationships between leaf economic, architectural and morphological traits, and gradients of fire and mammalian herbivory in grasses distributed across the African continent. Since much of the global variance in traits occurs within rather than between vegetation types (Chapter 2), I tested for associations between functional trait diversity within different vegetation types and the fire and grazing regime. Results reveal significant relationships between height and fire, between leaf economic traits and grazing, and identify functional groups based upon life history, habit and leaf chemical traits that are associated with different levels of grazing disturbance. However, none of the predictors could explain trait diversity.

Chapter 4. FUNCTIONAL TRAITS EXPLAIN SORTING OF SAVANNA GRASSES ALONG A GLOBAL RAINFALL GRADIENT

Species distributions are widely associated with moisture availability, which also has strong feedbacks to other ecosystem processes including fire regime and herbivore distribution. The mechanisms and traits that determine interspecific variation in the precipitation niche are however unresolved. I quantified the drought tolerance of grass species sampled along a global rainfall gradient. These results indicate that the spatial distribution of savanna grass species along rainfall gradients does not arise from a growth-survival trade-off. Instead, distributions are correlated with variation in canopy senescence under drought, which can be explained by variation in rates of stomatal closure and root traits.

Chapter 5. STOMATAL REGULATION EXPLAINS SENESCENCE UNDER DROUGHT FOR SOUTH AFRICAN SAVANNA GRASS SPECIES

I conducted another experiment investigating the relationships of stomatal conductance to senescence and the precipitation niche of African grass species. The experiment was conducted in South Africa under more natural conditions than plants experienced in the growth chamber study described in chapter 4. This work tested whether the relationships observed in the growth chamber were consistent with results found when plant physiological processes were also being regulated by natural fluctuations in light and temperature in an area where C_4 grasses naturally grow. The relationship observed between stomatal conductance and senescence were consistent with those observed in chapter four. However the same relationship was not observed between senescence and the precipitation niche of African grass species. This result emphasizes the importance of conducting studies under natural conditions as well as in controlled environments, and spatial scale is important when looking at trait environment relationships.

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CHAPTER TWO

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STATEMENT OF CONTRIBUTIONS

This chapter is in preparation as a manuscript. The initial ideas were developed by the candidate, Prof. Colin Osborne and Dr Gavin Thomas. The map which was used as the basis for species sampling was provided by Dr Caroline Lehmann. The phylogenetic tree was constructed by Dr Elisabeth Forrestel. All other data collection, data analyses and writing were carried out by the candidate. General editorial advice was provided by Prof. Colin Osborne, Dr Gavin Thomas, with comments from all the co-authors.

THE GLOBAL DISTRIBUTION OF FUNCTIONAL TRAITS WITHIN GRASSY BIOMES

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ABSTRACT

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. We made direct measurements on herbarium specimens to compile a global dataset of functional traits and the realised environmental niche for 279 of 841 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 distributions of traits in relation to gradients of soil properties and climate, while accounting for phylogenetic relatedness.

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), carbon-to-nitrogen ratio (C/N), specific leaf area (SLA) and leaf nitrogen content (LNC). Global-scale variation in leaf economic traits was primarily correlated with soil nutrients, while size related traits were associated with aridity. However, a large proportion of the trait variation occurred within different vegetation types, independent of large-scale environmental gradients.

Our work provides evidence among grasses for global relationships between leaf economic traits and soil fertility, and for an influence of aridity on traits related to plant size. However, 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, broadleaved, and needle-leaved trees; C_3 and C_4 grasses) have been widely used to group plants from geographically separate communities according to shared functional traits thought to convey competitive advantages in specific environments, regardless of species identity (Woodward, Lomas et al. 2004). However, there can be significant functional variation within plant functional types (Liu, Edwards et al. 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 et al. 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, Anderson 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 (Grime 1998), and a number of studies have supported this view (Garnier et al., 2016). Globally there are ~800 species of grass that characterise different 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 *et al.*, 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 (Grime, Thompson et al. 1997; Diaz, Hodgson et al. 2004; Wright, Reich et al. 2004). It has been proposed that trade-offs reflecting differences in the way plants acquire and allocate resources to the growth or conservation of tissues provide mechanisms that can determine distribution patterns across resource gradients (Herms and Mattson 1992; Fine, Miller et al. 2006).

In environments where resources are limited, dominant 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) (Grime, Thompson et al. 1997; Reich, Walters et al. 1997; Craine, Tilman et al. 2002; Westoby, Falster et al. 2002). These traits reflect the high cost of tissue loss to for example herbivory, for individuals where growth is resource limited (Coley, Bryant et al. 1985; Herms and 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 (Grime, Thompson et al. 1997; Reich, Walters et al. 1997; Craine, Tilman et al. 2002; Westoby, Falster et al. 2002) .

Functional traits that reflect different strategies of resource acquisition and allocation correlate strongly with resource availability at the community scale (Kraft and Ackerly 2010; Katabuchi, Kurokawa et al. 2012). Recent studies spanning environmental gradients have shown that similar sorting processes also drive economic trait distribution in predictable ways at a landscape scale (Asner, Martin et al. 2014; Fortunel, Paine et al. 2014). However, environmental trait distributions may not vary predictably across biomes and continents, and can be specific to areas of unique evolutionary history when considered at larger scales (Knapp, Smith et al. 2004; Lehmann, Anderson 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, Martin 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 using alternative methods suggest that ~40% of the Earth’s terrestrial surface is covered in grassy ecosystems (White *et al.*, 2000). These store large amounts of carbon, and support livelihoods and food security globally (Parr *et al.*, 2014). Grassy biomes are an ideal system for studying the global-scale relationships of functional traits with environment, as they cover 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.

METHODS

Species sampling

A global database of the species that characterise grassy biomes was compiled from regional maps of potential vegetation (Lehmann et al, unpublished). Within the map of Lehmann et al, grassy vegetation was defined as any vegetation unit in which grasses dominated the ground layer, and included grasslands, savannas and woodland. Within each grassy unit, the dominant grass species were extracted from the description and metadata associated with the original regional 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 unit from plot survey datasets (Lehmann et al, unpublished). In total, this map shows the distributions of 829 grassy vegetation types, characterised by 841 grasses identified to species level. This map was used as the basis for our species sampling.

To generate a manageable and representative data set for trait measurements we first randomly drew species without replacement from the global list. Random draws were weighted by the area over which each species is common, to ensure that globally important species were represented. A filter was then applied to remove any species that were not present, or not present in regions that they form a prevalent part of the vegetation, within the herbarium collection of the Royal Botanic Gardens, Kew. As many species as time would allow were measured giving a total of 279 species for study (Appendix S1 in Supporting Information).

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, Osborne et al. 2010) and reflects the return on previously acquired resources, since there is a trade-off between growing large, light-capturing, yet vulnerable leaves and producing strong, long-lived leaves (Westoby, Falster et al. 2002; Westoby and Wright 2006). Maximum leaf size is associated with different resource-use strategies (Ackerly, Knight et al. 2002). Plant height declines along gradients of decreasing moisture and/or nutrient availability (Wright, Reich et al. 2001), and is also thought to reflect different ecological strategies among species in relation to disturbance (Westoby 1998; Weiher, van der Werf et al. 1999). Taller plants compete more effectively for light (Tilman 1988; Cavender-Bares, Ackerly et al. 2004), 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 et al. 1983). Leaf tensile strength is an important form of defence against herbivory (Choong, Lucas et al. 1992) and is strongly correlated with leaf life span (Onoda, Westoby et al. 2011). Foliar C/N reflects levels of defensive compounds. A high level of foliar C/N often results from a high investment in carbon-based compounds that contribute to physical defence (e.g., cell-wall compounds such as lignin or chemical defence).

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 S2 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 S3 in Supporting Information). LNC is typically measured using dried leaf material and can be estimated directly from dried herbarium samples.

Trait measurements

Three replicates per species were measured for the following traits. Herbarium specimens were only sampled from areas where each species formed a dominant part of the vegetation. Herbarium sheets that were sampled were also selected, where possible, to represent the full 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 and C/N:* 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). Leaf concentrations of carbon and nitrogen (LNC) were measured and used to calculate the C/N. *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).

Values for maximum culm height, leaf length and leaf width were established from GrassBase, the Kew taxonomic database (Clayton et al. 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 2006) using the packages 'raster' (Hijmans 2015) and 'sp' (Bivand 2006). A global map of the total topsoil exchangeable bases was obtained from the Harmonized World Soils Database (IIASA 2008), and used to calculate the mean soil pH, percentage topsoil sand content (an indicator of drainage), 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 et al. 2005) via the r package ‘raster (Hijmans 2015)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 the seasonality of precipitation. 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 seasonality (Table 1).

	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

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).

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 et al. 2012 were employed to insert taxa for which there was no genetic data available using the pastis package in R (Thomas, Hartmann 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.

Statistical analyses

We first investigated the relationships among traits using a PCA to identify the main axes of variation between SLA, LTS, LNC, C/N, 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), Wright et al (2004), and Diaz et al (2016) 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 by regressing each trait against soil fertility, soil pH, soil % sand and the first six PC axes of the climatic variables. To evaluate whether traits differed systematically between continents, we fitted continent as a factor in PGLS models. The model residuals were checked for normality and logarithmically transformed where necessary. PGLS analyses were performed using the R package Caper (Orme et al., 2013).

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 (Pagel 1999). 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.

We performed variance partitioning using the R package nlme in R (Core 2016) to assess how much of the trait variation occurred within versus between sites, across 829 sites, with site being each of the grassy vegetation types defined by Lehmann et al (unpublished).

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 highest values of C/N and lowest LNC occurred across areas of the tropics (Fig. 1a-b), areas characterised by very low soil nutrients, low pH, high rainfall and consistently high temperatures. The lowest C/N and highest LNC occurred across the Eurasian Steppe (Fig. 1a-b), 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 (Fig. 1c). The toughest-leaved plants were in areas of Australia and the Eurasian Steppe (Fig. 1d), where SLA was also the lowest (Fig. 1c). The tallest and largest-leaved plants were in areas of the tropics, but particularly tropical Africa (Fig.1e-g).

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 Panicoideae being taller ($P<0.001$) (Fig. 2; Appendix S4 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 S4). Significant differences in LNC were also observed between grasses from Pooideae and Panicoideae clades, with Pooideae grasses having the highest LNC (Fig. 2; Appendix S4). Smaller lineages also contributed to the phylogenetic signal in all traits (Fig. 2; Appendix S4).

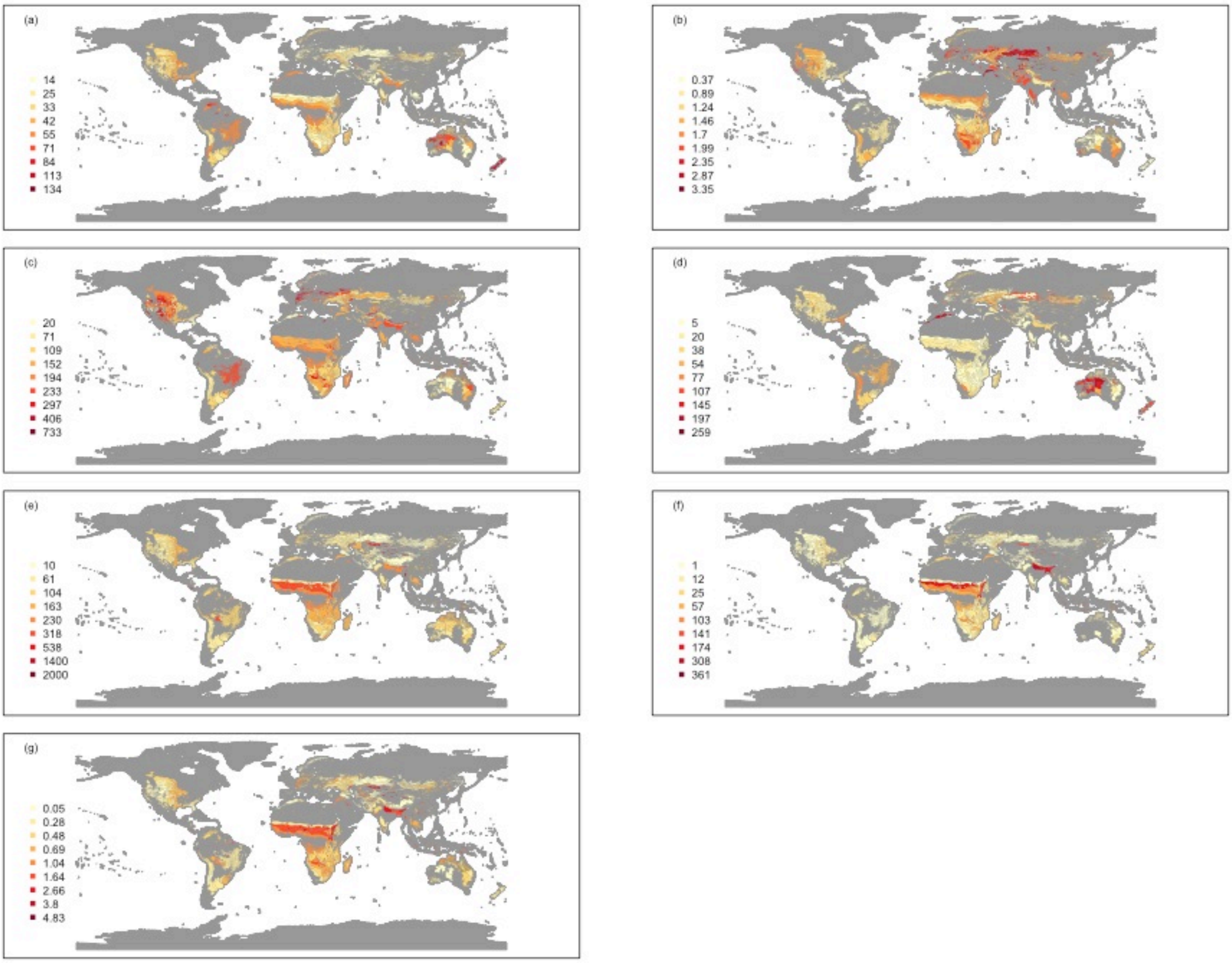

Figure 1. Global distributions of functional traits. Traits are: (a) C/N ratio, (b) leaf nitrogen content (%), (c) specific leaf area (cm^2/g), (d) leaf tensile strength (MPa), (e) maximum culm height (cm), (f) maximum leaf width (cm), and (g) maximum leaf area (cm^2). Mapping is based on the mean trait values for dominant species in each of the grassy vegetation types determined by Lehmann et al, (unpublished). Breaks in the colour ramp were set using the Jenks algorithm which seeks to reduce the variance within classes and maximize the variance between classes.

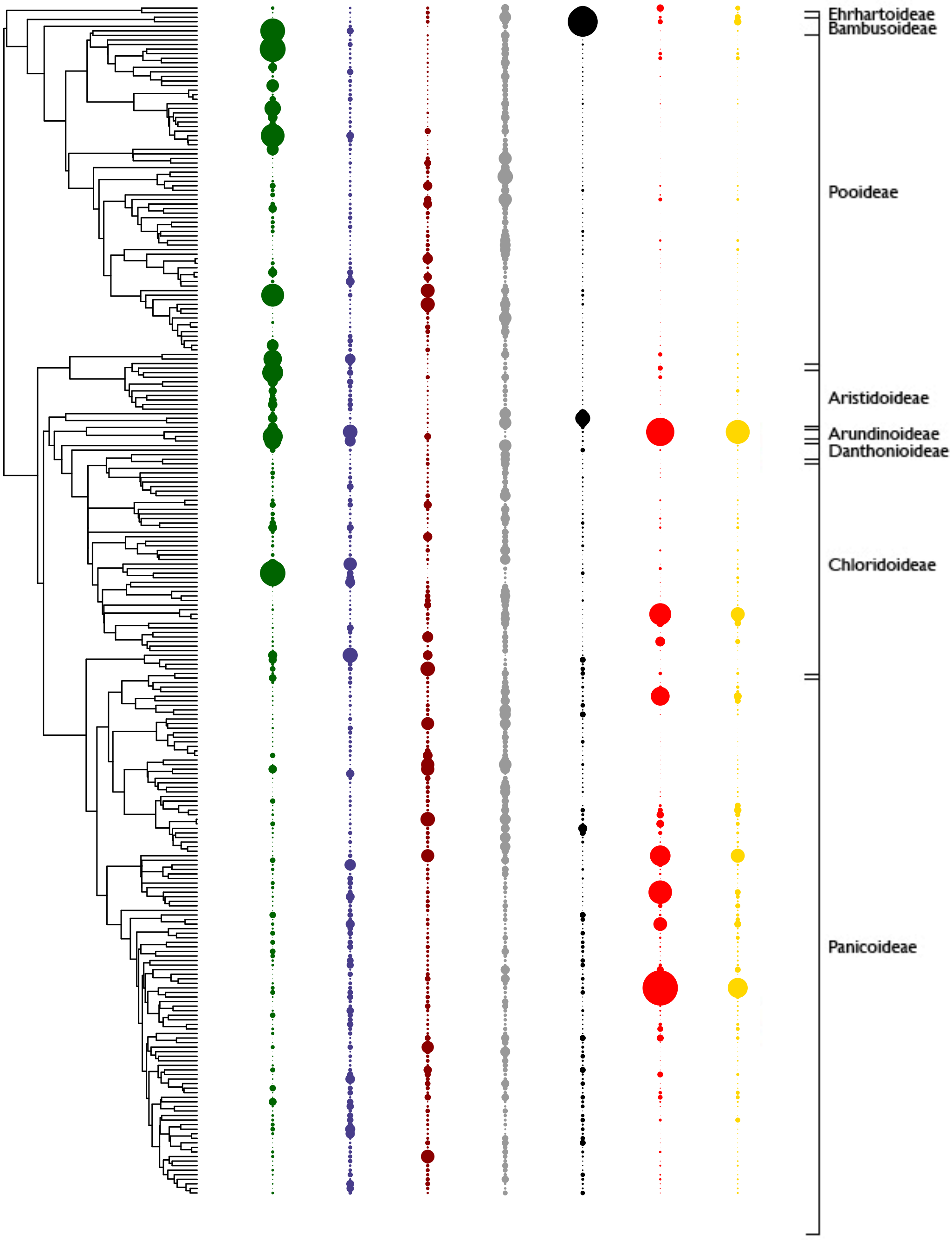

Figure 2. Functional trait values mapped across the phylogenetic tree. From left to right, traits are: leaf tensile strength, LTS (green), C/N ratio (blue), SLA (burgundy), % leaf nitrogen content, LNC (grey), maximum culm height (black), maximum leaf area (orange) and maximum leaf width (yellow) mapped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

Trait	λ	P (λ = 0)
LTS	0.77	***
N	0.59	***
C/N	0.57	***
SLA	0.14	*
Height	0.96	***
Leaf Area	0.24	n.s
Leaf Width	0.80	***

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 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, low C/N ratio and high 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 5a in Supporting Information). The loadings of traits on each axis are reported in Appendix 5b (Supporting Information).

Leaf economic traits all showed a statistically significant association with each other in the PGLS analysis. The SLA and LNC were positively correlated, as were C/N and LTS (Fig. 4; Appendix S6 Supplementary Information). In contrast, SLA and LNC were negatively correlated with both C/N and LTS (Fig. 4; Appendix S6). There was also a strong allometric scaling among size-related traits, which all showed positive relationships (Fig. 5; Appendix S6). A weaker relationship was observed between leaf width and C/N, SLA, LNC and LTS, and between LTS and maximum culm height (Appendix S6).

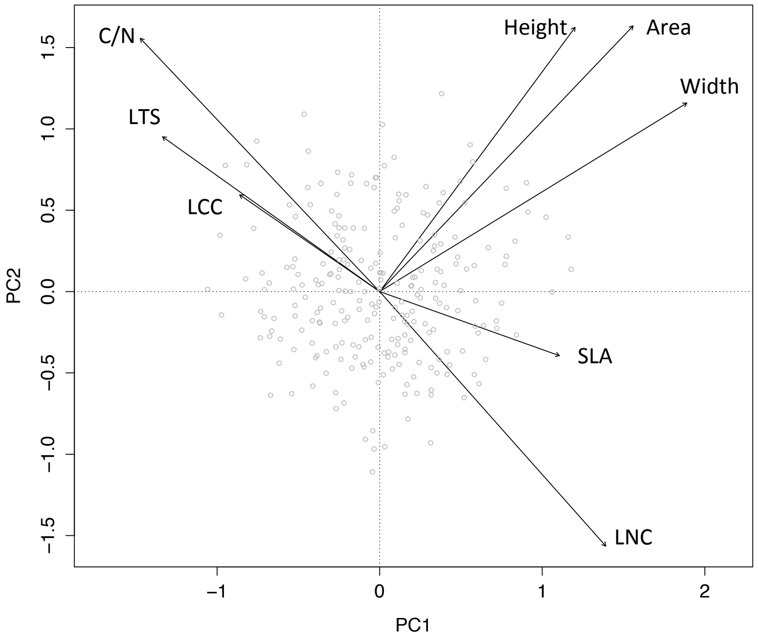

Figure 3. Principal components analysis (PCA) of traits, including specific leaf area (SLA), leaf carbon content (LCC), leaf tensile strength (LTS), carbon nitrogen ratio (C/N), leaf nitrogen content (LNC), maximum culm height, maximum leaf area and maximum leaf width. Orthogonal axes of trait variation are identified involving leaf economic traits and traits relating to size. Arrows represent the direction of increase of the trait values. All of the trait values were log transformed prior to analyses.

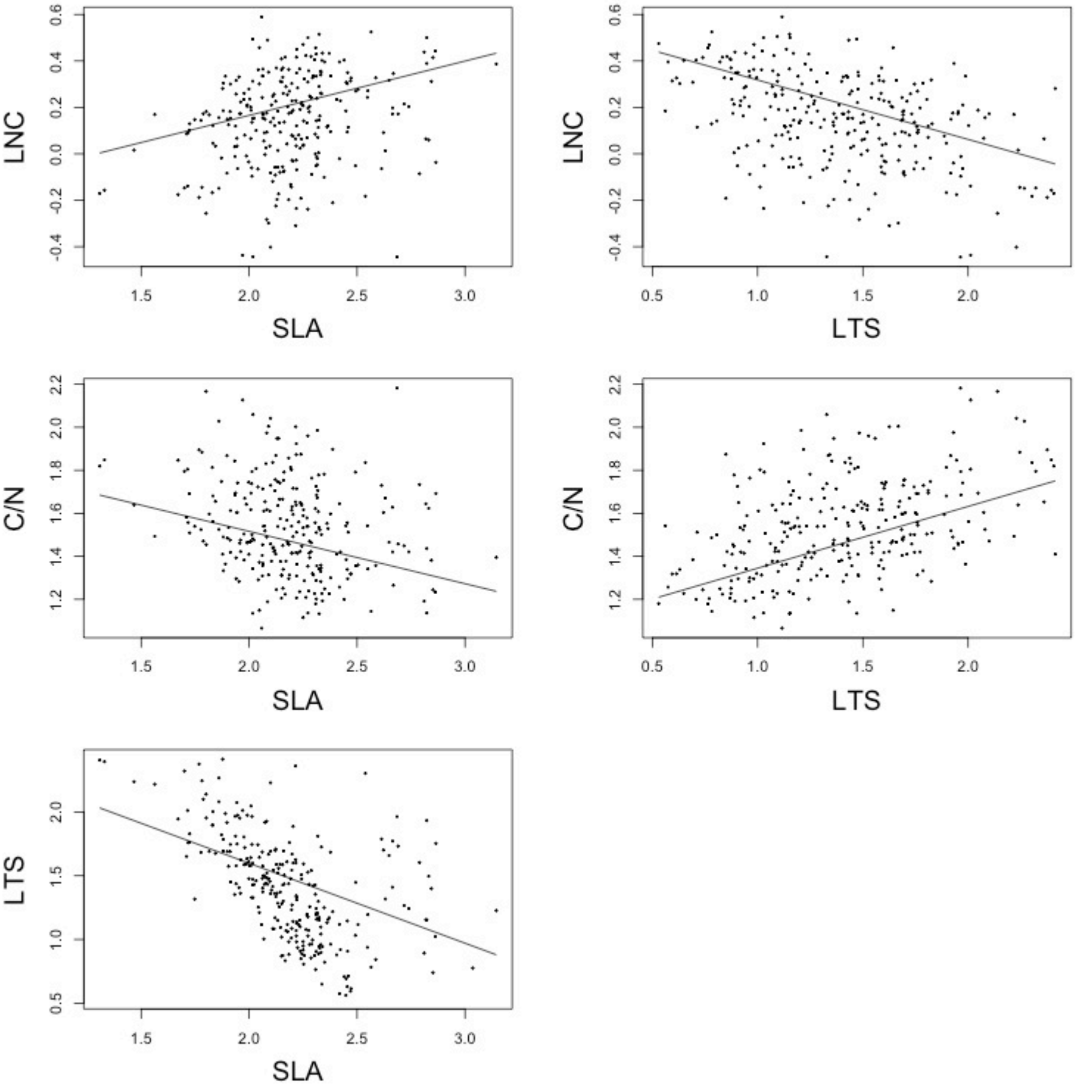

Figure 4. Relationships between pairwise combinations of the leaf economic traits, specific leaf area (SLA), leaf tensile strength (LTS), leaf nitrogen content (LNC) and C/N (C/N ratio). Regression lines result from PGLS models of pairwise traits. All trait values are logarithmically transformed, with units as in Figure 1.

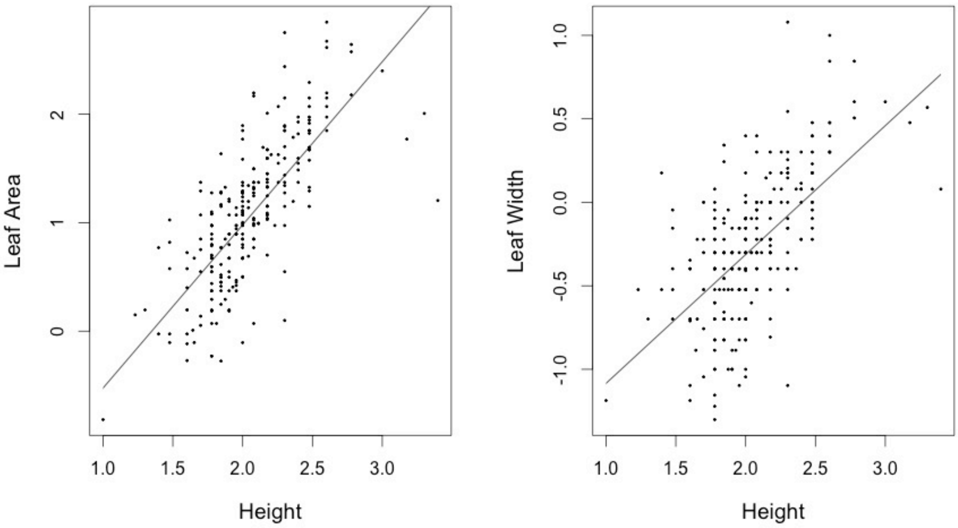

Figure 5. Relationship between pairwise combinations of the size traits: maximum leaf area, maximum culm height and maximum leaf width. Regression lines result from PGLS models of pairwise traits. All trait values are logarithmically transformed, with units as in Figure 1.

Traits and environment

ANCOVA revealed that, with the exception of the relationship between precipitation, LNC and C/N, there was no significant difference in the slopes of the pgls model fits for the different continents (Appendix 7 in Supporting Information). The interaction was therefore dropped from all subsequent analyses. The leaf economic traits that showed strongly significant associations with climate and soil were LNC ($r^2 = 0.20$) and C/N ($r^2 = 0.18$) (Table 3). Soil nutrients made a significant contribution to the explanatory power of the model ($P<0.001$) for both LNC and C/N, as did PC axes 2 (dryness and diurnal temperature) and 5 (temperature in combination with precipitation) ($P<0.01$ C/N) and PC 4 (temperature and isothermality) for LNC only ($P<0.05$) (Table. 3). Some of the variation in LTS and SLA was also explained by environmental gradients ($r^2 = 0.05$ and 0.03 respectively), with soil nutrients significantly contributing to the explanatory power of the model for LTS. Soil pH and % sand were significant predictors of SLA (Table 3). Size-related traits showed significant associations with the environment, maximum culm height ($r^2 = 0.05$), maximum leaf width ($r^2 = 0.08$) and maximum leaf area ($r^2 =0.06$), with PC2 (dryness and diurnal temperature) being a significant predictor of the variation in height and leaf width (Table 3). Leaf area and leaf width were also 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 ($\lambda = 0.64$), C/N ($\lambda = 0.65$), 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 sites, with site being each of the grassy vegetation types defined by Lehmann et al (unpublished). This showed that 60% of variation in LTS occurred within rather than between sites, and a large amount of within-site variation was also evident for SLA (95%), LNC (64%), C/N (56%), maximum culm height (55%), maximum leaf area (83%) and maximum leaf width (81%), suggesting that global- and regional-scale changes in environment are subsidiary to drivers of variation at the landscape and habitat scales.

	C/N			N			SLA			LTS		
	Slope	S.E	P	Slope	S.E	P	Slope	S.E	P	Slope	S.E	P
Soil nutrients	-0.03	0.01	***	0.02	0.01	***	0.02	0.01	**	-0.03	0.01	*
Soil pH	0.06	0.04	ns	-0.07	0.03	*	-0.13	0.05	**	0.04	0.07	ns
% Sand	0.00	0.00	ns	0.00	0.00		0.01	0.00	**	-0.01	0.00	ns
PC1	0.00	0.01	ns	0.00	0.01	ns	0.00	0.01	ns	0.01	0.01	ns
PC2	-0.02	0.01	**	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	-0.01	0.01	ns	0.00	0.02	ns
PC4	-0.02	0.01	ns	0.02	0.01	*	0.01	0.02	ns	-0.04	0.02	ns
PC5	0.03	0.01	**	-0.03	0.01	**	0.02	0.02	ns	-0.02	0.03	ns
PC6	0.00	0.02	ns	0.00	0.01	ns	0.00	0.02	ns	0.03	0.03	ns
λ	0.65			0.64			0.42			0.60		
r^2	0.18			0.20			0.03			0.05		

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

		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		
r^2	0.05			0.08			0.06		

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

DISCUSSION

Using a global comparative analysis of traits from around a third of the globally dominant grass species, we demonstrate that functional traits reflecting trade-offs in the acquisition and allocation of resources to growth and defence are significantly correlated with soil nutrients across the world's grassy biomes. We show that climate exerts a modest influence on some, but not all traits. However overall, global gradients in the abiotic environment explain a relatively small amount of trait variation across grassy biomes. Instead our analyses reveal large amounts of trait variation at smaller scales and strong phylogenetic patterns in the distribution of traits.

Trait relationships

We wanted to establish how leaf economics 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 (Diaz et al. 2016, Grime 1977). This finding provides 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, Bryant et al. 1985; Herms and Mattson 1992; Reich, Walters et al. 1997; Westoby, Falster et al. 2002; Diaz, Hodgson et al. 2004). An orthogonal axis of variation was identified relating to size and this is also consistent with previous studies (Westoby 1998; Diaz, Hodgson et al. 2004).

Leaf economic traits and environment

Next 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, Anderson et al. 2014). There was a marginally significant difference between continents in the responses of LNC and C/N to precipitation. However, for all other traits and environmental variables this was non-significant, showing that relationships between traits and environment are largely predictable 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, LTS and C/N) exhibited strong phylogenetic signal in both the individual traits and the residuals of the models. This finding is consistent with previous work showing phylogenetic signal in both the traits and habitat associations of grasses (Liu, Edwards et al. 2012; Visser, Woodward et al. 2012).

All economic traits were associated with soil fertility. However, LNC and C/N were also correlated with climate, with high values of LNC and the corresponding low values C/N found in dry climate regions with high diurnal temperature range – i.e. semi-arid or desert regions. 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, Williams et al. 1998; Wright, Reich et al. 2001). Arid regions also often coincide with areas of high soil fertility, which exerted a stronger effect on the variation in LNC and C/N 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 et al. 2013). Furthermore, our results show that both LNC and C/N ratio are 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 also driven by soil properties but not by climate. Soil fertility has previously been linked to toughness in leaves at smaller scales (Read, Sanson et al. 2005), which is consistent with theory that predicts better defended leaves in resource-limited habitats (Coley, Bryant et al. 1985). However, to the best of our knowledge the relationship of leaf toughness and soil characteristics over broader scales has not previously been reported. Toughening of the leaves caused by lignin production is commonly observed in plants from arid habitats (Read, Sanson et al. 2006), and it was therefore surprising that the PC axis describing precipitation did not have a significant effect upon LTS at a global scale. 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, Westoby et al. 2011). Our results provide new evidence that, at a global scale, soil nutrients are a more important predictor of LTS than climate.

SLA showed the weakest relationship of all the leaf economic traits to environment and was explained in part by a combination of all measured soil properties. Like LNC, SLA was positively correlated with soil nutrients, showing that faster growing, nutrient-demanding plants are found globally in areas of higher fertility. Soil pH and sand content were significant predictors of SLA but did not explain variation in any other traits, showing that 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 within-site variation. Variance partitioning showed that 95% of variance in SLA, 64% of variance in LNC, 65% of C/N and 40% of variance in LTS, occurred within-site. A large part of the variance in these traits therefore occurs at finer scales (landscape and habitat patch), a pattern also observed in other studies (Wright, Reich et al. 2004; Freschet, Cornelissen et al. 2010). Unexplained within-site variation 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 et al. 2011). Our data may therefore capture broad scale patterns but underestimate fine scale relationships between traits and soils.

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 correlated with climate, but not soil. Leaves perform several functions including light capture, water transport and defence, and 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, Scoffoni 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 was a significant predictor of 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. Grazing and aridity select for the same attributes (Coughenour 1985; Forrestel, Donoghue et al. 2015) and it is widely accepted that grasses of different stature share parallel responses to aridity and grazing. Tall grasses decrease in abundance following grazing and are associated with mesic habitats, while short grasses increase in abundance with increased grazing and aridity (De Bello, Leps et al. 2005; Diaz, Lavorel et al. 2007). 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 sites), suggesting that unexplained variation is driven by smaller scale predictors than climate. Differences in height imply a trade-off between competitive ability to capture light and tolerance to defoliation. Herbivory may therefore promote co-existence of grasses of varying height and differing tolerance to aridity following defoliation (Anderson, Kumordzi et al. 2013).

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. Traits linked to resource economics are correlated with global gradients in soil nutrients, whereas size-related traits are weakly correlated with climate. However, correlated traits do not necessarily share the same response to environment. Our global-scale results are consistent with theory formulated at the community scale about trade-offs in the allocation of resources to growth and defence. However, 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. Furthermore, 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.

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ACKNOWLEDGMENTS

This research was funded by Project Sunshine (The University of Sheffield). We thank David Simpson, Maria Vorontsova and Martin Xanthos at the Royal Botanic Gardens Kew for providing specimens.

APPENDICES

Appendix S1 List of all species included in the study.

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.

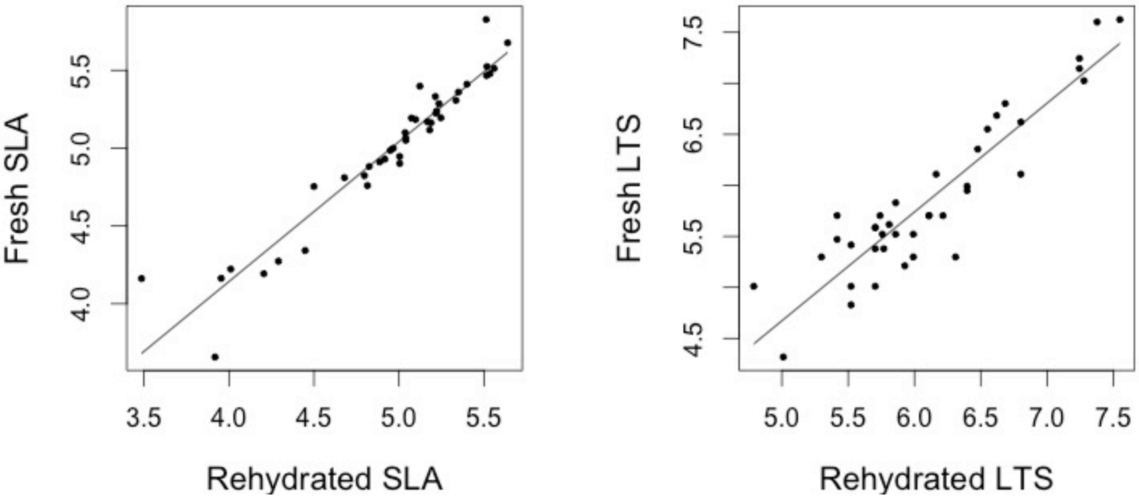
Tragus racemosus	(L.) All.
Triodia basedowii	E. Pritzel
Triodia longiceps	J. M. Black
Triodia pungens	R. Br.
Triodia wiseana	C. A. Gardner
Tristachya leiostachya	Nees
Urochloa mosambicensis	(Hack.) Dandy
Vossia cuspidata	(Roxb.) Griff.

Appendix S2 SUPPLEMENTARY METHODS

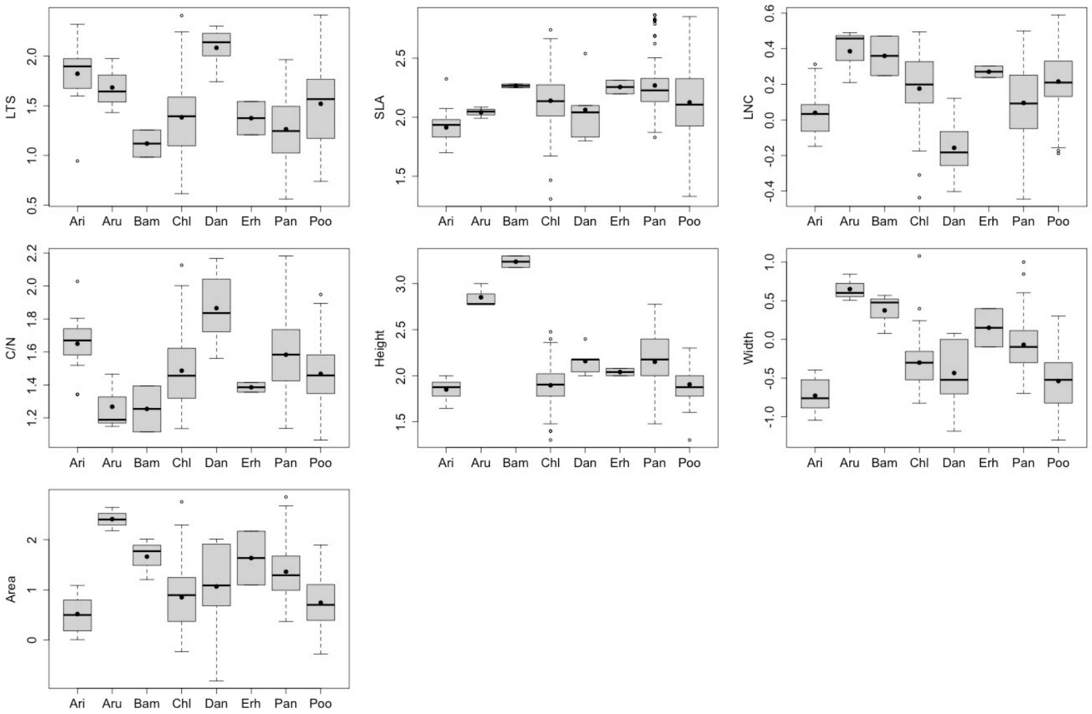
Measuring traits from herbarium specimens

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 S3 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 S4 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 5a 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), carbon to nitrogen ratio (C/N), maximum culm height, maximum leaf area and maximum leaf width.

	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8
Standard deviation	1.67	1.54	1.06	0.91	0.70	0.58	0.24	0.18
Proportion of Variance	0.35	0.29	0.14	0.10	0.06	0.04	0.01	0.00
Cumulative Proportion	0.35	0.64	0.78	0.89	0.95	0.99	1.00	1.00

Appendix 5b 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), leaf carbon content (LCC), carbon to nitrogen ratio (C/N), maximum culm height, maximum leaf area and maximum leaf width**.**

	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8
Height	0.31	0.45	0.15	-0.10	0.18	-0.79	0.13	-0.01
Leaf Area	0.40	0.45	0.07	-0.07	-0.05	0.31	-0.72	0.08
Leaf Width	0.48	0.32	-0.01	0.01	-0.19	0.44	0.65	-0.10
C/N	-0.38	0.43	-0.27	0.27	-0.09	-0.01	-0.07	-0.72
LCC	-0.22	0.17	-0.42	-0.84	-0.20	-0.01	0.04	0.08
LNC	0.36	-0.44	0.23	-0.39	0.08	-0.01	-0.11	-0.68
SLA	0.28	-0.11	-0.70	0.10	0.64	0.05	-0.01	0.01
LTS	-0.34	0.26	0.43	-0.23	0.68	0.31	0.12	-0.01

Appendix S6 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	**
C/N~LTS	0.287	0.030	0.35	0.26	***
C/N~SLA	-0.246	0.050	0.56	0.08	***
C/N~Leaf Width	-0.128	0.035	0.44	0.05	***
C/N~Height	0.012	0.048	0.52	0	ns
C/N~Leaf Area	-0.128	0.035	0.51	0	ns
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 S7 ANCOVA 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

		CN			N			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	DF	F value	P
Soil TEB	4	2.03	ns	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	0.59	ns	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	0.97	ns	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	0.94	ns	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	1.30	ns	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	0.56	ns	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	2.30	ns	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	3.38	*	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	1.96	*	4	1.74	ns	4	1.58	ns	4	0.80	ns	4	0.43	ns	4	2.04	ns	4	2.02	ns

CHAPTER THREE

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Grass functional traits are sorted by herbivory and fire at the continental scale

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STATEMENT OF CONTRIBUTIONS

This chapter is in preparation as a manuscript. The initial idea was developed by the candidate. Data collection was by the candidate but with a map showing the distribution of mammal species provided by Dr Gareth Hempson, trait data from Grassbase was provided by Maria Varontsova who also assisted with providing grass specimens for the candidate to measure. All data analyses and writing were carried out by the candidate. General editorial advice was provided by Prof. Colin Osborne and Dr Gavin Thomas.

GRASS FUNCTIONAL TRAITS ARE SORTED BY HERBIVORY AND FIRE AT THE CONTINENTAL SCALE

Authors: Emma Jardine, Gavin Thomas, Gareth Hempson, Maria Vorontsova,
Colin P. Osborne

ABSTRACT

Fire and herbivory are disturbances that can influence the distribution of species via the consumption of vegetation. Whether a system is controlled by fire or herbivory is strongly linked to aspects of soil nutrients and precipitation. How vegetation responds to fire and herbivory is expected to be explained by species functional traits. The relationships between fire, herbivory and plant functional traits have been studied at the community scale but it is not known if the observed relationships apply over larger scales. The lack of data documenting mammal distributions and abundances has previously hindered work investigating relationships of plant traits to grazing. We make use of a recently published map showing the historic distribution and abundance of mammals across sub-Saharan Africa and satellite data mapping fire intensity across the continent to test the relationships between these disturbances and plant functional traits. We also ask if species can be grouped into distinct functional types based upon their traits, and if these groups exhibit diverging responses to fire and grazing. Thirdly, we ask what are the relationships between fire, herbivory and community functional diversity?

We show that gradients of fire and mean annual precipitation are associated with leaf nitrogen content (LNC), C/N ratio and height, such that a low LNC, high C/N ratio and tall stature are found in areas of high fire and high precipitation. Leaf economic traits, but not size, are more strongly associated with gradients of grazing and soil nutrients than with fire, such that a high specific leaf area (SLA), high LNC, low C/N ratio and low leaf tensile strength (LTS) are associated with high soil nutrients, small mammals and low grazing intensity. Species form three groups based on their traits; these correspond to caespitose species, mat-forming grasses and annual species. The annual species are found in areas of lower soil nutrients and are grazed by larger mammals in comparison with caespitose grasses. We were unable to explain continental patterns of trait diversity using abiotic or biotic factors.

In conclusion, a considerable amount of trait variation exists within savanna grasses across the African continent, in large part driven by gradients of rainfall, fire, grazing and soil nutrients. Species which are grouped according to their functional, morphological and life history traits share common responses to grazing and soil nutrients. Variation in height is driven by gradients of fire and MAP. The distribution of traits that reflect different strategies of resource acquisition and use is driven more by soil fertility than grazing per se. Using this information in models of savanna functioning and distributions could improve predictions of how vegetation is likely to respond to changes in grazing and fire regimes.

Keywords

Fire, herbivory, functional traits, functional diversity, functional types

INTRODUCTION

Plant communities are partly structured by disturbances (Milchunas, Sala et al. 1988; du Toit 2003), including fire (Govender, Trollope et al. 2006) and herbivory (Carson and Root 2000; van Langevelde, van de Vijver et al. 2003). Although the outcomes of fire and herbivory are ecologically similar, with both acting as consumers of vegetation, they differ in their requirements, distribution and impacts upon community structure. Whether a system is controlled by fire or herbivory can depend on the interactions of these disturbances with climate and soil nutrients (Bond and Keeley 2005) and both can be highly selective in their consumption (Bond and Keeley 2005; Archibald and Hempson 2016). Interactions between climate, soil and disturbance can therefore filter the species in a community, resulting in convergence of functional traits (Keddy 1992; Keeley and Zedler 1998). This study investigates the relationships between climate, soil, herbivores, and fire and how these consumers influence the functional distribution and diversity of the dominant grass species across the African continent.

It is well known that fire can strongly influence plant community composition (Pausas 1999; Franklin, Syphard et al. 2005; Kahmen and Poschlod 2008), and across Africa fire is most frequent in areas of high precipitation with a seasonal pattern of rainfall. Ecosystems that are controlled by fire rely on an interaction between productivity, soil nutrients and the seasonality of rainfall to create an abundant yet dry, flammable fuel load (Krawchuk and Moritz 2011). Fire can vary in selectivity depending upon leaf moisture content, which is an important component of flammability and is strongly associated with the ignition of leaf material (Simpson, Ripley et al. 2016). However, fire can be very non-selective in the vegetation it consumes when there is a dry and continuously connected fuel bed, which can result in a highly homogenous set of traits when compared to trait diversity in grazed regimes (Collins and Smith 2006). Through the removal of plant biomass, fire creates a high light and low nitrogen environment (Knapp and Seastedt 1986; Vitousek and Howarth 1991), and frequent burning at the community scale selects for grasses with a high specific leaf area (SLA), low LNC and high leaf C/N ratio (Forrestel, Donoghue et al. 2014). These are all traits that facilitate rapid post fire recovery and a competitive strategy of resource acquisition and use.

Fire and herbivory differ in their distributions, with herbivory being the more dominant control in areas of lower rainfall with higher soil nutrients when compared to fire (Archibald and Hempson 2016). Mammalian herbivores can strongly influence community structure (Collins, Knapp et al. 1998; Olff and Ritchie 1998; Knapp, Blair et al. 1999). For example, repeated grazing by large mammals can lead to the creation of grazing lawns in areas of high nutrients (McNaughton 1985; Hempson, Archibald et al. 2015). Grazing lawns are characterized by predominantly short, mat-forming species, which spread via elongated rhizomes or stolons (Hempson, Archibald et al. 2015). At the global scale, grazing leads to an increase in short plants over tall plants, prostrate over erect plants, annual over perennial, and stoloniferous species (Diaz, Lavorel et al. 2007). Syndromes of leaf traits shared between plant species that exhibit a similar response to grazing have also been identified at the community scale, with plants that increase with grazing release exhibiting larger and lower density of stomata, higher stomatal pore index and lower LNC (Forrestel, Donoghue et al. 2015).

Community ecological theory predicts an interaction between productivity and herbivory based upon the allocation of resources to growth and defence (Coley 1988; Herms and Mattson 1992). Inherently linked to the distribution of plant traits that reflect trade-offs in growth and defence are the attributes of grazers, their selectivity, their body size and total herbivore biomass. The distribution of grazers of differing body size is constrained by forage quality, such that large herbivores can tolerate lower plant nutrient content but require larger quantities of forage than small herbivores (Illius and Gordon 1992). Large animals are therefore expected to be found in the most productive environments, irrespective of forage quality. Areas of low moisture but high soil nutrients support smaller herbivores, which require smaller amounts of higher quality forage, but these areas are not productive enough to support large species (Olff, Ritchie et al. 2002). The total biomass of mammals that can be sustained within a community is therefore also positively associated with productivity (East 1984).

The contrasting distributions of herbivores of differing body size and abundance are predicted to have contrasting outcomes for plant communities explained by trade-offs between allocation of resources to growth and defence. Growth-defence trade-offs provide a mechanism through which herbivores can determine distribution patterns across resource gradients (Fine, Miller et al. 2006). Selective grazing in unproductive environments by small mammals is predicted to favor unpalatable species with a high leaf tensile strength, C/N ratio and low LNC (Coley, Bryant et al. 1985; Reich, Walters et al. 1997; Craine, Tilman et al. 2002; Westoby, Falster et al. 2002). In these areas, environmental resources limit the replacement of photosynthetic tissues lost to herbivory, and an avoidance strategy of high C/N ratio and high leaf tensile strength is likely to promote dominance. On the other hand, non-selective grazing by the large mammals found in productive habitats is expected to favor competitive species with tall stature and high SLA, which can rapidly acquire environmental resources and allocate them to growth following defoliation (Westoby 1999).

These relationships between traits and the abiotic and biotic environment have been widely observed at a range of scales, however these relationships are often weak and may be more complex than models based entirely upon resource availability would predict. At the global scale, some traits that reflect differing strategies of resource-acquisition and resource-use show weak relationships to resource availability. For example, at the global scale the traits of co-existing species in grassy vegetation vary more within defined vegetation types than between them (chapter 2; Jardine et al., unpublished). Herbivores do not always exert consistent effects on trait filtering and can promote co-existence, however, studies of the relationships between species diversity and grazing have yielded mixed results, with some studies reporting an increase in species diversity with herbivory (Belsky 1992; Collins, Knapp et al. 1998), others reporting a negative association (Milchunas, Lauenroth et al. 1998; Howe, Brown et al. 2002), and some showing no relationship at all (Stohlgren, Schell et al.

1999; Adler, Milchunas et al. 2005). The effects of mammalian herbivores on plant species diversity probably depend upon the interaction of productivity, body size and abundance, and are likely to be positive when productivity is high and large mammals are present (Olff and Ritchie 1998). Large grazing mammals can increase species diversity in productive habitats by impacting upon the most competitive, dominant plant species, releasing less competitive species from competition for light (Huisman and Olff 1998), and by opening up gaps for recruitment (Knapp, Blair et al. 1999; Eskelinen and Virtanen 2005).

Understanding the relationships of these disturbances to the functional traits of species can add mechanistic understanding to observed patterns of species distribution and community assembly. In this paper we test the relationships between functional traits and functional diversity with gradients of fire and grazing across the African continent. Africa has been less impacted than other regions by mega-faunal extinctions and has a high prevalence of savanna grass fires. We ask the following questions. First, what are the relationships of plant functional traits to gradients of fire and herbivory across the African continent? We hypothesise that species from areas with frequent fire display traits associated with high productivity, rapid post fire recovery and a large fuel load (Table 3.1). We also expect that traits will correlate with gradients of grazer body size and grazing intensity, with large mammals and high grazer abundance being associated with high SLA, high LNC and low C/N. These are all traits that reflect rapid recovery from defoliation. Selective herbivory by small mammals will promote dominance of traits that reflect high levels of defence in unproductive environments, i.e. high C/N ratio, low LNC, high LTS and low SLA (Table 3.1). Secondly, we ask if species can be grouped into functional types based upon their traits, and if these groups are associated with specific environments, hypothesizing that species will form functional groups that exhibit diverging responses to fire and grazing. Thirdly, we ask what are the relationships between fire, herbivory and community functional diversity? We hypothesise that areas that burn the most will have low functional diversity. We also predict that mammals will influence species diversity, with effects that depend upon the interactions between soil nutrients, precipitation and mammal body size (Olff and Ritchie 1998)

Environment	Primary Control	Effect on Vegetation
High rainfall/low soil nutrients	Fire	Low trait diversity. Only tall, productive species dominate with high SLA, high C/N, high LTS and low LNC
Intermediate rainfall/ soil nutrients	Large mammals present (>1,000kg), high grazer biomass	The presence of large mammals is associated with an increase in mat-forming stoloniferous species or species with elongated rhizomes, traits that are associated with the formation of grazing lawns. High plant leaf trait and mammal size diversity is expected when large mammals are present, which release plants from competition allowing a wide range of leaf traits to co-exist and for taller and shorter species to co-exist.
Low rainfall/high soil nutrients	Small mammals (<1,000kg), low grazer biomass	Low trait diversity as only short unpalatable species (low SLA, low LNC and high C : N, high LTS) become dominant

Table 1. The hypothesized relationships between gradients of soil nutrients, grazer-body size, grazer abundance, mean annual precipitation (Olff and Ritchie 1998) and also fire and the functional traits of African grass species

METHODS

We use a comparative approach to test for correlations among plant traits and gradients of fire and grazing intensity, using species mean trait values and mean environmental data across the range of each plant species. We use a cluster analysis to test whether species can be assigned to distinct groups, whose members share similar responses to grazing and fire based on their functional attributes. We then use a functional diversity metric to investigate how the functional diversity of different vegetation types relates to the biotic and abiotic environment for these areas.

Grass species selection and occurrence

Dominant grass species across the African continent were identified from White (1983). Species were included in the study that had geo-referenced location records in GBIF and were represented by specimens in the Herbarium of the Royal Botanic Gardens, Kew. The sample included a total of 100 of the 192 dominant African grass species (Appendix 1).

All available geo-referenced occurences were extracted from the Biodiversity Information Facility web portal (http://www.gbif.org/) via the R statistical computing package rgbif (Chamberlain, Ram et al. 2015). Species names followed the taxonomy of the Kew grass synonymy database (Clayton, Vorontsova et al. 2006 onwards). In order to represent the small scales at which grazing and fire regimes can change, we discarded any longitude and latitude data that was not accurate to more than two decimal places. Finally the country of collection for each record was checked against the country that the co-ordinate reference fell in and, if these differed, the record was discarded. The point locations of records for each species were used to extract the associated environmental data from gridded datasets as described below.

Environmental data

All mapping of environmental variables was implemented in R (Core Development Team R

2006) using the package 'raster' (Hijmans 2015) and 'sp' (Bivand 2006). We used fire radiative power (FRP) as a proxy for fire intensity (Archibald, Lehmann et al. 2013). FRP is a measure of fire intensity that is inversely linked to fire frequency. A low frequency of fire allows a greater fuel load to accumulate, which results in high intensity fires when they do occur. We took values of FRP from the time series MODIS global monthly fire location product (MCD14ML) available from the years 2002-2015. FRP values (measured in megawatts per 1-km pixel) were extracted for all of the GBIF-derived species locations over all of the years of fire data. FRP values with a detection confidence of <50% were discarded. This data was then grouped by species, and the 95th quantile extracted. There is typically a bias towards low FRP values, due to the high variation in this measurement over the duration of a fire (Dwyer, Pinnock et al. 2000) and low values during the night. In order to remove this bias the 95th quantile was extracted and used used, as done elsewhere (Archibald, Lehmann et al. 2013)

Information on mammal distributions was obtained from a gridded dataset produced by (Hempson, Archibald et al. 2015). First we removed any mixed feeders and browsers from the dataset leaving only obligate grazers. Mixed feeders exhibit large amounts of spatial and temporal variability in grass removal, with some only feeding on grass very occasionally, when browse is unavailable. We were therefore unable to say which of these species have a sustained impact on removal of grassy vegetation. For each of the GBIF species locations, we extracted the grazing species that were present in the 0.5 degree grid cell that each point fell within. We calculated the mean body size of grazers (measured in kg) present in that cell. We also extracted the total biomass of mammals (measured in kg/km^2) for these grid cells and took a mean across each plant species range as a measure of grazing intensity experienced by each plant species. This is referred to as grazer abundance from here on.

A global map of the total topsoil exchangeable bases was obtained from the Harmonized World Soils Database (IIASA 2008), and used to calculate the total topsoil exchangeable bases (a measure of fertility, and hereafter referred to as "soil nutrients"). 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^+). A species mean based on the GBIF locations for each species was calculated and a measure of the mean annual precipitation across the range of each species was obtained from the Worldclim database in the same way (Hijmans, Cameron et al. 2005).

Trait Measurements

We measured traits that reflect different strategies of resource use and acquisition (SLA, LTS, LNC and C/N) that are predicted to show consistent responses to environmental gradients of fire and herbivory (Table 3.1). We also included morphological traits that are known to exhibit different responses to grazing (life history, rhizomes/stolons, plasticity of traits, stature and habit). Morphological trait data came from the Kew Grassbase dataset (Clayton, Vorontsova et al. 2006 onwards). The presence or absence of stolons, short rhizomes, elongated rhizomes, a mat-forming habit, caespitose habit and annual or perennial were treated as binary traits. For example, species were coded as 1 or 0 to indicate if stolons were always present, or if species were always caespitose. Some species were able to exhibit plasticity in these traits, which we hypothesize could be induced by grazing. We therefore included extra categories coded as 1 or 0 for species where rhizomes and stolons could be either present or absent and for species that could be either matt forming or caespitose.

Species level leaf trait measurements (SLA, foliar N content and foliar C/N ratio) were obtained from an existing dataset (chapter 2; Jardine et al. unpublished). These measurements had been made on material provided by the herbarium of the Royal Botanic Gardens, Kew. This dataset was supplemented by an additional 15 species from the list of African dominants that were collected from the Kew herbarium and measured following the same methods (chapter 2; Jardine et al., unpublished). Values of SLA, LTS, LNC, C/N ratio and maximum culm height were log-transformed before all species-level analyses.

Phylogeny

In order to carry out analysis that accounted for the evolutionary relationships between species, we used a phylogeny produce by Bayesian methods (chapter 2; Jardine et al., unpublished), which was pruned to include only the species present in our study. An additional 15 taxa were added to the phylogeny, using the function add.species.to.genus in the phytools package in R (Revell 2012). This places species at a random within a subclade defined by the most recent common ancestor of all other members of the same genus present in the phylogeny. The final tree used for analyses included 100 species (Appendix 2).

Statistical Analyses

Trait relationships with environment

We tested how the traits of each species relate to its realized environmental niche. The environmental niche was described by gradients of grazing, fire, soil nutrients and MAP summarized across the range of each grass species as described by GBIF occurrence data. Due to the high degree of co-variation among environmental variables, we performed a principal components analysis on plant species mean values for FRP, MAP, grazer abundance, grazer body size and soil fertility. This identified key axis of variation in the data, which were then used to investigate the relationships of traits with environment. (Fig 1; Appendix 3a & 3b).

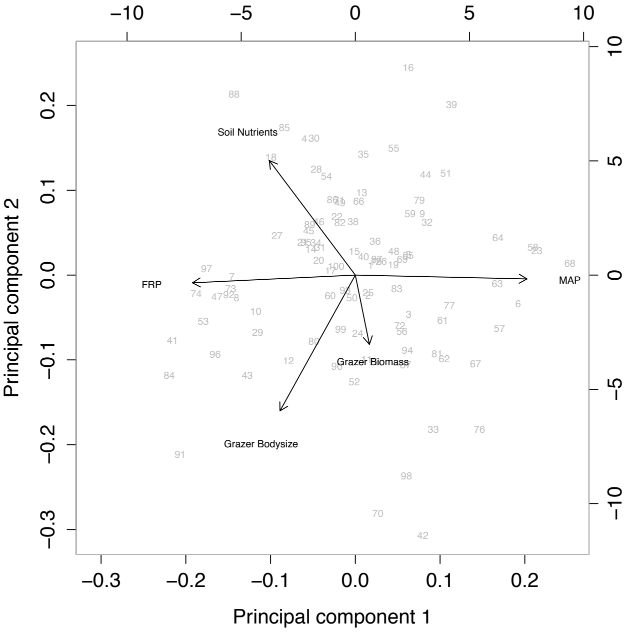

Figure 1. Principal components analysis (PCA) of the mean environmental factors for the range of each species including fire radiative power (FRP). Mean annual precipitation (MAP), soil nutrients, grazer bodysize and grazer biomass (a measure of grazer abundance). Arrows represent the direction of increase of the environmental values.

The first four of the five PC axes explained 96.4 percent of the total variance in the abiotic and biotic environments inhabited by each grass species. PC1 explained 41.6 percent of the variance and described variation in the environmental niche relating to rainfall and fire. At one end of this axis were species inhabiting areas of high rainfall and fires with a low FRP and, at the other end, low rainfall and high FRP. PC2 explained 21.8 percent of the environmental variation, relating to herbivore body size and soil nutrients. At one end was a plant environmental niche characterized by soils with low nutrients and grazing from animals with a large body size, whereas at the other end, the niche was characterized by fertile soils and grazing animals with a small body size. PC3 explained 19.9 percent of variation in the grass environmental niche, and represented an axis of abundance for the grazing mammal community, ranging from low to high. PC4 explained 13.3 percent of variance and was an axis ranging from small body size, low herbivore abundance and high soil nutrients to large body size, low soil nutrients and high herbivore abundance. The PC scores and loadings for each axis are shown in Appendix 3 and the first two principal components plotted in figure 1. We used a PGLS analysis implemented in the caper package in R (Orme 2013) to test the strength and direction of relationships between the traits and first four PC axes describing the environmental variation.

Trait syndromes

We used partitioning around medoids (PAM) to group species based on their trait values and morphological attributes. PAM uses a distance matrix to group species so that the sum of distances between an observation and all others in a cluster is minimised. The distance matrix for the PAM analysis was constructed as a Gower matrix of distances between all continuous and binary traits using the function daisy in the R package cluster (Maechler, Rousseeuw et al. 2013) . Trait measurements were standardized before making the matrix. We defined the number of clusters for the PAM analysis using the silhouette widths when the data was split into either one, two, three, four or five groups. Silhouette widths compare the distance of a species to others within its own cluster with the distance to species in other clusters. The average silhouette width was highest when the species were split into three clusters, meaning that the distance between clusters was maximized in this case relative to the internal distances within clusters.

Using the lda function in the package MASS in R (Venables and Ripley 2002), we used a linear discriminant function analysis to examine the strength with which each plant trait contributed to differences between the three clusters defined by PAM (Agrawal and Fishbein 2006). Life history was excluded from the LDA analysis as this trait was constant within clusters such that all species in cluster C were annuals and clusters A and B were entirely perennials. We used PGLS to see if the defined groups had any association with the environmental gradients described by the first four PC axis of environmental predictors. We also used PGLS and chi square tests (appendix 5) to test the significance of differences in trait values among the clusters.

Trait diversity

Vegetation types across Africa and the dominant species in each one were defined from White (1983). Species functional diversity in the 39 sub-Saharan grassy vegetation types (excluding Madagascar) that overlapped with our grazing data was calculated for SLA, LTS, maximum culm height, LNC and C/N ratio using Rao's quadratic entropy implemented in the R package FD (Laliberte and Legendre 2010). Rao's quadratic entropy is a measure of diversity in ecological communities which takes species dissimilarities into account (Rao 1982).

Environmental correlates

The body sizes of mammals present in each of these vegetation types was obtained from a gridded dataset (Hempson, Archibald et al. 2015) , and the diversity of mammal body size present in each vegetation type was also calculated using Rao's quadratic entropy (Rao 1982). Mammal diversity was weighted by grazer abundance. Mean values of MAP for the area covered by each vegetation type were extracted from Worldclim (Hijmans, Cameron et al. 2005) and soil nutrients from a global map of the total topsoil exchangeable bases was obtained from the Harmonized World Soils Database (IIASA 2008). We calculated the mean burned area for each vegetation type as a measure of fire disturbance. This was calculated as the grand mean of the total monthly area burned in each vegetation type between the years 2005 to 2011. Burned area was obtained from the ESA Climate Change Initiative global burned area product, a time series gridded dataset of area burned in meters square at a resolution of 10x10 degrees (Pettinari, Chuvieco et al. 2016).

Statistical analyses

We initially estimated the spatial autocorrelation in the data, using the trait response variable (trait functional diversity for each vegetation type) and the four environmental factors (MAP, burnt area, soil nutrients and mammal diversity). Spatial autocorrelation measures the similarity between samples for a given variable as a function of spatial distance. We constructed spatial correlograms which compute Moran's coefficients on distance classes based on the centroids of each vegetation type and the residuals of the traits and environmental factors. There was no evidence of spatially auto-correlated structure for any of the leaf economic traits, however a latitudinal gradient of diversity in height was observed. We therefore used a spatial generalized least squares (GLS) model that incorporates spatial structure in the error term of the regression model to assess the relationship between height diversity and the predictor variables. Different models of spatial structure (assuming either a spherical, exponential, Gaussian, linear or ratio structure) were tested, and the best fitting model was defined using the Akaike information criterion. For the leaf economic traits we used a linear model to look at the relationship between trait diversity and the biotic and abiotic environment. The full model was trait diversity ~ burnt area + mammal diversity + soil nutrients + MAP.

RESULTS

How do traits relate to environment?

First we wanted to investigate the relationships between plant functional traits and the realized environmental niche of each plant species, described in terms of abiotic and biotic factors across the species range. These relationships are reported in Table 2. There was a significant relationship between height and PC 1 ($r^2 = 0.15$, lambda = 0.217, $p = < 0.001$) and also between LNC and PC 1 ($r^2 = 0.216$, lambda = 0.57, $p = < 0.001$) or C/N ratio and PC 1 ($r^2 = 0.245$, lambda = 0.486, $p = < 0.001$, $p = < 0.001$). Species inhabiting areas of high MAP and low FRP had the lowest LNC, highest C/N ratio and greatest height (Fig 2). We also found significant relationships between all leaf traits and the gradients of soil nutrients and herbivore body size described by PC 2. However, there was no relationship between plant height and PC 2. Variation among SLA, LNC and C/N ratio were explained by PC 2: C/N ($r^2 = 0.245$, lambda = 0.486, $p = < 0.001$), and LNC ($r^2 = 0.216$, lambda = 0.57, $p = < 0.001$), SLA ($r^2 = 0.068$, lambda = 0.366, $P= < 0.01$). Plant species growing in areas of high soil nutrients where herbivores had a small mean body size were characterized by high LNC, low C/N ratio, high SLA and low LTS. In contrast, plant species growing in infertile areas under grazing from large bodied herbivores had low LNC, high C/N ratio, high leaf tensile strength and low SLA. Species values of LTS were also significantly related to PC 3 ($r^2 = 0.067$, lambda = 0.397, PC3 $p = <0.01$), with high LTS in areas of low grazer community abundance and low LTS in areas of high grazer abundance (Fig 2).

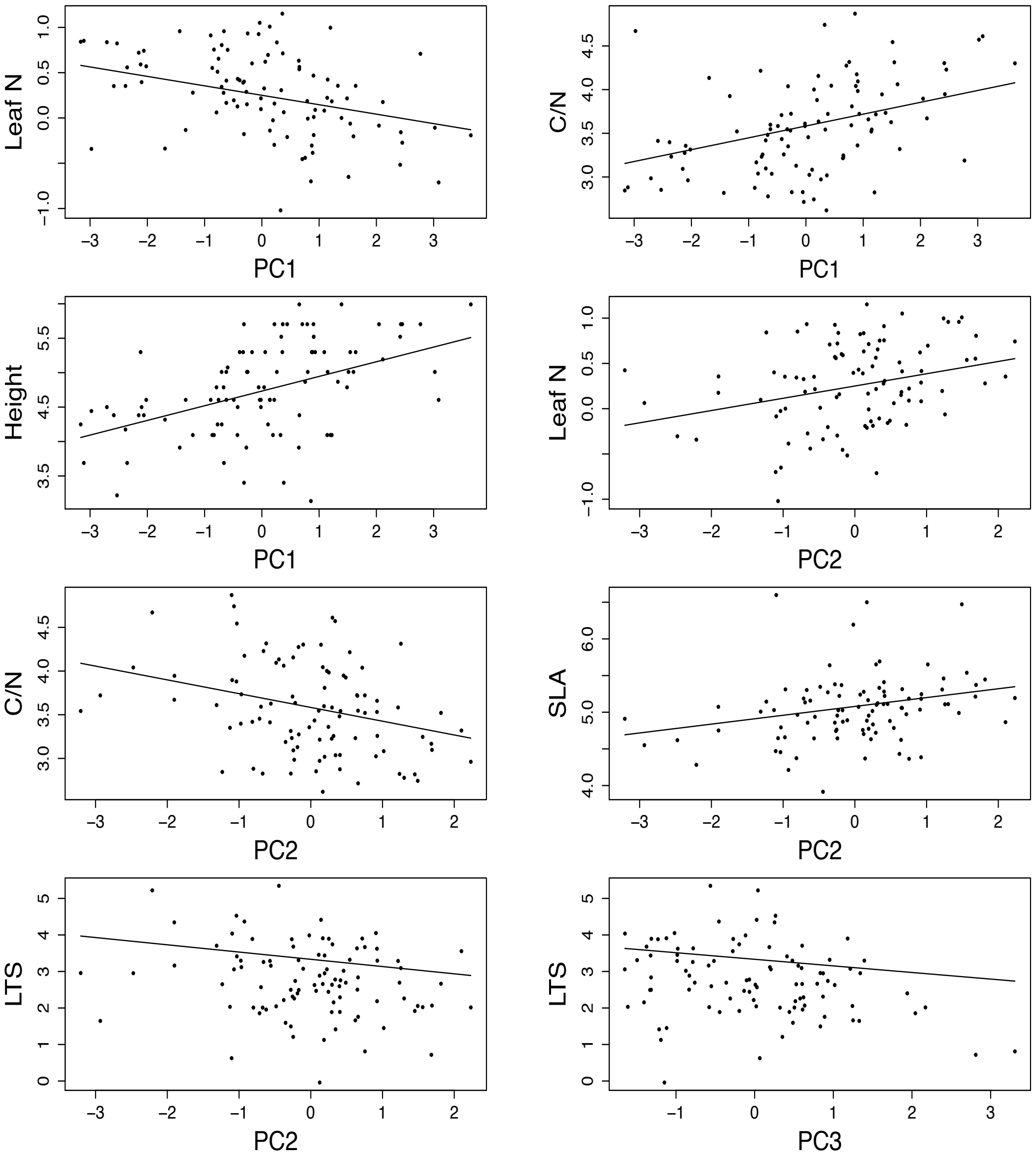

Figure 2. Relationships between the traits leaf N content (LNC), specific leaf area (SLA), maximum culm height (height) C/N (C/N ratio), leaf tensile strength (LTS) and PC axes representing environmental variation. Regression lines result from PGLS models of traits and the first four PC axes. The PC axes represent fire and MAP (PC1), grazer body size and soil nutrients (PC2), and grazer abundance and soil nutrients (PC3).

	PC1			PC2			PC3			PC4				
	Slope	SE	P	Slope	SE	P	Slope	SE	P	Slope	SE	P	r^2	λ
C/N	**0.140**	**0.032**	***	**-0.141**	**0.039**	***	-0.073	0.041	ns	-0.014	0.051	ns	0.245	0.486
LNC	**-0.112**	**0.028**	***	**0.122**	**0.034**	***	0.064	0.036	ns	-0.015	0.045	ns	0.216	0.570
SLA	0.024	0.033	ns	**0.122**	**0.043**	**	0.037	0.042	ns	0.058	0.054	ns	0.068	0.366
LTS	0.039	0.071	ns	-0.087	0.087	ns	**-0.286**	**0.091**	**	0.036	0.114	ns	0.067	0.397
Height	**0.195**	**0.043**	***	0.029	0.055	ns	-0.036	0.057	ns	0.024	0.070	ns	0.150	0.217
Elongated Rhizomes	0.008	0.015	ns	**0.045**	**0.021**	*	-0.029	0.022	ns	0.014	0.027	ns	0.029	0.000
Mat-forming	-0.011	0.018	ns	0.008	0.024	ns	-0.027	0.026	ns	0.068	0.031	*	0.023	0.000
Caespitose	0.008	0.023	ns	-0.014	0.031	ns	-0.001	0.033	ns	-0.077	0.040	ns	-0.001	0.000
Life History	0.024	0.027	ns	-0.052	0.034	ns	-0.015	0.035	ns	-0.032	0.044	ns	0.000	0.192
Short Rhizomes	-0.010	0.022	ns	-0.005	0.029	ns	0.028	0.030	ns	0.019	0.037	ns	-0.027	0.198
Plastic rhizomes	-0.003	0.009	ns	0.008	0.010	ns	0.015	0.011	ns	-0.002	0.013	ns	-0.014	1.000
Culms Plastic	0.003	0.015	ns	0.006	0.021	ns	0.029	0.022	ns	0.009	0.027	ns	-0.022	0.000
Stolons Plastic	-0.016	0.015	ns	0.028	0.021	ns	0.005	0.022	ns	-0.010	0.027	ns	-0.010	0.000

Table 2. Relationship between species means trait values and environmental predictors of geographical trait variation from PGLS analysis. The full model is defined as trait ~ PC1+PC2+PC3+PC4. PC1 is an axis of fire and MAP, PC2 of grazer bodysize and soil nutrients, PC3 an axis of grazing intensity and soil nutrients and PC4 of soil nutrients, grazer bodysize and abundance. Data were logarithmically transformed before tests. *P < 0.05; ** P < 0.01; *** P < 0.001; ns not significant. Significant relationships are highlighted in bold. All trait values were log transformed for the analyses.

Of the morphological and habit traits, the presence of elongated rhizomes was weakly but significantly associated with PC 2 ($r^2 = 0.029$, lambda = 0, $p = < 0.05$), and a mat-forming habit was weakly but significantly correlated with PC 4 ($r^2 = 0.023$, lambda = 0, $p = < 0.05$) (Fig 3). Mat-forming species and those with elongated rhizomes coincided with large mammals, high grazer abundance and low soil nutrients.

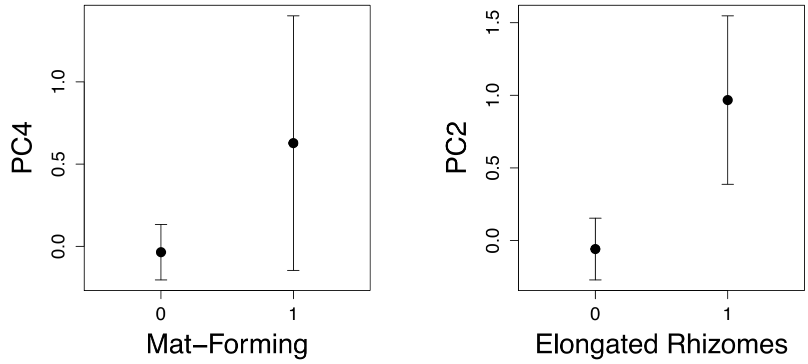

Figure 3. The relationships between morphological traits and environmental axes of variation. A value of 0 indicates that this trait is absent, while 1 means that it is present. Low values of PC 4 represent low herbivore abundance, small body size and high soil nutrients, while high values equate to high herbivore abundance, low soil nutrients and large mammal body size. Low values of PC2 represent presence of small-bodied mammals and high values large-bodied mammals.

Do species cluster into functional groups based upon their traits?

Species were assigned to three groups by clustering using PAM, and discriminant function analysis indicated how much each trait contributed to the differences among groups (Table 3.3). Traits that contributed substantially to at least one discriminant function were the mat-forming habit, the caespitose habit, and the presence or absence of rhizomes and stolons. Life history was constant across groups, such that clusters A and B were all perennials, and cluster C were all annuals. Cluster A included perennial species with a tall stature, caespitose habit with either short rhizomes or no rhizomes, a high C/N ratio and low LNC. Perennial species in cluster B could have either elongated rhizomes or stolons, and were short and mat-forming. Group C were all short, annual species lacking rhizomes or stolons with high LNC and low C/N.

	LD1	LD2	Cluster A	Cluster B	Cluster C	*P*
LTS	-0.141	-0.178	29.42	12.82	10.57	ns
SLA	-0.7	0.806	171.80	137.76	210.70	ns
LNC	**-0.039**	**0.505**	**1.32**	**1.51**	**2.13**	***
C/N ratio	**-0.312**	**-0.818**	**44.29**	**40.59**	**21.72**	***
Elongated Rhizomes	**0.127**	**-0.686**	**0.04**	**0.29**	**0.00**	*
Plastic Rhizomes	0.421	-1.16	0.01	0.14	0.00	ns
Stolons	**1.698**	**-0.815**	**0.03**	**0.57**	**0.07**	***
Plastic Stolons	-0.44	-1.577	0.07	0.00	0.00	ns
Height	**0.169**	**-0.852**	**164.16**	**74.29**	**88.21**	***
Plastic Culms	-0.123	0.28	0.04	0.14	0.07	ns
Matt Forming	**9.471**	**-0.31**	**0.00**	**0.86**	**0.00**	***
Caespitose	**-1.981**	**-0.274**	**0.96**	**0.00**	**0.93**	***

Table 3. The relationships between traits and trait clusters of dominant African grass species. Coefficients of linear discriminant functions (LDA's) indicate how much each trait contributes to the two factors generated by discriminant analysis. Mean trait values for each of the clusters A (caespitose species), B (mat forming species) and C (annual species) are shown. Whether each trait significantly contributed to differences between groups was tested by PGLS. Significant relationships traits are highlighted in bold.

None of the groups differed significantly in their relationships to PC 1, PC 3 or PC 4, but differed significantly in their distribution along PC 2, an axis of soil nutrients and grazer body size. Group B (perennial, mat-forming species) occupied a wide niche of grazing regimes and soil nutrients. Group C (annual species) occupied areas of significantly lower soil nutrients and larger grazer body size than group A (perennial, caespitose species) (Fig 4).

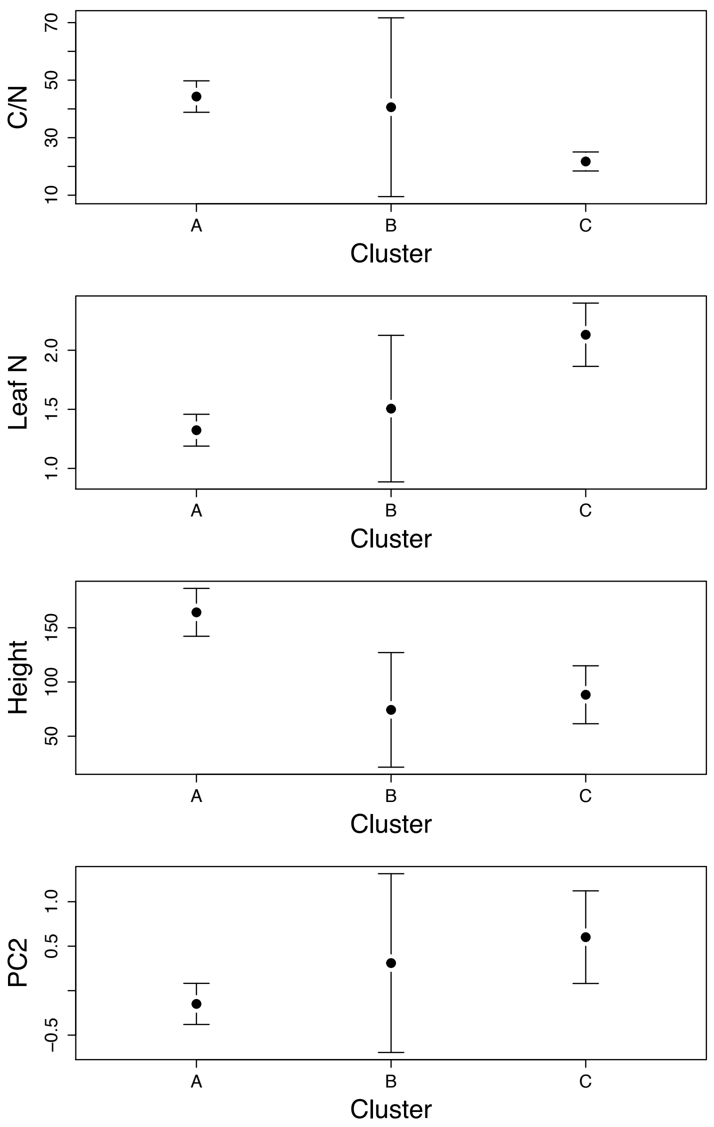

Figure 4. The relationship between A (caespitose grasses), B (mat-forming grasses) and C (annual grasses) with the traits C/N ratio, LNC content and maximum culm height. Also shown is the relationship between these cluster and PC axis 2. Low PC scores represent areas of high soil nutrients and small mammalian herbivore body size, High PC scores represent areas with larger mammals and low soil nutrients.

Trait Diversity

The relationships between the trait diversity of different vegetation types and potential biotic and abiotic factors are shown in Appendix 5. We were unable to explain the data using the predictors burnt area + mammal diversity + soil nutrients + MAP, although there did seem to be some patterns for some of the traits. For example, as predicted by our hypothesis, the diversity of height peaks at intermediate MAP and soil nutrients, in areas that burn less frequently when large mammals (>1,000 kg) were present and mammal diversity is high. However it is also possible to have low diversity of height within these environmental conditions. In vegetation types that lack large mammals and also those with the largest burnt area it is only possible to have low diversity in height.

The highest diversity of SLA also occurred at intermediate soil nutrients, however it was also possible for vegetation types at intermediate soil nutrients to have a low diversity of SLA. There were no discernible patterns for the diversity of LNC. However a high diversity of C/N ratio only occurs in areas that burn infrequently and where mammal diversity is high.

DISCUSSION

The aims of this study were to investigate how fire and grazing influence the continental distribution and diversity of grass functional traits, and whether species can be objectively grouped together into functional types associated with different fire and grazing regimes. Studies covering large geographic areas investigating the influence of grazing on trait variation are infrequent, due to a previous lack of information at macro-ecological scales about the distributions and abundance of mammal species. We have utilized recently available data that maps the distribution and historical abundance of grazing mammals at a continental scale. Using these data, we have been able to show the influence of both fire and historical grazing on the distribution of economic and morphological grass traits across sub-Saharan Africa.

In areas with a seasonal pattern of rainfall, fire is heavily dependent upon precipitation, with frequent, low intensity fires occurring in areas of high rainfall, where conditions create a large and combustible fuel load (Krawchuk and Moritz 2011; Archibald, Lehmann et al. 2013). We show that species from these areas have low LNC, and high C/N. These are traits that contribute to low rates of foliar decomposition, which is important for creating a large flammable fuel load (Knapp and Seastedt 1986; Aerts 1997). Forrestel, Donoghue et al. (2014) showed that plants in areas that burn frequently display traits associated with a high net assimilation rate, high nitrogen use efficiency and high photosynthetic rate (i.e. high values of SLA, LNC and stomatal size and pore index). These traits all confer competitive advantages in the high light, low nitrogen environment that is created after fires. However, although we show the same relationship between LNC, fire and MAP, we found no significant relationships between SLA, fire and MAP. Differences in results may be due to differences in the scale of analyses, or because other studies have excluded large grazers from experimental communities when studying the influence of fire on community composition. In considering fire and grazing in combination, we show that gradients of soil nutrients, grazer body size and mammal community grazer abundance are more important than fire regime in explaining continental scale variation in the distribution of SLA and also leaf tensile strength.

We found significant relationships at the continental scale between all leaf economic traits and gradients of grazer body size, abundance and soil nutrients. A decrease in SLA and LNC and increase in LTS and C/N ratio was observed with increasing grazer body size and decreasing soil nutrients. These results conflict with our predictions based upon community ecological theory that defence traits will be lowest in productive environments where a competitive strategy following defoliation is an important component of dominance. Instead our results indicate that it is soil fertility that mediates the allocation of resources to growth or defence related traits at the continental scale, and not grazing pressure. Much of the community ecological theory surrounding the allocation of resources to growth or resource conservation has been formulated through studies of tropical trees and insects (Coley, Bryant et al. 1985) or in temperate environments (Grime 1977) . We show that these predictions are not universally applicable to plants of different growth forms, subjected to mammalian herbivory, and growing in different climatic regions.

We also did not find a significant relationship between grazing and height at the continental scale, with plant height being driven primarily by fire and MAP. Decreases in height in response to local grazing have been widely reported in the literature (Sala, Oesterheld et al. 1986; McNaughton and Sabuni 1988), as has an increase in annual plant species in grazed areas (Diaz, Acosta et al. 1992; Diaz, Lavorel et al. 2007). Although we did not find direct pairwise relationships between height, life history and grazing, when species were grouped into functional types based upon their traits, we were able to identify three clusters of species that differed significantly in stature, life history and their relationship to soil and grazing. Short, annual species were associated with high grazing pressure and fertile soils. In contrast, tall, perennial, caespitose grasses were located in areas of lower intensity grazing and infertile soils. Short, perennial mat-forming grasses occupied a wide niche space that overlapped with both caespitose and annual plants. None of these groups differed in their relationships with fire and MAP at the continental scale.

Morphological traits were important in determining these functional groups and, of the leaf economic traits, C/N or LNC were important in distinguishing between caespitose and annual grasses. Stock, Bond et al. (2010) identified functional differences between caespitose and lawn grasses, with lawn grasses exhibiting higher foliar N levels than caespitose species. However Anderson, Kumordzi et al. (2013) did not find differences in leaf chemical traits between these groups. We show that the variation of LNC exhibited by grasses with a mat-forming habit was wide and overlapped with both annuals and caespitose species. We found that mat-forming grasses exhibited elongated (but not short) rhizomes, possessed stolons or expressed plasticity in the formation of rhizomes and stolons, most probably induced by grazing. These are traits that are all associated with the formation of grazing lawns. Large mammals such as white rhinoceros and hippopotamus play an important role in creating grazing lawns (Owen-Smith 1988; Cromsigt and te Beest 2014). Although mat-forming grasses occupy a wide niche space, we show that they can occur in areas grazed by mammals of larger body size than caespitose grasses. Annual species also occupy areas that are grazed by larger mammals when compared to caespitose grasses. Large mammals can open up gaps through trampling and the consumption of vegetation, which are important for plant recruitment (Knapp, Blair et al. 1999; Eskelinen and Virtanen 2005).

Finally, we were unable to find any models that would predict the relationship of community plant functional diversity to gradients of fire, soil, MAP or community grazer diversity. However, some patterns did emerge. In areas of frequent fire and high MAP, where large grazing mammals are absent, a high diversity of trait values is never observed. Areas of intermediate MAP, soil nutrients and high grazer diversity, where large mammal species are present, are the only conditions where high trait diversity occurs, probably due to the release from competition that happens when large mammals graze on tall species and create gaps for recruitment. However, it is also possible to observe low trait diversity under these conditions. Although other studies have shown that the effects of large mammals on species diversity depend upon productivity (Bakker, Ritchie et al. 2006), however this relationship was absent from our data.

CONCLUSIONS

We have shown that leaf economic traits of the African grass species measured in this study, but not height, are significantly correlated with continental-scale gradients of soil nutrients and grazer body size, and that height, LNC and C/N are associated with gradients in fire regimes. We show that the distributions of traits that reflect different strategies of resource acquisition and use are driven more by soil fertility than grazing per se, and that community ecological theory derived from studies of insects and trees does not apply across plant groups and different forms of herbivory. We objectively show the clustering of African grass species into functional types that share similar traits. These groups represent annuals, caespitose perennials and mat-forming grasses, with grazing and soil nutrients more important than fire in explaining the distribution of these functional groups. Our results show that there is considerable trait variation within savanna grass species across sub-Saharan Africa and that is in large part driven by gradients of rainfall, fire, grazing and soil nutrients. Common responses to environment are, however, shared between groups, meaning that predictions of how species may respond to future environmental change could usefully group species by life history, growth form, habit and traits that reflect foliar nutritional quality.

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ACKNOWLEDGMENTS

This research was funded by Project Sunshine (The University of Sheffield). We thank David Simpson, Maria Vorontsova and Martin Xanthos at the Royal Botanic Gardens Kew for providing specimens.

APPENDICES

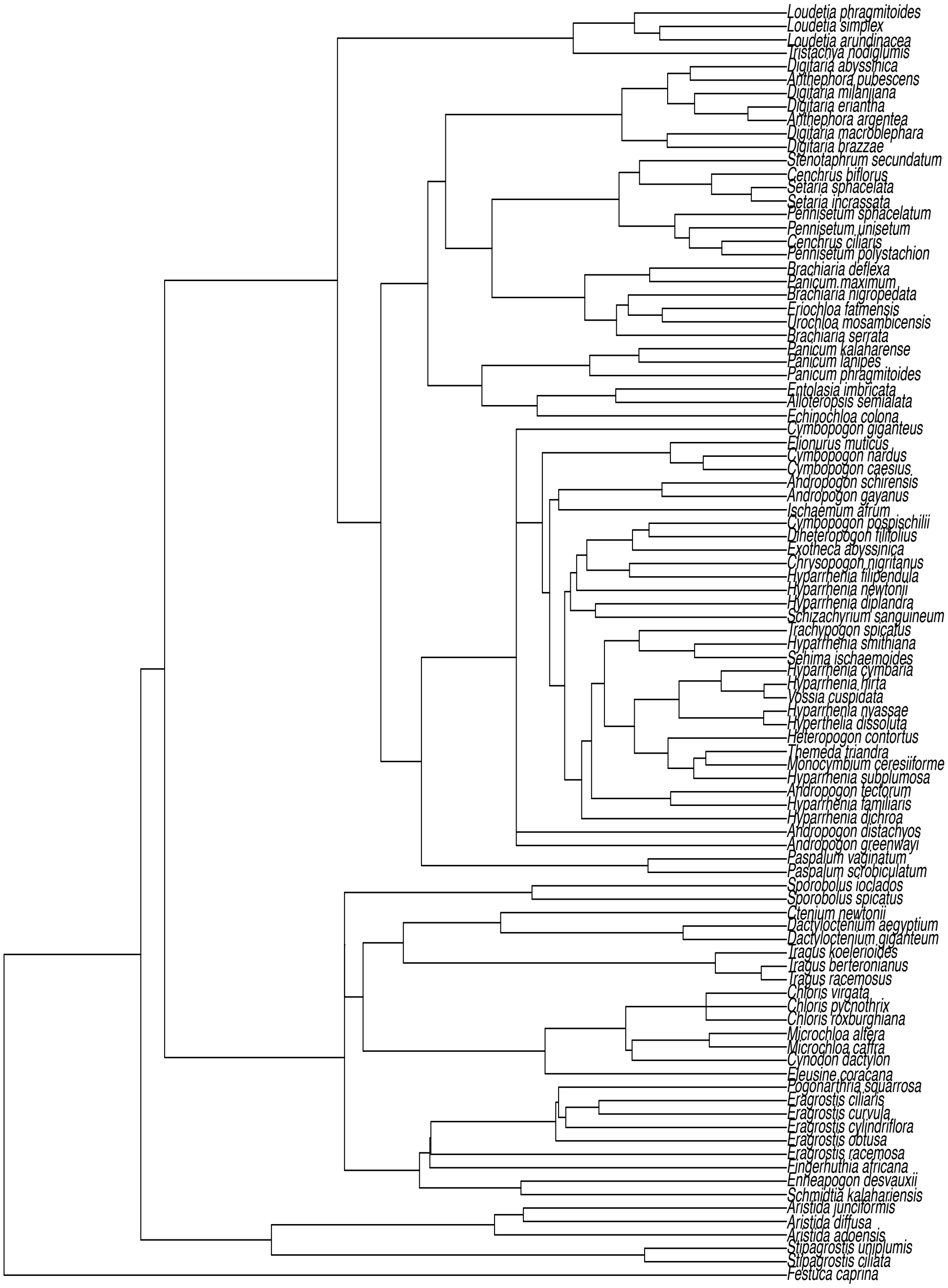
Appendix 1. The list of species included in this study

Name	Author
Alloteropsis semialata	(R. Br.) Hitchcock
Andropogon distachyos	L.
Andropogon gayanus	Kunth
Andropogon greenwayi	Napper
Andropogon schirensis	Hochst.
Andropogon tectorum	Schum. & Thonn.
Anthephora argentea	Goossens
Anthephora pubescens	Nees
Aristida adoensis	Hochst.
Aristida diffusa	Trin.
Aristida junciformis	Trin. & Rupr.
Aristida rhiniochloa	Hochst.
Brachiaria deflexa	(Schum.) C. E. Hubb. ex Robyns
Brachiaria nigropedata	(Fic. & Hiern.) Stapf
Brachiaria serrata	(Thunb.) Stapf
Bromus speciosus	Nees
Cenchrus biflorus	Roxb.
Cenchrus ciliaris	L.
Chloris pycnothrix	Trin.
Chloris roxburghiana	Schult.
Chloris virgata	Sw.
Chrysopogon nigritanus	(Benth.) Veldkamp
Ctenium newtonii	Hack.
Cymbopogon caesius	(Hook. & Arn.) Stapf
Cymbopogon giganteus	Chiov.
Cymbopogon nardus	(L.) Rendle
Cymbopogon pospischilii	(K. Schum.) C. E. Hubb.
Cynodon dactylon	(L.) Pers.
Cynodon incompletus	Nees
Dactyloctenium aegyptium	(L.) Willd.
Dactyloctenium giganteum	B. S. Fisher & Schweickerdt
Digitaria abyssinica	(A. Rich.) Stapf
Digitaria brazzae	(Franch.) Stapf
Digitaria eriantha	Steud.
Digitaria macroblephara	(Hack.) Paoli
Digitaria milanjiana	(Rendle) Stapf
Diheteropogon filifolius	(Nees) Clayton
Echinochloa colona	(L.) Link
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 obtusa	Munro ex Ficalho & Hiern
Eragrostis racemosa	(Thunb.) Steud.
Eragrostis superba	Peyr.
Eriochloa fatmensis	(Hochst. & Steud.) Clayton
Exotheca abyssinica	(Hochst.) Anderss.
Festuca caprina	Nees
Fingerhuthia africana	Lehm.
Heteropogon contortus	(L.) Beauv. ex Roem. & Schult.
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 smithiana	(Hook.) Stapf
Hyparrhenia subplumosa	Stapf
Hyperthelia dissoluta	(Nees) Clayton
Ischaemum afrum	(J. F. Gmel.) Dandy
Loudetia arundinacea	(A. Rich) Hochst. ex Steud.
Loudetia phragmitoides	(Peter) C. E. Hubb.
Loudetia simplex	(Nees) C. E. Hubb.
Microchloa altera	(Rendle) Stapf
Microchloa caffra	Nees
Monocymbium ceresiiforme	(Nees) Stapf
Panicum kalaharense	Mez
Panicum lanipes	Mez
Panicum maximum	Jacq.
Panicum phragmitoides	Stapf
Paspalum scrobiculatum	L.
Paspalum vaginatum	Sw.
Pennisetum polystachion	(L.) Schult.
Pennisetum sphacelatum	(Nees) T. Dur. & Schinz
Pennisetum unisetum	(Nees) Benth.
Pogonarthria squarrosa	(Roem. & Schult.) Pilger
Schizachyrium sanguineum	(Retz.) Alston
Schmidtia kalahariensis	Stent
Sehima ischaemoides	Forsk.
Setaria incrassata	(Hochst.) Hack.
Setaria sphacelata	(Schumach.) Stapf & C. E. Hubb. ex

	Moss
Sporobolus ioclados	(Trin) Nees
Sporobolus spicatus	(Vahl) Kunth
Stenotaphrum secundatum	(Walt.) Kuntze
Stipagrostis ciliata	(Desf.) de Winter
Stipagrostis uniplumis	(Licht.) de Winter
Themeda triandra	Forsk.
Trachypogon spicatus	(L.) Kuntze
Tragus berteronianus	Schult.
Tragus koelerioides	Aschers.
Tragus racemosus	(L.) All.
Tristachya nodiglumis	K. Schum.
Urochloa mosambicensis	(Hack.) Dandy
Vossia cuspidata	(Roxb.) Griff.

Appendix 2. Phylogeny showing relationships between all taxa included in this study.



Appendix 3a The proportion of variance explained by each axis of a principal components analysis of the species means over their distribution of the environmental variables FRP (fire radiative power), MAP (mean annual precipitation), grazer biomass, mean grazer bodysize and soil nutrients.

	PC1	PC2	PC3	PC4	PC5
Standard deviation	1.4413	1.0447	0.9962	0.8142	0.41965
Proportion of Variance	0.4155	0.2183	0.1985	0.1326	0.03522
Cumulative Proportion	0.4155	0.6337	0.8322	0.9648	1

Appendix 3b Loadings of each environmental variable on each of the PC axes identified following principal components analysis of the species means over their distribution for FRP (fire radiative power), MAP (mean annual precipitation), grazer biomass, mean grazer bodysize and soil nutrients.

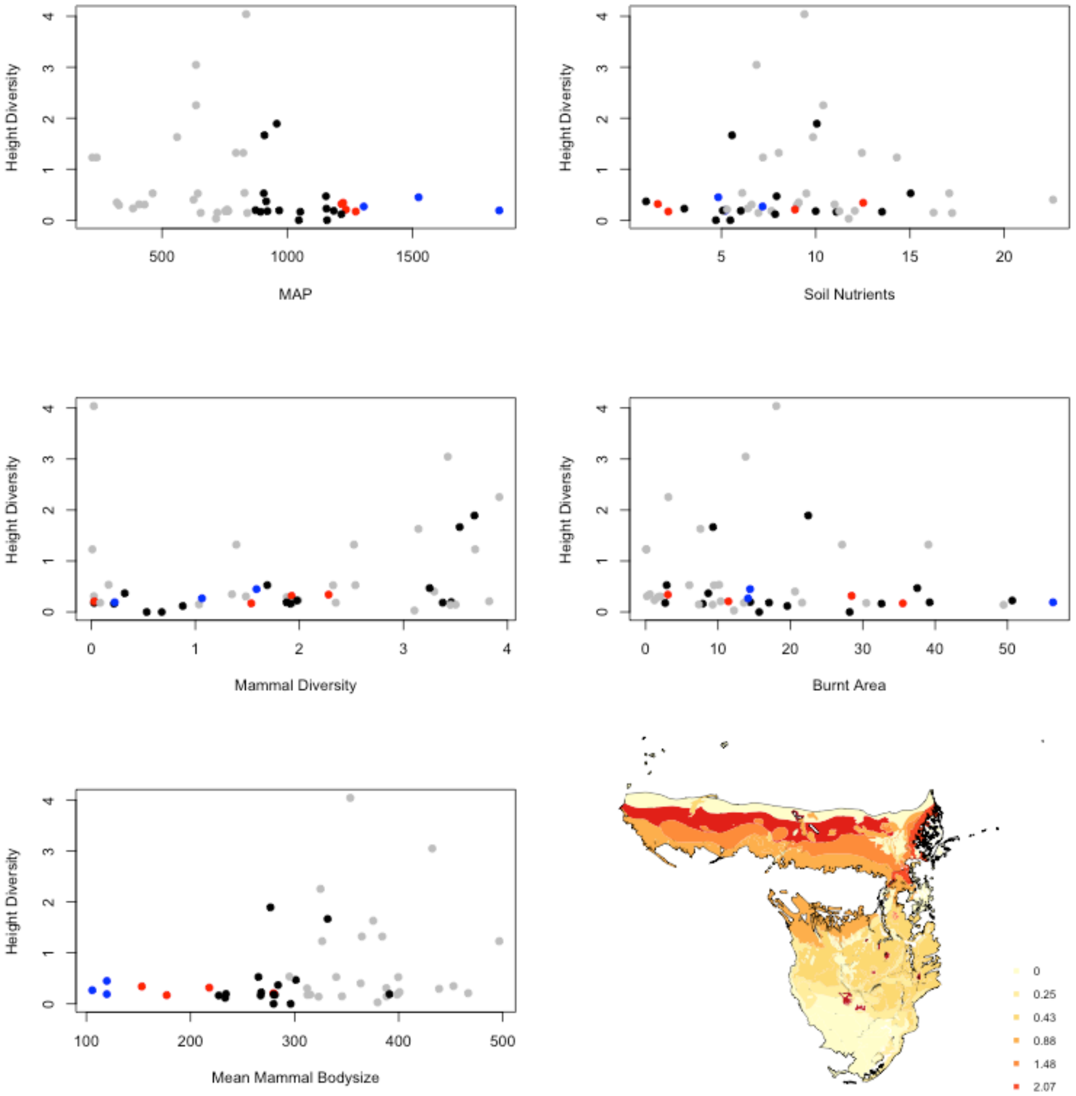
	PC1	PC2	PC3	PC4	PC5
MAP	0.65	-0.02	0.01	0.17	-0.74
Grazer Biomass	0.05	-0.36	0.92	-0.15	0.04
Grazer Bodysize	-0.29	-0.71	-0.16	0.61	-0.09
FRP	-0.62	-0.04	-0.03	-0.44	-0.65
Soil Nutrients	-0.33	0.60	0.36	0.61	-0.16

Appendix 4. Chi Square test showing which traits significantly contribute towards differences in functional groups defined by cluster analysis

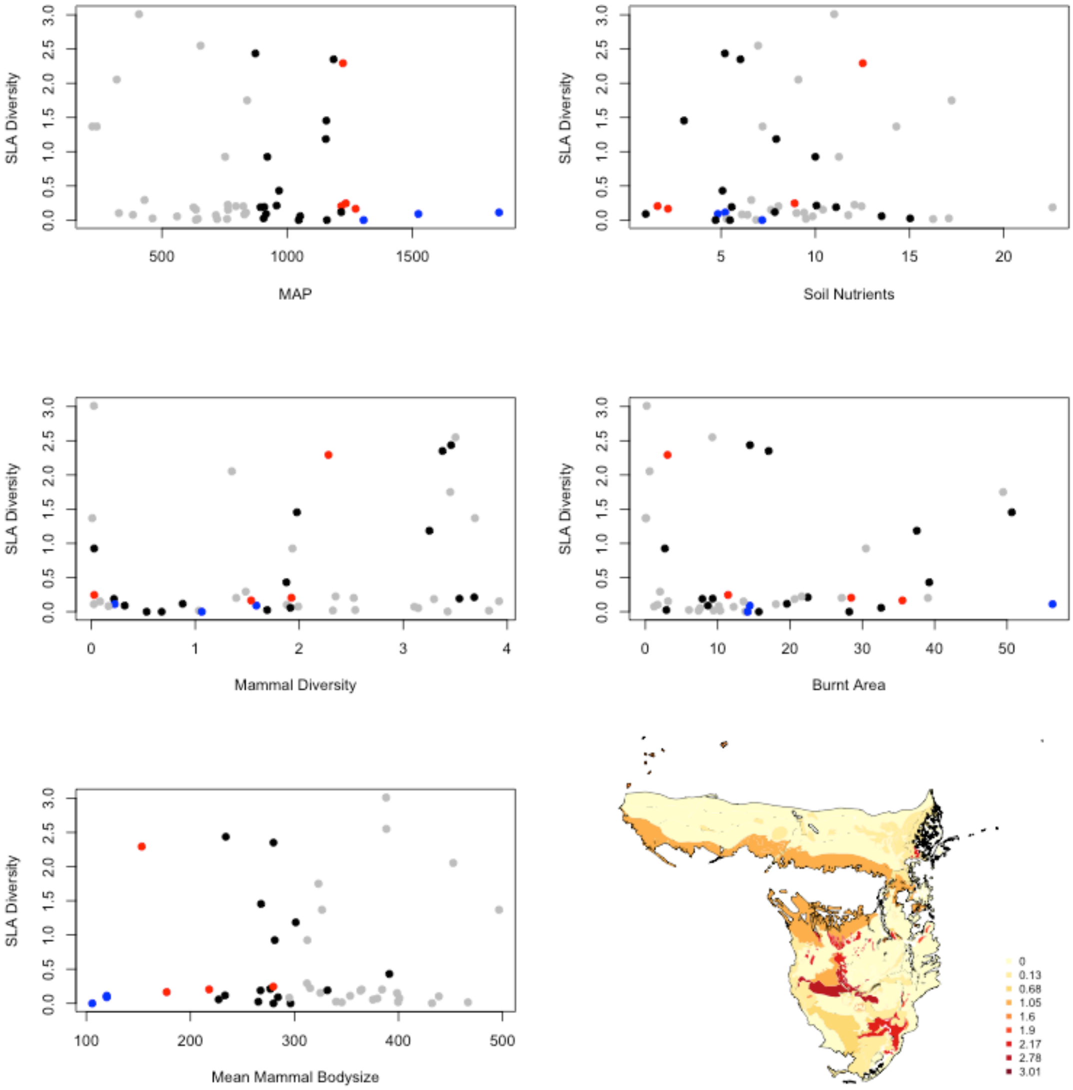
	x^2	df	p
Elongated Rhizomes	8.622	2	*
Stolons	27.77	2	***
Caespitose	57.816	2	***
Plastic Culms	1.459	2	ns
Short Rhizomes	3.172	2	ns
Life History	95	2	***
Plastic Rhizomes	5.5443	2	ns
Plastic Stolons	1.498	2	ns
Matforming	80.514	2	***

Appendix 5. The relationship of plant trait diversity (calculated using Rao's quadratic entropy), for (a) Height (b) Specific leaf area (SLA) (c) leaf nitrogen content (d) C/N ratio and (e) leaf tensile strength (LTS) to MAP (mean annual precipitation), soil nutrients, mammal diversity (calculated using Rao's quadratic entropy), burnt area and mean mammal body size. Each point on a scatter point represents each of the vegetation types across sub Saharan Africa. Scatter plots are coloured by the largest mammal present in that vegetation type. Grey = white Rhino, black = Hippopotamus, red = buffalo and red = Roan. Maps show the diversity of traits with breaks in the colour scale were set using Jenks algorithm.

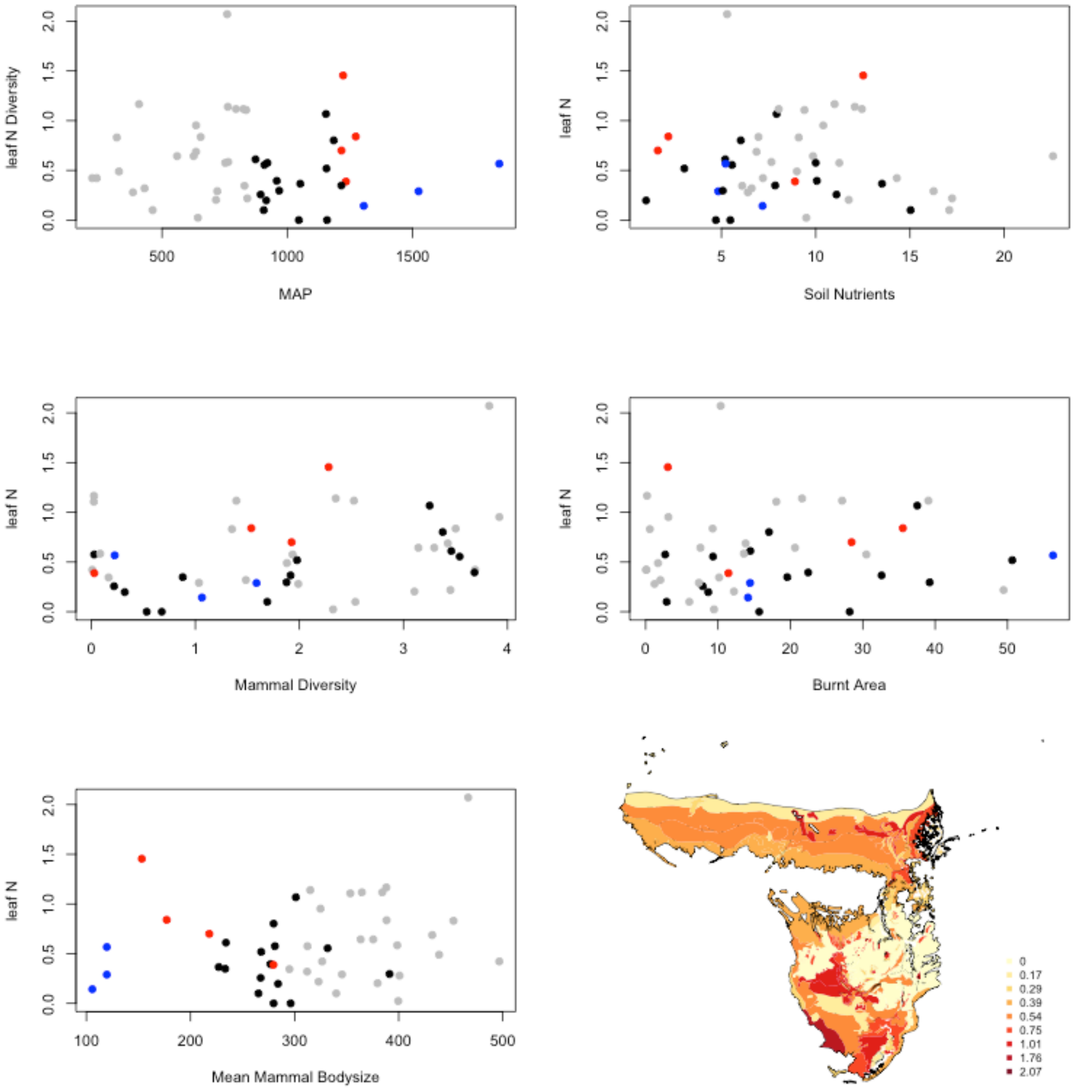
(a).



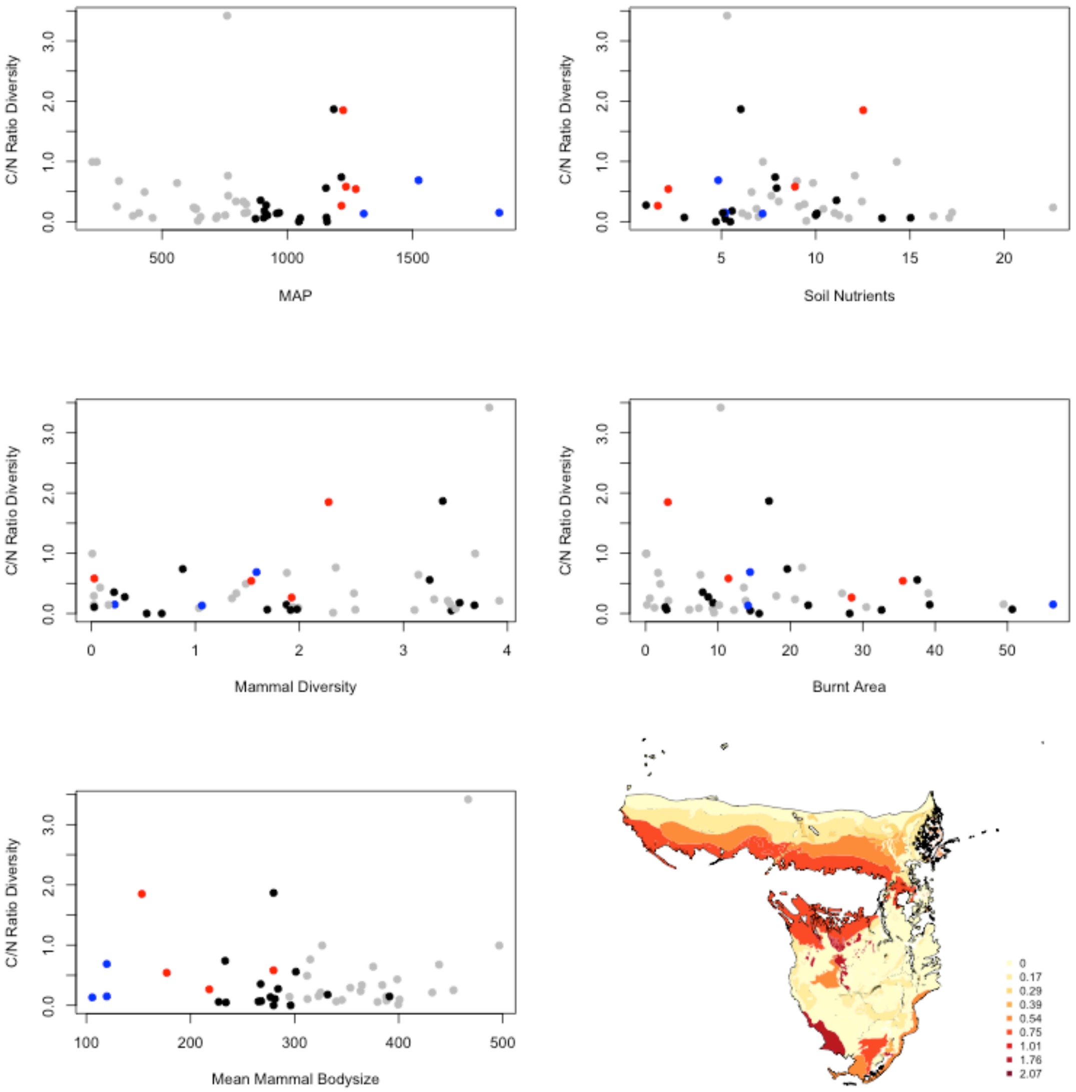
(b).


SLA Diversity
0.0
0.5
1.0
1.5
2.0
2.5
3.0
500
1000
1500
MAP
SLA Diversity
5
10
15
20
Soil Nutrients
SLA Diversity
0
1
2
3
4
Mammal Diversity
SLA Diversity
0
10
20
30
40
50
Burnt Area
SLA Diversity
100
200
300
400
500
Mean Mammal Bodysize
0
0.13
0.68
1.05
1.6
1.9
2.17
2.78
3.01

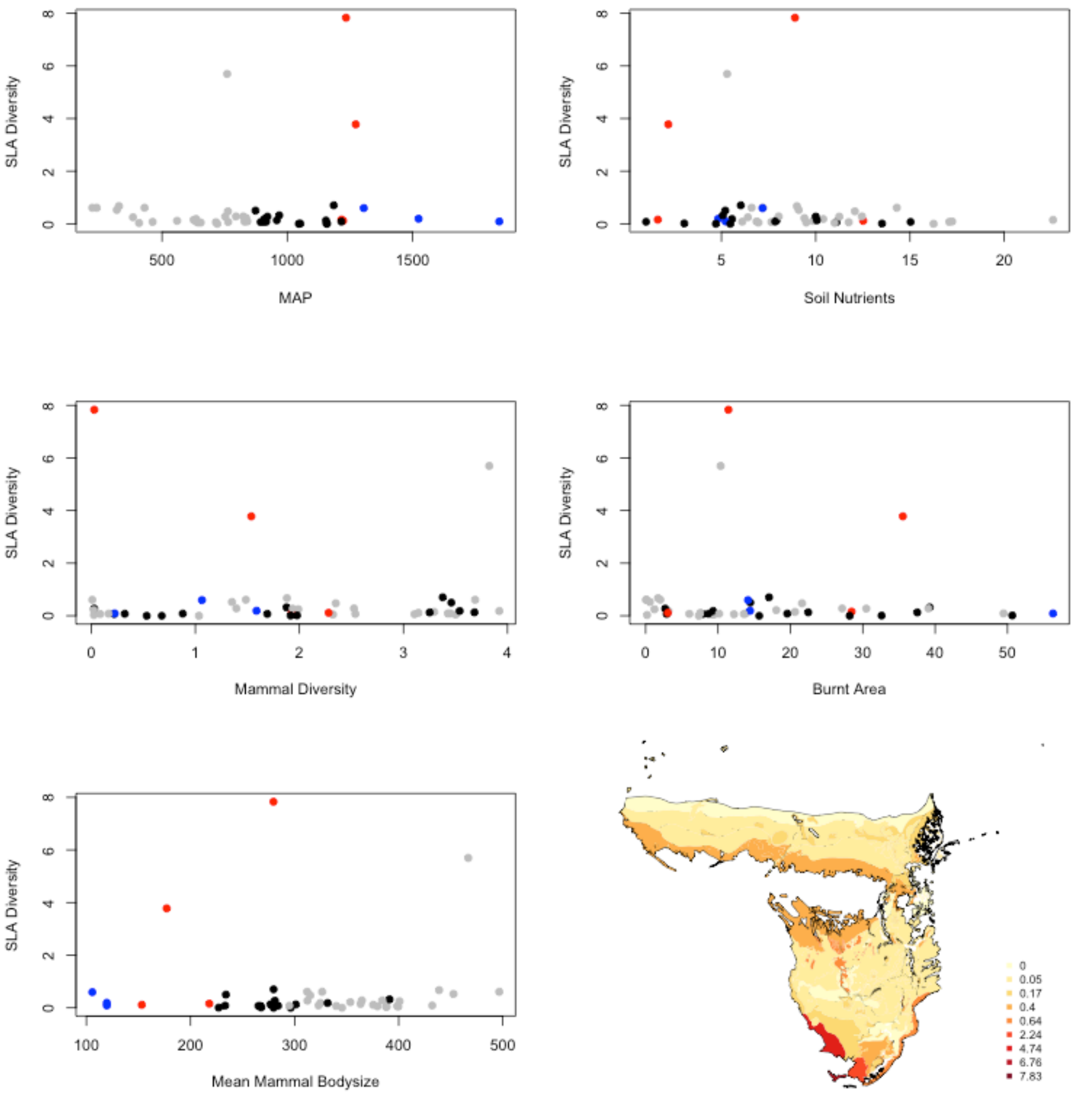
(c).


leaf N Diversity
0.0
0.5
1.0
1.5
2.0
500
1000
1500
MAP
leaf N
5
10
15
20
Soil Nutrients
leaf N
0
1
2
3
4
Mammal Diversity
leaf N
0
10
20
30
40
50
Burnt Area
leaf N
100
200
300
400
500
Mean Mammal Bodysize
0
0.17
0.29
0.39
0.54
0.75
1.01
1.76
2.07

(d).


C/N Ratio Diversity
0.0
1.0
2.0
3.0
500
1000
1500
MAP
5
10
15
20
Soil Nutrients
0
1
2
3
4
Mammal Diversity
0
10
20
30
40
50
Burnt Area
100
200
300
400
500
Mean Mammal Bodysize
0
0.17
0.29
0.39
0.54
0.75
1.01
1.76
2.07

(e).


SLA Diversity
0
2
4
6
8
500
1000
1500
MAP
SLA Diversity
5
10
15
20
Soil Nutrients
SLA Diversity
0
1
2
3
4
Mammal Diversity
SLA Diversity
0
10
20
30
40
50
Burnt Area
SLA Diversity
100
200
300
400
500
Mean Mammal Bodysize
0
0.05
0.17
0.4
0.64
2.24
4.74
6.76
7.83

CHAPTER FOUR

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STATEMENT OF CONTRIBUTIONS

This chapter is in preparation as a manuscript. The initial idea and experimental design was developed by the candidate, Prof. Colin Osborne and Dr Gavin Thomas. All data collection, data analyses and writing were carried out by the candidate with general editorial advice by Prof. Colin Osborne and Dr Gavin Thomas.

FUNCTIONAL TRAITS EXPLAIN SORTING OF SAVANNA GRASSES ALONG A GLOBAL RAINFALL GRADIENT

Emma C. Jardine, Gavin H. Thomas, Colin P. Osborne

ABSTRACT

Understanding the global distributions of plants is a major challenge in macro-ecology. While species distributions are closely associated with moisture availability, the mechanisms and traits that determine interspecific variation in the precipitation niche are unresolved. This requires the drought tolerance of species to be quantified. We hypothesised that species sampled across global rainfall gradients would show variation in survival under drought and growth rate under wet conditions, and that these facets of plant performance could be explained by functional traits governing water acquisition and conservation.

Drought relations are especially important for plants dominating seasonally dry ecosystems like savannas. Our experiment therefore subjected 18 species of savanna grasses to a lethal drought under controlled environmental conditions. The number of days until death was quantified for each species along with measurements of growth rate, root traits, leaf traits and aspects of hydraulic function expected to influence drought tolerance.

We identified a trade-off between the growth rate under wet conditions and mortality under drought. However this could not explain the position of ecological niches along rainfall gradients. These were instead correlated with rates of canopy senescence. We identified two strategies in relation to drought. Species that stayed green as the water potential declined, and those that senesced more quickly but could extend survival under water deficits via drought tolerant meristems. Plants with the "stay green" strategy occupied drier habitats and had the longest survival under drought, which was facilitated by narrow root diameter and isohydric stomatal behaviour. Plants that senesced more quickly had wider roots, an anisohydric strategy, and occupied wetter habitats.

These results show a growth-survival trade-off in relation to drought among savanna grass species, but indicate that this does not explain their spatial distribution along rainfall gradients. Instead, species distributions in low rainfall regions are correlated with slower canopy senescence under drought, which can be explained by more pronounced stomatal closure in a drying soil and narrower roots.

Keywords
drought, survival, senescence, grasses, traits, precipitation

INTRODUCTION

Explaining why species occupy different environments is a central goal of ecology and understanding how functional traits mediate plant-climate relationships provides a way of answering this question. At a global scale, primary productivity and species distributions are correlated with gradients of rainfall (Leith 1975; Currie and Paquin 1987). These patterns are thought to be mediated by trade-offs in species functional traits (Woodward 1987; Reich 2014). In habitats where water is not a limiting resource, competition excludes species and the ability to rapidly acquire resources and grow quickly is expected in the dominant species (Grime 1977; Craine 2009). However, soil water deficits in arid habitats are expected to exclude plants that are not adapted to drought. Significant variation in drought performance has been observed among species, with those found in drier environments surviving longer under conditions of drought than species adapted to mesic conditions (Sack 2004; Engelbrecht, Kursar et al. 2005). This suggests that productivity under well-watered conditions and survival during drought events are both important factors in determining species distributions along rainfall gradients.

Photosynthetic traits are closely associated with both the growth and survival of species (Poorter and Bongers 2006), and there is strong co-ordination between photosynthetic characteristics and hydraulic function (Brodribb, Holbrook et al. 2002). When stomata are open for photosynthetic gas exchange, water loss through transpiration is inevitable, making stomatal conductance and hydraulic function inherently linked (Brodribb and Jordan 2008). The structure of a plant’s hydraulic system places physical limits on the transport of water (Brodribb 2009), and any of the traits that are prerequisites for fast growing, resource-acquisitive species therefore depend on a plant's water transport system. For example, high hydraulic conductance is needed for rapid water transport from roots to leaves and for maximising stomatal conductance, which controls productivity when water is unlimited (Nardini and Salleo 2000).

Stomatal regulation has been proposed as the primary mechanism mediating plant mortality under drought (McDowell, Pockman et al. 2008). A continuum between opposing hydraulic strategies has been described, with isohydric species at one end, and anisohydric species at the other. As soil water potential decreases under conditions of drought, isohydric species maintain midday water potential regardless of drought conditions by rapidly closing their stomata (McDowell, Pockman et al. 2008). This strategy avoids hydraulic failure caused by cavitation (air entering the xylem vessels). The cost of stomatal closure is diminished carbon intake and, if a drought lasts longer than carbohydrate reserves, then carbon starvation is likely (Katul, Leuning et al. 2003). Anisohydric species, by contrast, allow midday water potential to decline as available soil water declines, allowing continued photosynthetic carbon assimilation. However, under intense and prolonged drought, anisohydric species may suffer hydraulic failure caused by cavitation. A trade-off has been described between hydraulic safety and efficiency (Skelton, West et al. 2015) which may provide adaptive benefits in areas of differing rainfall regime, depending on the length and predictability of drought events.

The link between mortality and hydraulic performance in different environments relies upon the premise that hydraulic failure causes death. However, many species of grass, forbs and shrubs have meristems either below ground or just above the soil which can regrow after aboveground parts have senesced (Overbeck and Pfadenhauer 2007). In such cases, the rates of stomatal closure may be associated with growth rate but decoupled from plant survival under conditions of declining soil moisture. A number of other mechanisms may also decouple or modify the stomatal behaviour-mortality relationship. For example, leaf rolling allows gas exchange to continue, whilst reducing water loss (Knapp 1985; Kadioglu and Terzi 2007). Canopy senescence can be induced by drought stress and plays a major role in survival by remobilizing nutrients that have been accumulated in leaves. When this is accompanied by leaf shedding, water losses through transpiration are also avoided (Munne-Bosch and Alegre 2004). Leaf shedding is commonly found among species of mediterranean-type and seasonal subtropical environments, and both deciduousness and a thickened tap root strongly predicts survival along a rainfall gradient in tropical trees (Ackerly 2004; Poorter and Markesteijn 2008).

Grassy savannas, in which the ground cover is dominated by C_4 grass species, cover 20% of the vegetated land surface in regions where seasonal droughts occur, and occupy a very broad precipitation range from ~200 mm MAP to ~3000 mm MAP (Scholes and Archer 1997). These climate relationships make them an excellent system for studying large-scale patterns of drought performance. Climate has long been considered a major factor in determining the global distributions of grass species (Hartley 1952; Taub 2000), and different phylogenetic clades have associations with areas of contrasting aridity at both global and regional scales (Edwards and Smith 2010; Visser, Woodward et al. 2012), indicating that functional trait interactions with moisture availability may be a key factor in determining their habitat associations. However, we currently have limited knowledge of the adaptive functional traits underpinning these large-scale patterns. This study aims to understand the functional traits underlying species differences in mortality during drought and to link these with the patterns of rainfall influencing spatial distributions of C_4 savanna grasses. We hypothesize a trade-off between growth rate and mortality of C_4 grasses, which correlates with species distribution patterns. We measure time until senescence and death, stomatal conductance, water relations, leaf rolling, root diameter, specific leaf area (SRL), relative growth rate (RGR). We expect that species from arid areas will adopt an isohydric strategy, maintaining shoot water potential and green leaves, and surviving longer under drought via early stomatal closure, and characterised by a slow growth rate, low specific root length and narrow root diameter. Conversely, we expect plants from mesic habitats to adopt an anisohydric strategy, exhibiting a fast growth rate, and having high specific root length and wide root diameter.

METHODS

Species selection and environmental variables

We designed a controlled environment experiment to test for differences in survival under drought and measured the functional traits that might explain variation in survival. We sampled a total of 18 C_4 grass species, chosen to include representatives from six independently derived lineages containing C_4 species, (Appendix 1), and also species whose distributions represent a wide gradient of MAP (mean annual precipitation) and precipitation seasonality. All of the species selected for study were determined as locally dominant based upon information from vegetation maps e.g. (White 1983). Seasonality was quantified by subtracting the annual maximum mean monthly precipitation from the annual minimum mean monthly precipitation, based on data from WorldClim (Hijmans, Cameron et al. 2005)

The realised precipitation niche of each species was quantified by mapping occurrence data from GBIF onto the climatic data from WorldClim. Point occurrence data from GBIF were cleaned so that the analysis only included records where the co-ordinate reference was recorded to two decimal places or more, and where the GBIF country code matched the country of the co-ordinate reference. The remaining numbers of geo-referenced records for each species are listed in Appendix 1. Habitat data from floras were cross checked for each species to ensure plants from shallow, eroded soils or wetlands were excluded, as precipitation data would not accurately reflect the water available in the habitats of these species.

Drought Experiment

Seeds were germinated in a controlled environment (MLR 352H, Sanyo, Osaka, Japan), on moist filter paper on petri dishes with a 16-hour day length, a day/night temperature of 25/20 °C and 60% humidity. Hard-coated seeds with a low germination rate were soaked in water that had boiled, then been left to cool for 10 minutes, followed by being soaked in cold water for 24 hours before being put into the chamber. Seedlings were transplanted once they had one fully expanded leaf into

1-litre pots (length, 5cm; width, 5 cm; height, 40cm) containing John Innes No.1 compost and 2.5 grams of slow release fertiliser granules (Miracle Gro, Scotts, Marysville, Ohio, USA). The experiment took place in a controlled environment chamber (MTPS 120, Conviron, Winnipeg, Manitoba, Canada). A randomised block design was used to ensure there was no bias in the experiment due to environmental heterogeneity. Plants upon which trait measurements were to be taken were also assigned a random harvest number to indicate the order in which they should be measured throughout the drought.

After transplanting, plants were grown on in the chamber with a day length of 16 hours and a day/night temperature of 22/18 °C. Humidity was maintained at 60% and the photosynthetic photon flux density (PPFD) at plant canopy height measured at 500 μmol m^{-2} s-^{1}, giving a daily integrated photon flux of 28.8 mol m^{-2} d^{-1}. Atmospheric CO_2 in the chamber was the current ambient level. Plants were allocated to a drought and a control treatment, and all were first watered to field capacity every three days for four-five weeks. After this time, watering was completely stopped for individuals in the drought treatment. Controls continued to be watered every three days.

Shoot Senescence and Plant Death

Depending upon germination success, up to 20 individuals per species were assessed for senescence and death (Appendix 1). Six individuals per species were kept well-watered as controls. Plants were visually assessed for shoot senescence and plant death on alternate days. Senescence was counted as the days between transplanting and the full senescence of outer leaves and culms exposed to the external environment. The time until death was the number of days between transplanting and the point when there was no greenness visible on any of the leaves, culms or internal meristems, including those protected within leaf sheaths. To ensure death had occurred, plants were then re-watered and observed for two weeks to check for re-growth. Any plants that showed re-growth were discarded from the survival analyses. The difference between senescence and death was used as a measure of the time that meristems were able to stay viable in a dormant state, from now on referred to as meristem survival.

Trait Measurements

For each species, one individual from the drought treatment and one control was measured every three days. Between six and ten individuals were measured per species over time, depending on how long it took for each species to close its stomata. An open gas exchange system (LI-6400, LI-COR Inc, Lincoln, Nebraska, USA) was used to measure stomatal conductance on one of the newest fully expanded leaves from each individual. The leaf was clipped into the chamber and allowed to equilibrate for less than 3 minutes, to gain a “snapshot” of physiological behaviour under growth conditions. The chamber was set to a block temperature of 22 degrees, PPFD of 500 μmol m^{-2} s^{-1}, flow rate 300 μmol/s and CO_2 400 ppm to match the growth environment. Measures of stomatal conductance were always made between five and seven hours after the lights in the chamber turned on. For each species a threshold of gs <5 mmol m-2 s-1, was used to define stomatal closure and the pre-dawn water potential at this threshold, (Ψ_{crit}) was recorded following (Craine, Ocheltree et al. 2013).

After making stomatal conductance measurements, midday water potential was measured and a black plastic bag placed over the plant to keep it in humid, dark conditions, allowing measure of pre-dawn water potential (PDWP) to be taken the following morning. Leaf water potential is a measure of the resistance pathway for water movement, and is also a function of soil water availability, evaporative demand and soil conductivity. Pre-dawn water potential is an indication of soil-available water, as at this time the water potential of the leaf is in equilibrium with the soil. Water potentials were measured by removing one leaf, which was immediately placed in a Scholander pressure bomb (Model 600, PMS Instrument Company, Albany, Oregon, USA). Midday water potential was plotted against pre-dawn values, and a linear model fitted for each species, whose slope (σ) was used to indicate the relative sensitivity of plant hydraulic conductance to declining water availability (i.e.

hydraulic vulnerability to water deficits (Martinez-Vilalta, Poyatos et al. 2014). The intercept of this relationship (Λ) was taken as a simple measure of the maximum transpiration rate per unit of hydraulic transport capacity under well-watered conditions (Martinez-Vilalta, Poyatos et al. 2014)

Root measurements were made on six of these individuals per species. All of the growing media was removed by washing the roots, and the area, diameter and total length scanned using a root image analysis system (WinRHIZO, Regent Instruments, Quebec City, Canada). The roots were then dried at 70 °C for 24 hours, the dry mass determined and used to calculate specific root length (SRL = length / dry mass). Measures of size standardized mean relative growth rate at the 20th percentile (RGR) and specific leaf are (SLA = area / dry mass) for each species were taken from a previous, published experiment, in which plants had been grown to a similar size and developmental stage under resource-rich, well-watered conditions (Atkinson, Mockford et al. 2016).

Phylogenetic Reconstruction

For each the 18 species included in the study, sequences were obtained from Genbank for the chloroplast markers *trnLF, trnKmatK*, *ndhF* and *rbcL* (see Appendix 1). Each marker was individually aligned using Muscle (Edgar 2004) and manual adjustments made. The four datasets were then concatenated resulting in an alignment with 6476 base pairs. The best-fitting models of molecular evolution for each of marker were estimated using PartitionFinder (Lanfear, Calcott et al. 2012). The *HKY+I* model for the site *rbcL*, GTR+G for *trnKmatK* and *ndhF* and HKY+G model for *trnLF* were applied to produce a time-calibrated phylogenetic tree through Bayesian inference implemented in BEAST2 (Bouckaert, Heled et al. 2014). A log-normal relaxed clock was used, with priors on divergence times modelled by a Yule process. A single run consisting of a single MCMC chain were run for 10,000,000 generations. Convergence of the runs was assessed in Tracer and the first 10% of the run discarded as burn-in. All the trees sampled after burn-in were pooled, and common ancestor node heights were plotted on a maximum clade credibility tree, which was used for comparative analyses (Appendix 2).

Statistical analyses

All statistical analysis was conducted using R 3.0.2 (R Core Team 2013), using species means for each of the traits and environmental predictors. We used a principal components analysis (PCA) to look at the relationships between all traits, MAP and precipitation seasonality that might predict death and senescence. We conducted all analyses both including and excluding σ and Λ. We did not have these measures for all species and, as these traits were unimportant in explaining variation in either death or senescence, they were excluded from the PCA's in order to maximise sample size. Due to the smaller sample size for measurements of Ψ_{crit}, this trait was also not included in any of the PCA's. The PCA for the predictors used to explain variation in time until death included senescence, RGR, SLA, SRL, root diameter, leaf rolling, precipitation seasonality and MAP. Predictors included in the PCA used for the analysis of senescence were the same, but excluded senescence.

We used a phylogenetic generalised least squares model (PGLS) implemented in the caper package in R (Orme 2013) to assess the relationships between death and the scores from the first two axes of the PCA of predictors, and also between senescence and the scores from the PC axes. We also used PGLS analysis to look at the relationships between death, senescence and meristem survival with each individual trait and with precipitation seasonality and MAP. PGLS accounts for phylogenetic autocorrelation in model residuals that is expected due to common ancestry.

RESULTS

Mortality

At the end of the experiment, all the control plants that had been kept well-watered were alive. We wanted to know if interspecific variation in the number of days until death could be predicted by traits and climate. We performed a PCA to identify the main axes of variation in traits and climate and used the PC scores from this in a PGLS model to see if the main axes of variation could explain time until death. The first axis of the PCA explained 36% of the variation and the second explained 22%.

Traits loading most heavily on PC1 were senescence, MAP and RGR, with species having a slow growth rate, slow senescence and low MAP being to the left of this axis and species with a fast growth rate, fast senescence and high MAP on the right (Appendix 3a & 4a). Traits loading most heavily on PC2 were SLA, SRL and root diameter (Appendix 3a & 4a). Variation in days until death was strongly and significantly explained by PC1 (adjusted $r^2 = 0.369$, $\lambda = 0$, $P = < 0.05$, slope = -2.098, SE = 0.786, n = 13) (fig.1), but not PC2 (adjusted $r^2 = -0.08697$, $\lambda = 0$, P = ns, slope = 1.014, SE = 1.378, n = 13).

Next, we wanted to know more about the contributions of individual traits and climatic variables to variation in drought mortality, so performed a series of PGLS analyses on time until death, testing for relationships with each potential predictor (Table 1). As expected from the PCA analysis, a trade-off was evident between days until death and RGR, with slow growing species surviving the longest under drought conditions ($r^2 = 0.256$, $\lambda = 0$, $P = <0.05$, slope = -0.003, SE = 0.001, n = 13). In contrast, a positive relationship was evident between death and senescence, with plants that stayed green the longest also surviving the longest under drought ($r^2 = 0.334$, $\lambda = 0.275$, $P = <0.01$, slope = 0.417, SE = 0.135, n = 18) (Fig 4.1).

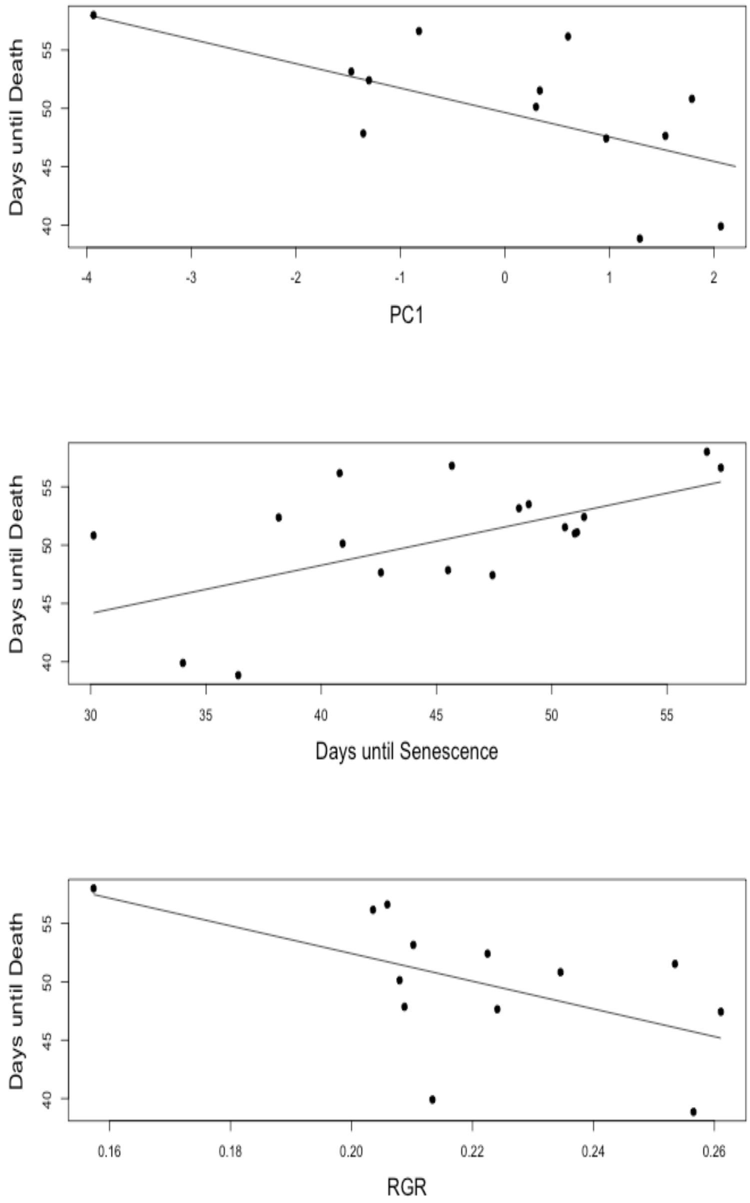

Figure 1. Relationships between the number of days until death and PC1 (adjusted $r^2 = 0.369$, $\lambda = 0$, $P = < 0.05$), number of days until senescence ($r^2 = 0.334$, $\lambda = 0.221$, $P = < 0.01$), and relative growth rate ($r^2 = 0.256$, $\lambda = 0$, $P = < 0.05$).

Two strategies were identified in relation to greenness: species for which the senescence of external aerial parts corresponded to plant death; and species that were able to extend survival beyond leaf senescence via persistent meristems. Although death was not directly associated with variation in climatic variables across all the species in the experiment, if the analysis was confined to those species with persistent meristems, meristem survival after full senescence was strongly and significantly related to MAP ($r^2 = 0.49$, $\lambda = 0$, $p = <0.05$, slope = 0.007, SE = 0.003, n = 9) (fig.4.2). Species that exhibited the longest meristem survival after leaf senescence occupied environments with a high MAP, but there was no significant relationship between meristem survival and seasonality.

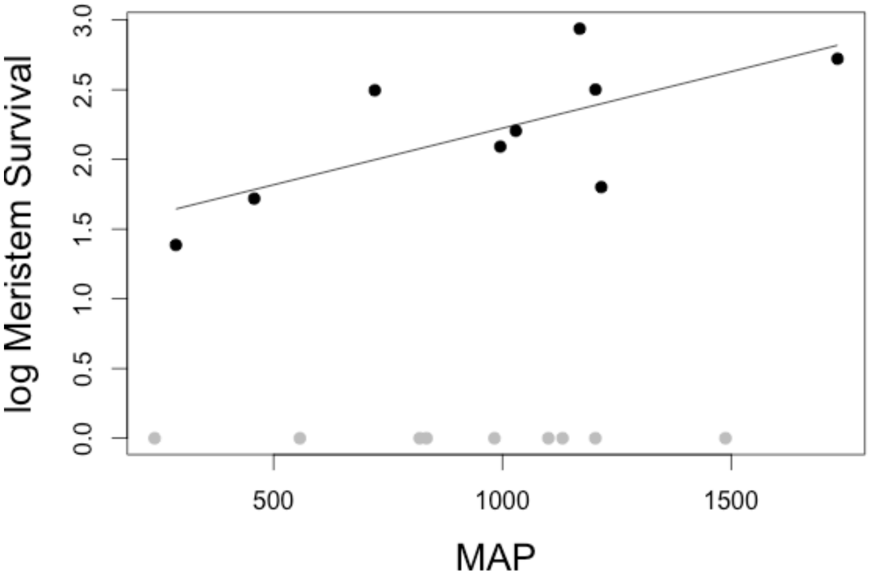

Figure 2. The relationship between meristem survival and climatic variables. Meristem survival was log transformed. Species with meristems that survived after senescence of exterior aerial parts (i.e. drought tolerators) are shown in black. In contrast, species in which meristems did not survive any longer than exterior aerial parts (i.e. drought avoiders) are shown in grey. The line shows the relationship between meristem survival and MAP for drought tolerators ($r^2 = 0.49$, $\lambda = 0$, $p = < 0.05$).

	Death				**Senescence**				**Meristem Survival**			
	r^2	**P ($\lambda = 1$)**	**λ**	**n**	**r^2**	**P ($\lambda = 1$)**	**λ**	**n**	**r^2**	**P ($\lambda = 1$)**	**λ**	**n**
MAP	0.023	ns	0	18	**0.203**	*	**0**	**18**	**0.490**	*	**0**	**10**
Seasonality	-0.011	ns	0	18	0.154	ns	0	18	0.187	ns	0	10
Root diameter	0.126	ns	0	18	**0.253**	*	**0**	18	-0.068	ns	0	10
σ	0.283	ns	0	12	-0.089	ns	0	12	0.275	ns	0	7
Λ	-0.099	ns	1	12	0.023	ns	1	12	0.044	ns	1	7
Ψ_{crit}	0.211	ns	0	8	0.211	ns	0	8				
SRL	-0.034	ns	0	18	-0.057	ns	0	18	-0.107	ns	0	10
SLA	0.113	ns	0	13	-0.088	ns	1	13	-0.118	ns	0	8
RGR	**0.256**	*	**0**	**13**	0.043	ns	1	**13**	0.111	ns	0	8
Senescence	**0.334**	**	0.275	**18**								

Table 1. The relationships between death, senescence, meristem survival and the traits hypothesised to predict their variation. Significant relationships are highlighted in bold, ‘*’, $P<0.05$; ‘**’, $P<0.01$; ‘***’, $P<0.001$. λ is a measure of phylogenetic signal in the residuals of the model. A value of 1 indicates strong phylogenetic signal. A value of 0 indicates no phylogenetic signal.

Shoot Senescence

Having established that senescence was a strong predictor of death, we then tested if variation in senescence could be explained by traits and climate. We again performed a PCA to identify the main axes of variation in traits and climate, but excluded senescence this time. The first axis of the PCA explained 35% of the variation in traits and climate, while the second explained 23%. Traits loading most heavily on PC1 were MAP, RGR and precipitation seasonality (rainfall of wettest minus driest month). On the left hand side of PC1 were slow-growing species from dry, unseasonal environments, and to the right were fast-growing species from wet, seasonal habitats. SLA, SRL and root diameter loaded most heavily on PC2 (Appendix 3b). The loadings of traits on each axis are shown in appendix 4b. Variation in shoot senescence was explained by PC1 (adjusted $r^2 = 0.355$, $\lambda = 0$, $P = <0.05$, slope = -3.069, SE = 1.156, n = 13). However, the relationship between senescence and PC2 was not significant (adjusted $r^2 = 0.0373$, $\lambda = 0$, P = ns, slope = 2.197, SE = 1.840, n =13). When looking at the relationship of senescence to individual predictors (Table 1), MAP was important in explaining interspecific variation in rates of senescence under drought (adjusted $r^2 = 0.203$, $\lambda = 0$, $P = < 0.05$, slope = -0.010, SE = 0.004, n=18), as was root diameter (adjusted $r^2 = 0.253$, $\lambda = 0$, $P = < 0.05$, slope = -54.847, SE = 21.120, n = 18) (fig. 3). Species that senesced quickly during the experimental drought treatment occupy wetter habitats and have larger root diameter (fig. 3). We also found that species with the ability to re-sprout from meristems (drought tolerators) had significantly wider root diameter than species that stayed green throughout the drought (drought avoiders) (F = 4.32, $p < 0.05$) (fig. 4.)

We found a negative but non-significant overall relationship between shoot senescence and Ψ_{crit}, with species that senesced quickly always having a low Ψ_{crit}, meaning that stomata stay open for longer with declining water potential (adjusted $r^2 = 0.211$, $\lambda = 0$, p = n.s, slope = 11.256, SE = 6.640, n = 8) (fig. 2). Species that stayed greener for longer showed a wider range of stomatal responses. However *Aeluropus lagopoides* and *Sporobolus indicus* were outliers to the overall pattern. These were the only species in the experiment to exhibit leaf rolling, and their leaves were unrolled to take the measurements of stomatal conductance. Leaf rolling, however, effectively reduces transpiration by decreasing the boundary layer conductance of the leaf, enabling stomata to stay open longer. When these two species were excluded from the analysis, the relationship became very strong ($r^2 = 0.8269$, $\lambda = 0$, $p = < 0.01$, slope = 18.844, SE = 3.780, n = 6), with species that senesced quickly leaving stomata open for longer (fig. 3).

Traits and Climate

We found no significant relationships between individual traits and MAP (Appendix 5).

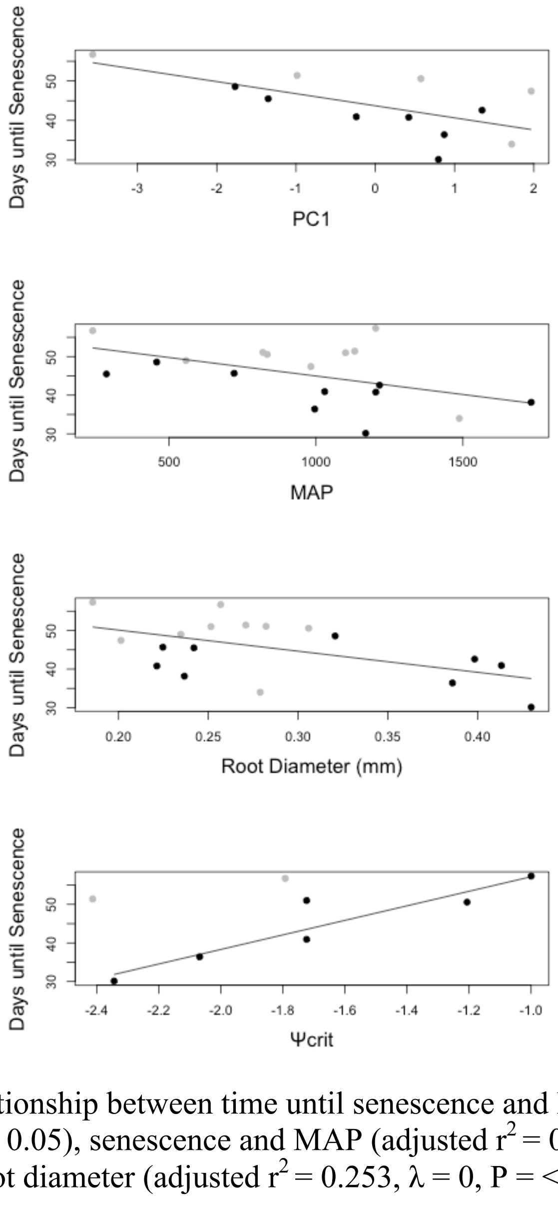

Figure 3. The relationship between time until senescence and PC1 (adjusted $r^2 = 0.355$, $\lambda = 0$, $P = < 0.05$), senescence and MAP (adjusted $r^2 = 0.203$, $\lambda = 0$, $P =*$), and senescence and root diameter (adjusted $r^2 = 0.253$, $\lambda = 0$, $P = <0.05$. In the top and

middle panels, species with meristems that were able to survive after senescence of exterior aerial parts (i.e. drought tolerators) are shown in black. Species without drought tolerant meristems (i.e. drought avoiders) are shown in grey. The relationship between Ψ_{crit} and days until senescence does not including leaf-rolling species ($r^2 = 0.827$, $\lambda = 0$, p = <0.01). In the bottom panel these species are shown in black and species that roll leaves are shown in grey.

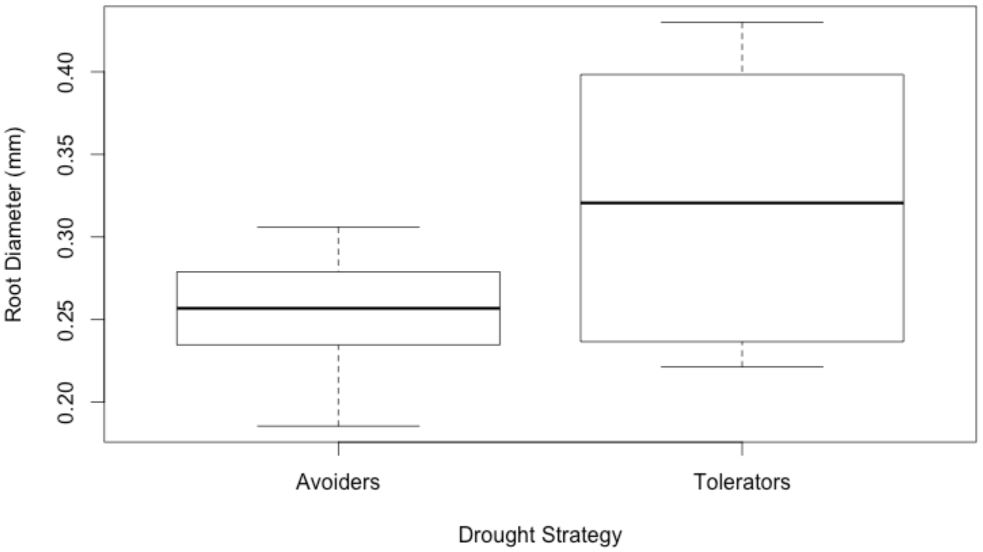

Figure 4. The difference in mean root diameter for drought tolerators (species with drought resistant meristems and the ability to resprout following full senescence) and drought avoiders (non-resprouting species). ANOVA revealed significant differences between the two strategies (F = 4.32, $p < 0.05$).

DISCUSSION

Growth Survival Trade-off

We evaluated whether mortality under drought could predict the position of species along a rainfall gradient and measured functional traits that may explain variation in mortality. Species varied widely in the time until death under drought, and there was a trade-off between rapid growth and longevity, such that species sorted along a gradient between rapid growth but early mortality, and long survival but slow growth rate. A trade-off between RGR and mortality caused by light as the limiting resource is well established among tree species in closed-canopy forests (Grubb 1977; Hubbell and Foster 1992; Poorter and Bongers 2006; Joseph Wright, Kitajima et al. 2010).

Previous work has also shown that drought and shade tolerance are both co-ordinated across species and are linked to hydraulic performance (Markesteijn, Poorter et al. 2011). The relationships between photosynthetic productivity and hydraulic performance are also well established at the leaf scale (Nardini and Salleo 2000; Brodribb, Holbrook et al. 2002). However, we think our experiment is the first to identify a growth-survival trade-off for plant growth and drought mortality.

Fast growth is competitively advantageous, and traits that are necessary for the rapid acquisition of resources and fast growth are incompatible with those required for drought survival. Physiological traits, for example high conductance to gas diffusion, are inextricably linked to a high transpiration rate and low water use efficiency (Orians and Solbrig 1977) because stomata control both carbon uptake and water loss. Furthermore there is a trade-off in the investment of resources to leaf versus root tissues (Orians and Solbrig 1977), which could generate a growth-survival trade-off because higher leaf allocation causes fast growth (Poorter and Remkes 1990; Ruiz-Robleto and Villar 2005), whereas greater root allocation is associated with drought tolerance (Markesteijn and Poorter 2009). At face value, these relationships might be expected to underpin the sorting of species along rainfall gradients characterized by an inverse relationship between the length and productivity of the wet, growing season, on one hand, and the duration and intensity of drought events on the other. However, this hypothesis was not supported by our data.

Drought Strategies

Neither growth nor mortality were related statistically to species distributions along global rainfall gradients. A similar pattern has also been observed in tropical forests whereby drought survival in itself is not correlated with MAP, because survival is primarily determined by deciduousness (Poorter and Markesteijn 2008). We found that senescence was strongly related to MAP, with plants that stayed greener for longer living in drier areas. We identified two strategies for survival. Some species maintained green shoots throughout the drought and full senescence of exterior parts coincided with plant death, which we refer to as "drought avoiders" because they avoid the adverse effects of water deficits by maintaining plant water potential as the soil dries. Drought avoiders use traits that increase access to water or delay water loss, for example narrow roots, which have a high surface area to volume which means that a larger volume of soil can be explored for the equivalent investment in root mass. Drought avoiders also exhibited early stomatal closure, to maintain plant water status and retain leaves throughout the drought (Levitt 1980). Other species, "drought tolerators" senesce quickly, however, survival could be extended via drought resistant meristems. These species do not maintain photosynthetic tissues throughout drought, but are able to persist via meristems that can remain alive at low water potential, and have are expected to have traits that facilitate resprouting.

The identification of these two strategies explains the absence of a relationship between death and MAP. Staying green for longer was the best strategy for survival as soil dried, and plants that stayed green for longer lived the longest. In habitats where water is limited, retaining green shoots as soil dries has the advantage that new leaves do not need to grow when rains occur, enabling photosynthesis to rapidly resume. In contrast, a drought tolerance strategy has the advantage of maintaining traits that are important for growth when rain is plentiful, for example high stomatal conductance. However the physiological properties needed for rapid growth are incompatible with drought avoidance, and it seems likely that drought is therefore avoided in an apparently dormant state throughout a long and predictable dry season.

Within the drought tolerating group of species, meristem survival under drought was associated with MAP but not seasonality. Species from the wettest habitats had the longest surviving meristems as soil dried. Savannas in high rainfall environments are associated with a predictable dry season and frequent fires, whereas the dry season in arid savannas is less predictable and fires are less frequent (Govender, Trollope et al. 2006). Rapid growth in mesic savanna produces large amounts of biomass to fuel fires and senescence during the dry season cures the fuel for fires. The ability to re-sprout from meristematic tissue is thus an important trait in structuring fire-controlled and drought-prone systems (Pausas, Pratt et al. 2016). Resprouting shrubs in drought and fire prone Mediterranean ecosystems have a wider root diameter (Paula and Pausas 2011), where carbohydrates are stored to support regrowth after disturbance (Schutz, Bond et al. 2009). Fire frequency and MAP are strongly related in savanna ecosystems. Ripley et al. (2015) found that resprouting rate after fire was correlated with the frequency of fires that a species experienced and also that those groups of plants with a higher proportion of green biomass *before* the fire re-sprouted the slowest *after* fire. These groups (Aristida and Danthonioideae) are known to inhabit dry environments (Visser et al. 2012). We found that drought avoiding grass species with drought tolerant meristems have wider root diameter than drought avoiders. Paula and Pausas (2011) found that differences in root structure between sprouting and non-resprouting shrubs reflect different foraging strategies, whereby non-resprouters more efficiently explore the upper soil layer via thinner, more branching roots. However, the root structure of resprouting species enabled carbon storage and deep soil penetration. It is therefore expected that grasses from wet, fire prone environments with a predictable dry season will senesce rapidly under drought. However, have the ability to rapidly resprout following drought and fire, facilitated by drought and fire resistant meristems, wide roots and stored underground carbon reserves.

Traits and Drought Performance

A trend for decreasing root diameter with lower rates of canopy senescence was observed across all species. The ability to effectively take up water via roots is an important part of drought tolerance (Rieger and Litvin 1999), not just in terms of storage, but also in determining maximum rates of gas exchange (Brodribb and Feild 2000). Water transport in roots of small diameter is more efficient than in wider roots. This is because soil-root hydraulic conductivity is increased by having a larger surface area in contact with the soil, (Rieger and Litvin 1999) and is expected to increase water acquisition as soil dries (Wasson, Richards et al. 2012).

Of the other traits measured, although the sample size was small, our results indicate that stomatal regulation may also be important in remaining green for longer, but only after species that rolled leaves were removed from the analysis. The rolling of leaves reduces boundary layer conductance and enables stomatal conductance on the rolled leaf surface to remain higher for longer as the drought progresses (Taylor, Ripley et al. 2014). This is because a microclimate is created within the rolled leaf, where humidity and boundary layer resistance near the leaf surface are increased (Kadioglu and Terzi 2007). By rolling leaves, plants are able to remain photosynthetically active under drought, reducing the risk of carbon starvation, whilst limiting water loss (Knapp 1985).

We found no direct relationship between Ψ_{crit}, root diameter, nor any other functional traits with MAP. Other traits are also important in drought performance, but were beyond the scope of this study. For example, osmotic adjustment helps to maintain cell turgor and therefore sustain physiological processes, including stomatal opening and photosynthesis under drought (Blum, Mayer et al. 1983; Ludlow and Muchow 1990). A great diversity of co-varying traits co-exist in dry habitats (Hernandez, Vilagrosa et al. 2010), and different combinations of these traits can act to achieve the same effect.

In conclusion, this study identifies a trade-off between rapid growth and survival under drought. However, neither growth nor survival could explain species distribution along rainfall gradients because this was predominantly associated with senescence and meristem persistence. Species whose leaf canopies stayed green for longer were associated with arid environments, whereas species that senesced quickly but persisted for longer without leaves grew in wet regions. Based on these findings, two strategies were identified in response to declining soil water: (1) drought avoiders that retained green photosynthetic shoots throughout drought; and (2) drought tolerators with quickly senescing shoots but with the ability to extend survival via drought resistant meristems. It was this ability of some species to extend their survival via drought resistant meristems that resulted in a decoupling of the growth survival trade-off from MAP. However, staying green for longer resulted in the longest survival, and traits that facilitated this were small root diameter and either leaf rolling or isohydric stomatal regulation. Plants that senesced quickly had wider root diameter and anisohydric stomatal regulation. The traits of plants that senesced quickly enable rapid growth but are incompatible with survival under drought. However, we hypothesise that drought tolerant meristems and wide roots are likely to facilitate resprouting after drought and fire in areas of seasonally high MAP and frequent fires. Our results suggest that the global distributions of savanna grasses can be predicted by variation in rates of senescence and meristem survival, root traits and stomatal strategy.

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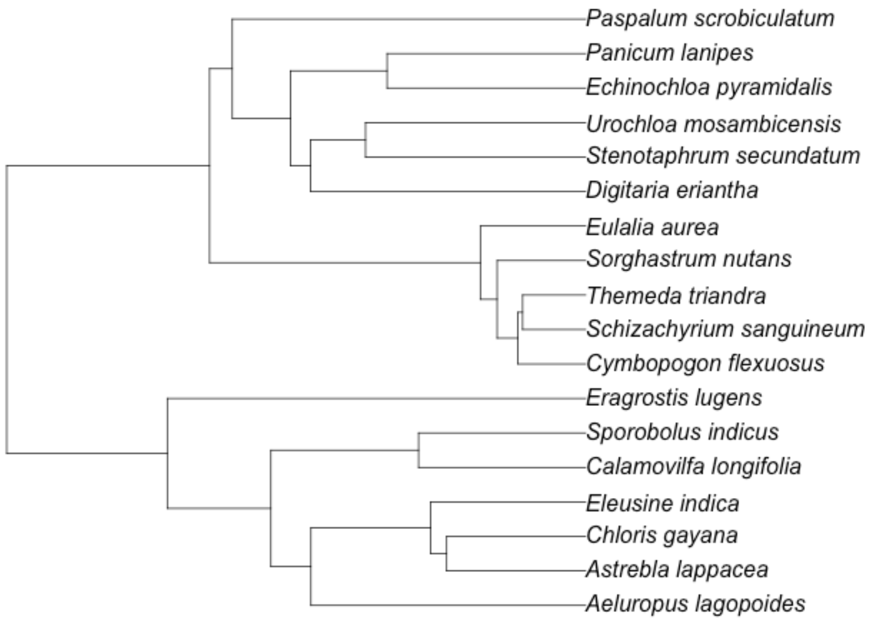
ACKNOWLEDGMENTS

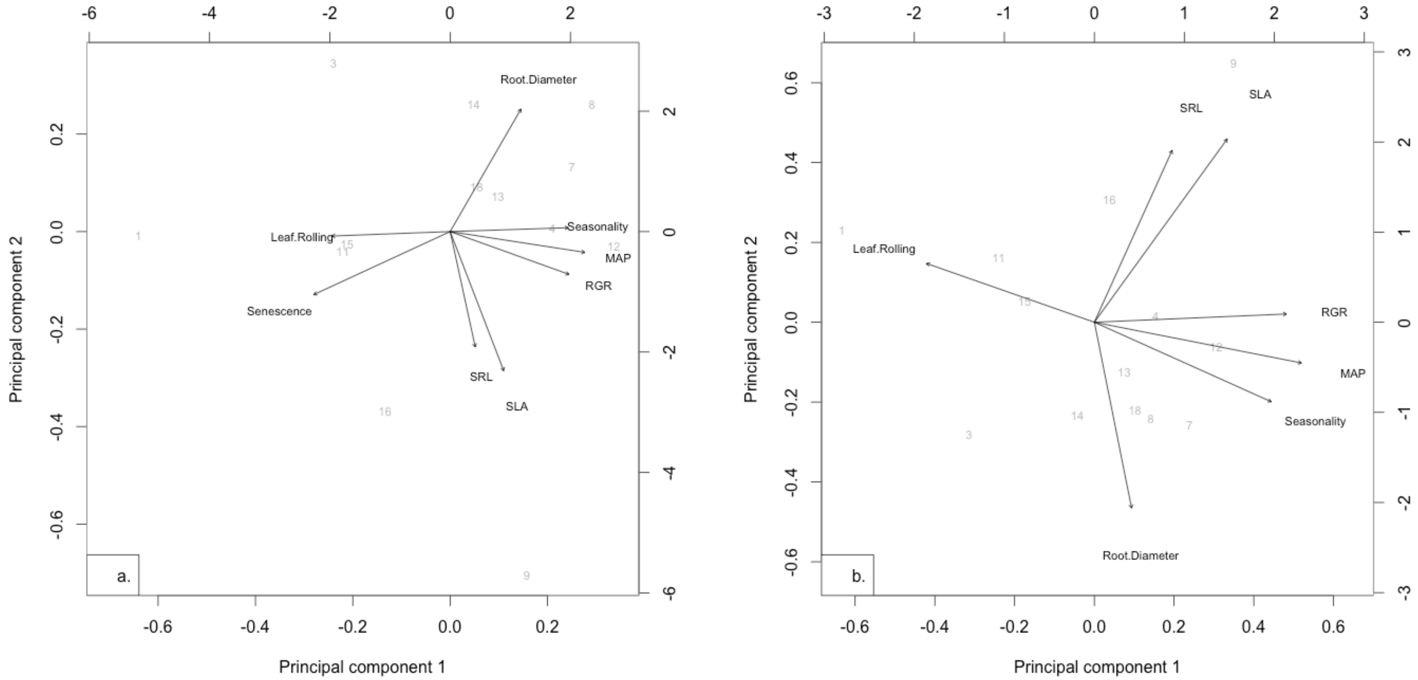
This work was funded by Project Sunshine (University of Sheffield).

APPENDICES

Species	***No. Individuals***	***No. Geo-referenced records***	***Genbank Accession Numbers***			
			rbcL	***ndhF***	***matK***	***trnL***
Aeluropus lagopoides (L.) Thw.	20	34		GU359591.1		GU360013.1
Astrebla lappacea (Lindl.) Domin	9	306	JN681651.1	JN681599.1	AF144589.1	GU360009.1
Calamovilfa longifolia (Hook.) Scribn.	19	10	EF125104.1	GU359716.1		EF137559.1
Chloris gayana Kunth	20	419	AM849409.1	AM849205.1	AF164424.1	KR738428.1
Cymbopogon flexuosus (Nees) W. Watson	19	16	KP087913.1	AF117404.1	KT309064.1	DQ004971.1
Digitaria eriantha Steud.	20	303	HE573375.1	HE573497.1	HE574068.1	KP057660.1
Echinochloa pyramidalis (Lam.) Hitchc. & Chase	17	269	KR737524.1		KR735143.1	KR738671.1
Eleusine indica (L.) Gaertn.	17	345	EF125108.1	AM849151.1	AF144580.1	EF156691.1
Eragrostis lugens Nees	20	198		GU359704.1		GU990387.1
Eulalia aurea (Bory) Kunth	6	2812	AM849410.1	AM849213.1	HE574011.1	
Panicum lanipes Mez	7	58				AY142732.1
Paspalum scrobiculatum L.	10	565	LN907994.1	LN908157.1	LN906759.1	AB817562.1
Schizachyrium sanguineum (Retz.) Alston	12	273	LN908005.1	LN908168.1	LN906770.1	DQ004993.1
Sorghastrum nutans (L.) Nash	15	318	FR821342.1	FR821360.1	FR821326.1	DQ005001.1
Sporobolus indicus (L.) R. Br.	17	332	HE575834.1	HE575785.1	HE575870.1	EF156732.1
Stenotaphrum secundatum (Walt.) Kuntze	16	369	EF125139.1	AY029684.1	KC123431.1	EU939985.1
Themeda triandra Forsk.	15	434	LN908022.1	LN908185.1	LN906787.1	DQ005005.1
Urochloa mosambicensis (Hack.) Dandy	15	370		FJ486516.1		GU594532.1



Appendix 2. The phylogenetic tree showing relationships between the taxa included in this study produced by Baysian inference.



Appendix 3. (a) Principal components analysis of the traits root diameter (mm), mean annual precipitation (MAP), precipitation seasonality (rainfall of wettest minus driest month), RGR (relative growth rate), specific root length (SRL), specific leaf area (SLA), number of days until dull senescence and whether species rolled leaves under drought. PC scores were used in subsequent analyses to predict variation in the number of days until death between species (b) the principal components analysis of traits root diameter (mm), mean annual precipitation (MAP), precipitation seasonality (rainfall of wettest minus driest month), RGR (relative growth rate), specific root length (SRL), specific leaf area (SLA) and whether species rolled leaves under drought. PC scores were used in subsequent analyses to predict variation in the number of days until full senescence between species

Appendix 4a. Loadings of each traits on each of the first two PC axes identified following principal components analysis of the traits relative growth rate (RGR), specific leaf area (SLA), specific root length (SRL), root diameter, leaf rolling, senescence and the environmental variables MAP (mean annual precipitation), and seasonality of rainfall.

	PC1	PC2
RGR	0.402	-0.184
SLA	0.181	-0.599
SRL	0.085	-0.496
Root Diameter	0.240	0.527
Leaf Rolling	-0.400	-0.019
MAP	0.456	-0.090
Senescence	-0.461	-0.271
Seasonality	0.400	0.017

Appendix 4b. Loadings of each traits on each of the first two PC axes identified following principal components analysis of the traits relative growth rate (RGR), specific leaf area (SLA), specific root length (SRL), root diameter, leaf rolling and the environmental variables MAP (mean annual precipitation), and seasonality of rainfall.

	PC1	PC2
RGR	0.474	0.025
SLA	0.328	0.555
SRL	0.192	0.520
Root Diameter	0.092	-0.562
Leaf Rolling	-0.414	0.178
MAP	0.511	-0.123
Seasonality	0.436	-0.241

Appendix 5. The relationship between traits and mean annual precipitation.

	R^2	*P*	λ
Root diameter	-0.0534	ns	0
σ	-0.0602	ns	0
SRL	0.1159	ns	0
SLA	-0.0802	ns	0
RGR	0.0728	ns	0
Λ	0.0738	ns	0
Ψcrit	-0.2487	ns	0

CHAPTER FIVE

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Stomatal regulation explains senescence under drought in savanna grasses

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STATEMENT OF CONTRIBUTIONS

The initial idea and experimental design was developed by the candidate, Prof. Colin Osborne, Prof. Brad Ripley and Dr Gavin Thomas. All data collection, data analyses and writing were carried out by the candidate with general editorial advice by Prof. Colin Osborne and Dr Gavin Thomas.

STOMATAL REGULATION EXPLAINS SENESCENCES UNDER DROUGHT FOR SOUTH AFRICAN SAVANNA GRASSES

Authors: Emma Jardine, Brad Ripley, Gavin Thomas, Colin P. Osborne

ABSTRACT

In chapter four we found a trade-off between the retention of green leaves throughout drought via stomatal closure and leaving stomata open yet senescing with declining water availability. These results were obtained in a controlled environment. In real plant communities and ecosystems plants are simultaneously subjected to fluctuations in multiple biotic and abiotic factors that can influence trait responses to environment.

We conducted a common garden experiment where savanna grasses were subjected to a controlled drought in an area of South Africa where they naturally grow to test whether our findings in chapter four were replicated under more natural conditions where plants experienced natural fluctuations in light and temperature as the wet season moved into the dry.

Our results confirm under weather conditions that plants which stay green for longer under drought do so via early stomatal closure whereas plants that senesce quickly leave stomata open for longer. We identify a trade-off between remaining green under drought and continuing carbon uptake through stomatal regulation, which applies across controlled and more natural settings and lends support to our previous findings. We do not however find the previously identified relationships between senescence and MAP which may be due to the scale of the analyses or the limited range of MAP sampled.

KEYWORDS

Senescence, stomatal closure, precipitation, drought

INTRODUCTION

Plants have evolved physiological responses to help them adapt to and survive times of excess solar radiation, extremes of temperature, nutrient limitation, herbivory or drought. Many studies investigating the physiological behavior of plants in response to environmental conditions are performed in controlled environments in growth chambers, as this allows variation in traits that are due to factors other than the treatments applied to be minimized. Under more natural conditions, plants are simultaneously subjected to alterations in nutrient availability, photoperiod, temperature, light intensity and biotic interactions, all of which can induce plastic responses in measured traits and environmental interactions via acclimation or developmental plasticity (Kozlowski and Pallardy 2002). In reality plants may exhibit a complex network of interlinked responses to environmental variation (Valladares and Pearcy 1997). Studies of how plants respond to their environment under natural conditions are therefore important to complement those conducted in growth chambers, and are necessary to fully understand the trait responses of plants to environmental variation that may explain contrasting species distributions.

Phenotypic differences have been demonstrated between lab grown and field grown plants, with lab grown plants exhibiting faster growth rates, higher nitrogen concentrations and altered morphology (Poorter, Fiorani et al. 2016). Furthermore plants in the field grow at higher densities than those grown in pots, are affected by competitive interactions with other plant species and also by interactions with pests and symbionts. Responses to environmental manipulation that have appeared strong under laboratory conditions can be weakened when studied in the field (Long, Ainsworth et al. 2006). However, other studies conducted in controlled environments (Taylor, Ripley et al. 2011) have yielded very similar results to those found in the field (Taylor, Ripley et al. 2014). In a previous study conducted in a growth chamber, we showed that variation in senescence is important in explaining a species position along a global rainfall gradient and that variation in senescence could be explained by differences in stomatal regulation under drought. It is however unclear to what extent our observations will translate to a more natural setting in the seasonal sub-tropics or warm temperate regions, where the onset of drought in the dry season coincides with falling temperature and shortening daylength. Throughout evolution environmental cues may be coopted. For example in the temperate zone senescence may be cued by day length, however deciduousness is thought to be an adaptation to low temperatures.

Senescence can result from aging. However, functionally it is also an adaptation whereby nutrients are relocated from one part of a plant to another and can be induced by not only drought but other environmental cues such as nutrient limitation, waterlogging, too much or too little light and extremes of temperature. In perennial species, remobilisation of nutrients to young leaves or to storage tissues allows the plant to either retain young leaves or regrow completely from stored reserves after rain (Diamantoglou and Kull 1988; Yang, Zhang et al. 2002; Munne-Bosch and Alegre 2004). An alternative strategy is to tolerate drought and maintain water potential via stomatal closure, in order to maintain photosynthetic tissues that can become photosynthetically active immediately upon cessation of drought without the need for regrowth from stored reserves. These different responses may provide advantage under different rainfall regimes. For example senescence is a long term and irreversible response and so may be the best strategy for surviving a long, intense drought. Stomatal closure is rapidly reversed and so is likely to be more advantageous for surviving short and intermittent drought.

In chapter 4, we found a trade-off between the retention of green leaves throughout drought via stomatal closure on one hand, and leaving stomata open yet senescing with declining water availability on the other. These results were obtained through an experiment using a global sample of savanna species in a controlled environment, where plants had no natural variation in daily photoperiod or temperature that may also influence these processes of stomatal regulation and leaf senescence. We therefore conducted another experiment under more natural conditions, to test whether the relationships we observed between stomatal regulation, senescence and rainfall regime with plants grown in a controlled growth chamber, are consistent with results found when plant physiological processes were also being regulated by natural fluctuations in light and temperature. We were interested in measuring savanna grass species and so the experiment was conducted in the Eastern Cape of South Africa, close to where the C_4 grass species studied grow naturally.

METHODS

Plant materials and growth conditions

A total of 48 individuals representing eight species were collected from the wild from two sites at Middelburg (25°46.6°S29°28.0°E) and Grahamstown (33°19.8°S26°31.4°E), in the Eastern Cape of South Africa between March and April 2016. These sites were chosen because they differed in their rainfall regime. Species were selected that were the dominant, perennial grasses at each site, and also that represent major phylogenetic clades because different clades are known to have different associations with precipitation across South Africa (Visser, Woodward et al. 2012). Grasslands in the vicinity of Middelburg are characterized by a mean summer annual rainfall of 654 mm and winter drought, a mean annual temperature of 15.8°C and, on average, 28 days of frost. In contrast, grasslands near Grahamstown receive a mean annual rainfall of 545 mm with a bimodal distribution peaking in October–November and February-March, a mean annual temperature of 16.5 °C and a mean annual frost incidence of 5 days per year (Mucina 2006).

Plants were dug up with as many of the roots in tact as possible, soaked in a bucket of water for 5 minutes, all leaves were cut off and plants placed into plastic bags for no more than 24 hours before being planted into 7 litre pots filled with topsoil from the Eastern Cape. A total of twelve individuals per species were divided equally among control and drought treatments in a randomised block design. All plants were watered to field capacity every three days and allowed to establish for a period of between 4-6 weeks in the polytunnel at Rhodes University Grahamstown, before initiating a controlled drought. Temperatures experienced by plants in the polytunnel are slightly higher than those experienced in the field, but are representative of natural conditions (Ripley, Donald et al. 2010). Although the abiotic conditions of the polytunnel closely resembled those of the field, they do not include the effects of competition for light, water and nutrients that would be encountered in the field. The experiment was conducted at the onset of the dry season in an area of South Africa where species would naturally be subjected to water deficit. The experiment took place in an area where C_4 savanna grasses naturally occur.

Managing the drought

The soil water content at field capacity was calculated to be 22%. Three spare plants of each species had all soil washed from their roots and were weighed to obtain a mean plant weight for each species calculated. This plus the weight of the pot was subtracted from the total weight of each potted plant in the experiment in order to calculate the weight of soil and water at field capacity (water being 22% of this) for each individual. After the initiation of the drought, each individual was weighed every three days and water added so that the percentage soil water content was reduced by from 22% by 19.6%, over the course of 21 days. Throughout the drought, controls were watered to field capacity every three days.

Trait Measurements

Leaf gas exchange was measured on the youngest fully expanded leaves of tillers using a portable open gas exchange system (LI-6400; LI-COR Bio-sciences, Lincoln, NE, USA). The chamber was set to a block temperature of 25 degrees C, PPFD of 1000 μmol m^{-2} s^{-1}, flow rate 300 μmol/s and CO_2 400 ppm to match as closely as possible the growth environment in the polytunnel. Leaves were clipped into the IRGA and left for readings to stabilise before each measurement was taken. All physiological measurements were made between 8.30 am and 2.30 pm at five time points between days 0 and 21 of the drought and always on one of the newest fully expanded leaves from each individual.

To measure leaf senescence, a 20-point quadrat was placed over the plant and the uppermost leaf that touched each point was visually assessed for percentage leaf senescence. A mean of senescence across these 20 leaves was then used as a measure of whole canopy senescence for each individual on each day of measurement. Measurements were made at five evenly spaced time points throughout the drought and we took the species mean whole canopy senescence on day 21 of the drought as our measure of whole canopy senescence.

Environmental data

The precipitation niche of each species within the African continent was calculated using data obtained from the Biodiversity Information Facility web portal (http://www.gbif.org/) via the R statistical computing package rgbif (Chamberlain, Ram et al. 2015). For each geo-referenced record of each species, a number of cleaning steps were undertaken on the data, as described in chapter three. The mean annual precipitation (MAP) for each of the cleaned points was extracted from the WorldClim database (Hijmans, Cameron et al. 2005) using the R package 'raster' (Hijmans 2015) and the mean for each species calculated as a measure of the precipitation niche within Africa.

Statistical Analyses

A large, published grass phylogeny (Spriggs, Christin et al. 2014) incorporating 3595 taxa, constructed from 14 separate phylogenies (each representing a main grass lineage) was pruned to include only the taxa in our study (fig1). This tree was used in a pgls analysis which accounts for phylogenetic autocorrelation implemented in the R package caper (Orme 2013), to look at the strength and direction of relationships between rates of senescence and stomatal closure, stomatal closure and MAP, and senescence and MAP.

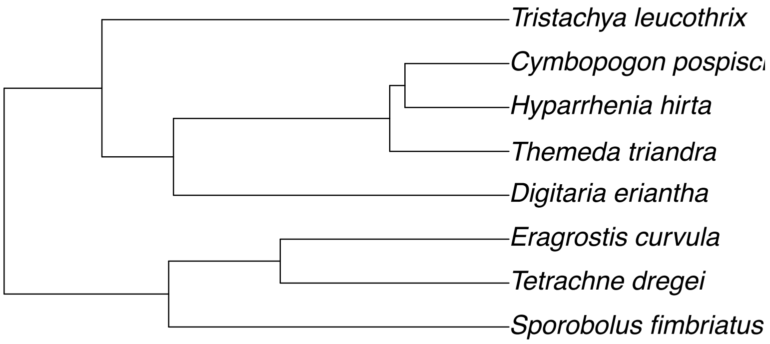

Figure 1. Phylogenetic tree showing the relationships between taxa included in this study.

RESULTS

Stomatal conductance and senescence for the drought treatment and controls are shown in figures 2 and 3 respectively. At the end of the drought, ANOVA revealed that all of the plants subjected to the drought treatment had senesced more than the control plants (F = 11.56, p = <0.01). However, species differed in their responses to the drought, with whole canopy senescence on day 21 varying from 13 % to 48%. Species also differed in their stomatal regulation (fig 2). For example, after 21 days of drought *Cymbopogon pospischilii*, one of the more slowly senescing species, did not differ from the control in its stomatal conductance. However for *Sporobolus fimbriatus*, a quickly senescing species, the difference was very pronounced (fig 3).

We first wanted to see whether variation in rates of senescence under drought could be predicted by stomatal closure. We used the species mean ratio of the stomatal conductance of the controls to drought treatment on day 21 of the drought as our measure of rate of stomatal closure. Our measure of senescence was the percentage whole canopy senescence that drought treated plants displayed on day 21 of the drought. There was a strong and significant relationship between senescence and stomatal regulation, with plants that senesced the fastest under drought leaving stomata open for longer ($r^2 = 0.496$, $\lambda = 0$, p = *, slope = 2.865, SE = 1.020). (fig 4). Next we tested to see whether variation in senescence and stomatal regulation could explain a species precipitation niche across the African continent There was a non significant relationships between senescence and MAP ($r^2 = 0.206$, $\lambda = 1$, p = ns).. There was also no relationship between stomatal closure and MAP ($r^2 = -0.1$ $\lambda = 0$, p = ns).

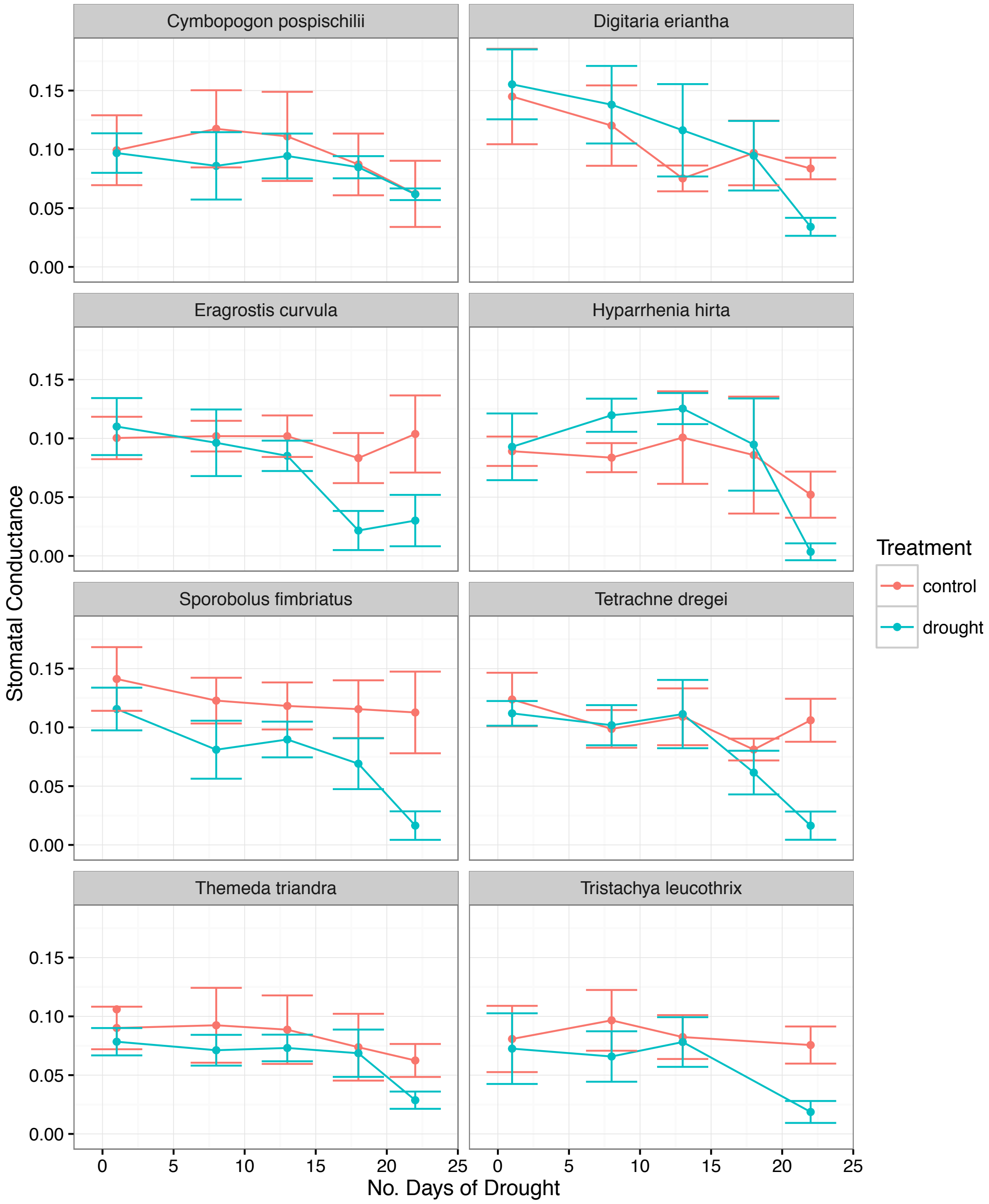

Figure 2. Stomatal conductance of the droughted and control plants throughout the controlled drought.

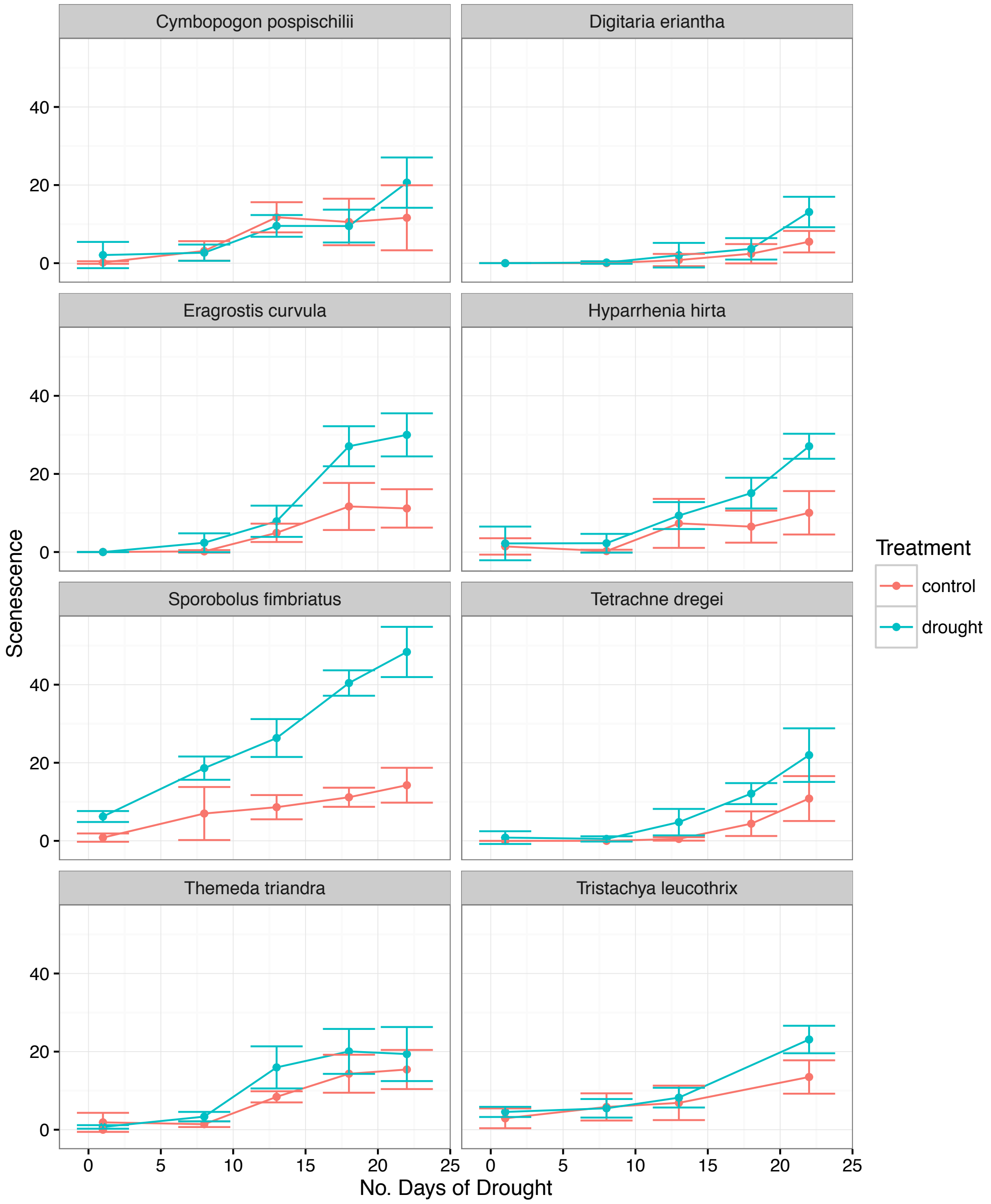

Figure 3. The percentage whole canopy senescence for the drought and control plants throughtout the controlled drought.

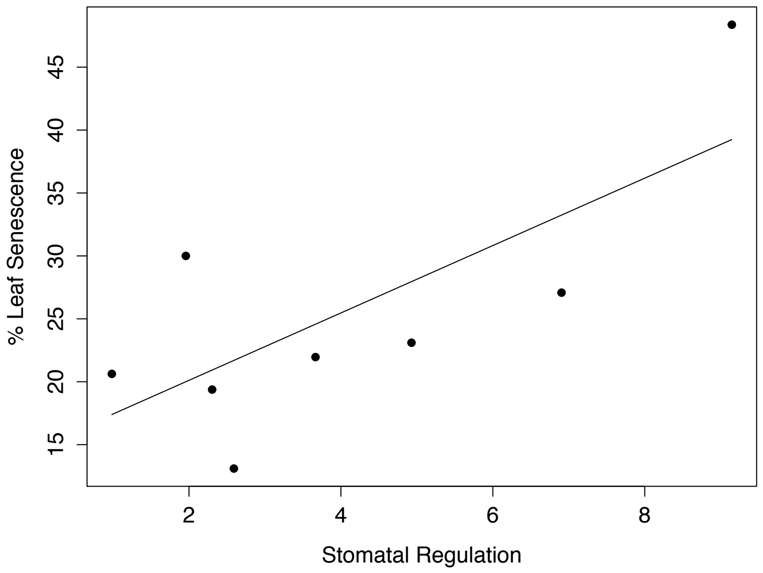

Figure 4. The relationship between percentage whole canopy leaf senescence to stomatal closure on day 21 of the drought ($r^2 = 0.496$, $\lambda = 0$, p = *, slope = 2.865, SE = 1.020). We used the species mean ratio of the stomatal conductance of the controls to drought treatment on day 21 of the drought as our measure of rate of stomatal closure. Our measure of senescence was the percentage whole canopy senescence that drought treated plants displayed on day 21 of the drought.

DISCUSSION

Through a common garden experiment we have shown that the global relationship previously observed between stomatal closure and senescence under drought in a controlled environment, operates against a background of changing season. This mechanism is operational for a regional flora moving from a wet summer season to dry winter season, with daily fluctuations in photoperiod and temperature, in an area where C_4 savanna grass species naturally grow. Dry seasons are important in structuring savanna grassland because they are the time when the biomass produced during the rainy season cures to make abundant fuel for fires. All savanna grasses must therefore be able to survive periodic drought events.

ABA is a hormone involved in the responses of plants to abiotic stresses, which includes drought (Fujii and Zhu 2009). ABA plays a role in both inducing both short-term reactions to water deficit such as stomatal closure, or more long-term responses, such as senescence or dormancy, and we identify a trade-off between these two responses. Although leaf shedding is often viewed as sensitivity to drought, both senescence and stomatal regulation increase plant survival under drought (Volaire and Norton 2006; Fujii and Zhu 2009). Leaf senescence, which involves the transfer of nutrients from the senescing leaves to meristems. An extreme form of this is summer dormancy (Volaire and Norton 2006), whereby nutrients are relocated from storage-organs to fuel regrowth with the cessation of drought. Early stomatal closure under drought represents an alternative strategy that helps to maintain the water potential of new and old photosynthetic tissues and reduce the risk of hydraulic failure, but at the cost of continued carbon assimilation (McDowell, Pockman et al. 2008).

In chapter four we revealed a significant relationship between variation in rates senescence under drought explained by differences in stomatal closure, and a species position along a global rainfall gradient, calculated as the mean MAP for the global range of the species. Plants that stay greener for longer via early stomatal closure, occupy drier environments and quickly senescing species are found in wetter environments at the global scale. We did not uncover the same relationship between senescence and MAP for species across Africa. One possible explanation for this is the smaller sample size for this study. Alternatively, these contrasting results may be due to the different scale of the analysis. Results from previous work included species covering a rainfall gradient from 240 mm per year to 1732. The mean annual precipitation for species in this study ranged from just 558mm to 880mm. It is possible that sampling a broader range of MAP is needed to see the relationship.

Our results add mechanistic detail to understanding plant responses to drought. We identify a trade-off between remaining green under drought and continued carbon uptake through stomatal regulation that applies across controlled and more natural settings. However, we show that the strength of trait-environment relationships may differ depending upon the scale of analysis.

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CHAPTER SIX

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GENERAL DISCUSSION

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STATEMENT OF CONTRIBUTIONS

The general discussion was written by the candidate with editorial advice from Prof. Colin Osborne and Dr Gavin Thomas.

GENERAL DISCUSSION

DISCUSSION

The traditional explanation for why different species are found in contrasting habitats is that species are adapted to a particular niche (Hutchinson 1957; Wright 2002; Chase and Leibold 2003; Silvertown 2004). On the other hand, neutral theories assume that all organisms are equal, and that only stochastic processes e.g. random dispersal, the birth and death of individuals, and the number of organisms in a community are important in community assembly (MacArthur and Wilson 1967; Hubbell 2001). From an adaptationist point of view, plant traits are important in explaining why different species grow in contrasting environments. Conversely, if neutral processes are more important, traits may be of little value in predicting how vegetation will respond to global change. Furthermore if only neutral processes explain ecosystem assembly we might not expect vegetation to respond to global change at all. The ways in which functional traits adapt species to environmental factors are therefore of the utmost importance. However, the significance of environmental factors in driving functional gradients within biomes and across continents remains poorly understood. Growth survival trade-offs have proved important in explaining plant-environment relationships, but work in this area has to-date focussed intently upon leaf economics (Grime 1977; Herms and Mattson 1992; Reich, Walters et al. 1997; Craine, Tilman et al. 2002; Westoby, Falster et al. 2002). When relationships are not observed between economic traits and environment, it does not however mean that other relationships do not exist; these other axes of variation which may be important in explaining contrasting species distributions are far less well understood.

The aims of this thesis were to identify which dimensions of the niche are important in sorting plant species across spatial gradients and to identify which traits are important in adapting species to these niche dimensions at global scales. I asked the following questions: How do traits that reflect different strategies of resource acquisition and use respond to climate and soil nutrients? I explore whether the distributions of traits are influenced by disturbance in the form of fire and grazing. I also ask which traits beyond the leaf economic spectrum are important in explaining a species precipitation niche. The main findings of this thesis are summarised in figure 1, which shows the relationships between traits, and between traits and the environment.

Identifying traits that explain variation in relation to environment in as many settings as possible has been described as the holy grail of ecology and the results of this study reveal that LNC or C:N ratio have predictable responses to disturbance, soil and climate across regions of different biogeographic and evolutionary history. However, other highly correlated traits (SLA, LTS) do not necessarily share the same relationships with environment (chapters two and three) and, at global scales, evolutionary history provides a stronger explanation of economic trait variation than contemporary environment (chapter 2). The thesis also reveals new dimensions of trait variation at the global scale. I show that the position of a species along a rainfall gradient is correlated with variation in canopy senescence under drought, and that the traits determining rates of senescence are stomatal regulation and root architecture (chapters four and five). Crucially these results indicate that understanding the distribution of functional traits requires a coordinated consideration of traits, evolutionary history and environment.

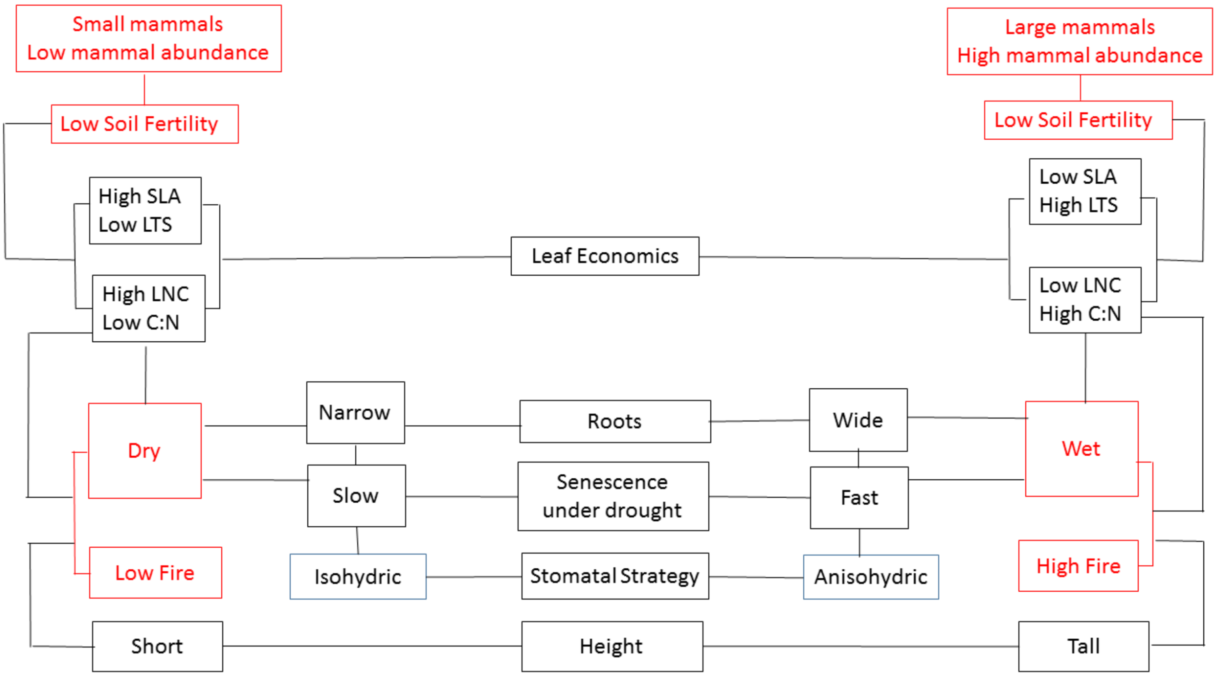

Figure 1. The relationships between traits, shown in black, and environment, shown in red. Boxes that are linked are shown in this thesis to be related to one another. High fire refers to fire frequency, wet or dry refers to MAP, hieght describes plant maximum culm height.

Precipitation is a primary driver of variation in aboveground productivity across biomes

(Knapp and Smith 2001) and it was therefore expected that traits reflecting a competitive strategy of resource acquisition and use would be positively correlated with gradients of MAP. Across the African continent, species from wet, productive and fire prone environments exhibit tall stature, indicative of a competitive strategy for light capture (chapter 3). However, at global scales the relationship between height and climate alone is much weaker when fire is not also considered (chapter 2). Leaf nitrogen or C/N ratio responds to gradients of MAP (although these traits are more strongly related to soil nutrient). However, despite being highly correlated with LNC and C:N ratio, SLA and LTS are not explained by climate and only weakly associated with gradients of soil and grazing (chapters two and three). SLA is a widely measured plant trait that is often used as a proxy for growth rate, and a high SLA is commonly accepted as a key trait reflecting a competitive strategies of resource acquisition and use (Wright, Reich et al. 2004). However, a recent study at the global scale found no relationships between SLA and growth rate (Paine, Amissah et al. 2015). Furthermore, Forrestel, Donoghue et al. (2017) found no association between SLA and rainfall gradients, showing instead that traits can act in different combinations, resulting in the same relationship between precipitation and productivity across regions .

A species niche is multi-dimensional, therefore the conclusion that climate is unimportant in determining growth – survival trade-offs responsible for species distributions should not be drawn from these results. This raises the question of what role climate has to play in influencing growth and survival along climatic gradients and which traits or trait combinations reflect this? In chapters four and five I investigated other axes of variation beyond the leaf economic spectrum to test whether physiological responses to drought could better explain a species position along a global rainfall gradient than economic or size related traits. I identified a trade-off between growth and survival under drought that was not correlated with rainfall. I instead showed that variation among species in their rates of senescence was correlated with species position along a rainfall gradient, and that senescence could be explained by stomatal regulation and leaf rolling. Stomatal closure and/or leaf rolling are both strategies that enable species to maintain photosynthetic tissues with declining water potential and therefore enhance survival during drought. Plants have evolved a variety of physiological innovations to tolerate or avoid drought and this may also include osmotic adjustment. Osmotic adjustment (the accumulation of solutes in cells as water potential declines) can sustain photosynthesis via turgor maintenance throughout progression of a drought. Osmotic adjustment has been implicated in maintaining stomatal conductance, photosynthesis, leaf water potential and delaying leaf rolling under drought (Hsiao, Otoole et al. 1984; Chaves and Oliveira 2004). This provides a physiological mechanism whereby some photosynthesis can be maintained under water deficit and may be associated with the moisture environment of a species range (Ludlow, Chu et al. 1983).

Considerable variation between species has been observed in their ability to osmotically adjust (Turner 1979) and individuals may also vary in osmotic adjustment between elongating and expanded leaves (Munns, Brady et al. 1979). In grasses, enclosed meristems are protected from evaporative desiccation by older leaf sheaths, but elongating leaves are also able to tolerate declining water potential via osmotic adjustment (Munns, Brady et al. 1979; Riazi, Matsuda et al. 1985; Volaire and Thomas 1995; Volaire, Thomas et al. 1998). In chapter four, I showed that grasses exhibit two alternative strategies in response to drought; maintaining green shoots with declining water availability, or senescence of aerial parts but with survival extended via drought tolerant meristems contained within older sheaths. Future work that would enhance our understanding of traits that contribute to drought tolerance would investigate the role of osmotic adjustment in i) meristem survival under drought and ii) whether osmotic adjustment in expanded leaves enables plants to keep stomata open for longer as available water declines than plant which do not osmotically adjust.

Early stomatal closure with drought is one mechanism I identified through which plants can retain green tissues with declining water potential and the stay green strategy was also facilitated via narrow roots. An alternative strategy was displayed by grass species that have wide roots, exhibit fast senescence of aerial parts under drought but were able to delay mortality via drought tolerant meristems. These alternative strategies that contribute to determining a species hydrological niche, in turn, are known to have feedbacks to other ecosystem processes including fire and productivity. Fire is strongly correlated with gradients of MAP, as a highly productive environment is required to produce abundant fuel for fires. However a dry season to cure the fuel is also necessary for ignition and flammability, and leaf senescence is used as a phenological indicator to predict fire spread across landscapes (Cheney, Gould et al. 1998). At the global scale, I showed (chapter four) that rapidly senescing species with drought resistant meristems were found in wetter environments when compared to species that stay green, and in chapter three I showed that fire increases with increasing MAP. Drought patterns are intrinsically linked with fire in nature. This means that differences in plant strategies could be driven as much by fire as by MAP and, in fire controlled ecosystems can only be understood when considered together.

The ability to re-sprout from underground reserves and drought tolerant meristems after senescence of aboveground parts, is an important adaptation to surviving prolonged periods without water (Zeppel, Harrison et al. 2015), but also occurs in response to tissue loss through fire (Bond and Midgley 2001; Clarke, Lawes et al. 2013). Drought deciduous plants endure the dry season with carbon and nutrient reserves stored in sub surface crowns and roots. This adaptation to a non-growing season is critical for regrowth after fire and drought. Regrowth following the loss of above ground parts is dependent on upward translocation from belowground reserves (Canadell and Lopez-Soria 1998; Schutz, Bond et al. 2009), and the storage of reserves is therefore an important component of re-sprouting following drought, fire, frost or defoliation by herbivores. Shrubs that re-sprout following fire and drought do exhibit larger below ground reserves of carbohydrates than species that regenerate via seed (Knox and Clarke 2005; Schwilk and Ackerly 2005). Future work should concentrate on understanding how allocation of carbohydrates to stored reserves contributes to resprouting. It would be expected that plants from wet environments that exhibit fast senescence under drought would allocate more non-structural carbohydrates to root storage than species with a stay green strategy. Species from arid habitats that retain photosynthetic tissues under drought via stomatal closure would deplete carbon reserves under water deficit to prevent carbon starvation whilst stomata are closed.

The relationships uncovered between plant traits and climate, soil and disturbance indicate that plants are adapted to their environment. However, evolutionary history and biogeography can also shape patterns of trait distribution. Trait-environment relationships may differ in areas of different biogeographic history and results from chapter two indicate that evolutionary history provides an important explanation of the distribution of trait variation at global scales. There may therefore not be a single set of traits that can be used to make generalizations about increased fitness in a given environment across areas of different biogeographic and phylogenetic history (Forrestel, Donoghue et al. 2017) . For example, although comparisons of the response of functional traits to grazing are similar across continents with similar grazing histories (Diaz, Noy-Meir et al. 2001), comparisons of the response of individual traits to grazing reveals divergent responses between areas that differ in biogeography and grazing history (Forrestel, Donoghue et al. 2015). Other studies have shown that the relationships of individual traits to grazing, can change along climatic gradients (De Bello, Leps et al. 2005), and it seems likely that relationships of leaf traits to grazing may not be general across all settings (Vesk, Leishman et al. 2004). Results from chapter three do however identify plant functional groups defined by life history, presence of rhizomes and differences in leaf N that display diverging response to grazing, that have previously been shown to have a positive response to grazing at global scales (Diaz, Lavorel et al. 2007).

CONCLUSIONS

In order to improve our understanding of how ecosystems may respond to global change it is necessary to identify which dimensions of a species niche are important in determining species distributions and the traits that underpin these relationships. SLA and LTS are widely accepted as traits reflecting trade-offs between fast growth rate and the conservation of resources, and variation in these traits is predicted to be caused by differences in resource availability. I provide evidence that, at global and continental scales, SLA and LTS do not predict how species respond to climate, soil or disturbance. Conversely, I show that, at the same scales, variation in LNC or C/N ratio is linked to gradients of fire, MAP, soil nutrients and herbivory, and that multiple global change drivers collectively influence plant N and C in predictable ways. This suggests that the distribution of leaf chemical traits can provide a foundation upon which to view ecological patterns and processes across regions and biomes differing in climate, disturbance regime and evolutionary history. At global scales even the economic traits with the strongest relationships to soil and climate (LNC and C/N ratio), still exhibit between 70 – 75% unexplained variation. Through this study I identify other axes of variation that are important in explaining a species niche. Root architecture and stomatal traits relating to a fast/ slow continuum explain different strategies of senescence that explains growth and survival along a rainfall gradient. These results contribute to the identification of the traits and trait combinations that can improve our understanding of ecosystem assembly and improve predictions of how species and ecosystem may respond global change.

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