Functional differences between dominant grasses drive divergent responses to large herbivore loss in mesic savanna grasslands of North America and South Africa

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Summary

1. Grazing and fire are disturbances integral to the evolution and maintenance of savanna grasslands. Humans are altering or completely eliminating these disturbance regimes at a global scale, with important consequences for savanna ecosystem structure and function. It is unknown whether the alteration of these disturbance regimes will have similar effects on grass communities of savanna grasslands in different geographic regions that vary in their biogeographic and evolutionary histories, as well as in the diversity of extant grazers.

2. Here, we examined the effects of large herbivore loss on different aspects of grass community structure – taxonomic, phylogenetic and functional – across a range of fire frequencies in C_4-dominated mesic savanna grassland sites of North America (Konza Prairie Biological Station, Kansas, USA) and South Africa (Kruger National Park). The goal of the study was to determine whether the loss of large herbivores exerted a consistent effect on the grass communities of two physiognomically similar grasslands with different biogeographic and grazing histories.

3. The removal of large herbivores resulted in divergent responses in the grass communities at Konza and Kruger that was consistent across fire treatments. At Konza, there was a rapid and significant response to grazing exclusion while the response was muted and transient at Kruger. Functional syndromes associated with grazing resistance were generally conserved across sites, and it was the functional strategies of the dominant species at each site that drove the divergent responses. Further, our study supports the hypothesis that grazing and aridity may be selective forces that act in parallel as those species that were grazing resistant also occupied drier niches.

4. *Synthesis.* Our study demonstrates that savanna grassland communities with different biogeographic and grazing histories respond differently to the removal of large herbivores and that climate, fire and grazing are interactive forces in maintaining savanna grassland diversity and function. We show that the functional attributes of the dominant grasses, which are in part driven by the biogeographic and grazing history experienced, are the most relevant in predicting the response of savanna ecosystems to the loss of large herbivores.

Key-words: biogeography, determinants of plant community diversity and structure, disturbance, dominant species, drought, fire, functional traits, grass community, grazing, phylogeny

Introduction

Grazing and fire are integral drivers of grassland and savanna dynamics globally (McNaughton 1983; Milchunas, Sala & Lauenroth 1988; Fuhlendorf & Engle 2001). In mesic

(subhumid) grasslands and savannas (hereafter referred to as savanna grasslands, *sensu* Scholes & Walker 1993), these disturbances interact in important ways to influence the composition of vegetation and key ecosystem functions, such as primary productivity and nutrient cycling (Hobbs *et al.* 1991; Milchunas & Lauenroth 1993; Collins *et al.* 1998). Humans are altering these disturbance regimes at a global scale, often

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by reducing fire frequency and large herbivore diversity or by completely eliminating fire and grazing, with important consequences for savanna grassland structure and function. What remains unknown is whether the effects of alterations in these disturbances are similar or different for savanna grasslands in different geographic regions, which can vary considerably in their biogeographic and evolutionary histories and in the diversity of large herbivore grazers that they support. Most experimental studies of grazing and fire response are limited to a single study site and/or focus on the taxonomic response (Knapp *et al.* 2004; Eby *et al.* 2014; Koerner *et al.* 2014; Staver & Bond 2014). Understanding how savanna grassland plant communities will respond to alterations in fire and grazing necessitates a comparative approach that examines multiple facets of community structure and function (Lavorel & Garnier 2002; Diaz *et al.* 2004; Knapp *et al.* 2004). Incorporating phylogenetic and functional approaches could provide a more mechanistic and predictive understanding of the causes and consequences of observed community responses to altered disturbance regimes (Webb *et al.* 2002; Fukami *et al.* 2005; Helmus *et al.* 2010; Cavender-Bares & Reich 2012; Forrestel, Donoghue & Smith 2014). Furthermore, studying multiple facets of diversity in a comparative framework can elucidate generalities and historical contingencies across regions in community responses to disturbance (Adler *et al.* 2004, 2005; Diaz *et al.* 2007; Forrestel, Donoghue & Smith 2014), providing a link between the evolutionary history of a region and contemporary responses to disturbance.

The herbaceous community in savanna grasslands is generally dominated by C_4 perennial grasses that define the physiognomic structure of the system and provide fuel for fire and a primary food source for many large ungulate grazers (Knapp *et al.* 1998). These grasses co-evolved with large herbivore grazers which play a major role in structuring these grass communities (Stebbins 1981; McNaughton 1983; Huntly 1991; Hobbs 1996; Bouchenak-Khelladi *et al.* 2009). Thus, the modification of grazing regimes and loss of native large herbivores will likely drive changes in grass community structure and function (Cromsigt & te Beest 2014). Large herbivore grazing has been found to increase plant community richness and diversity (McNaughton 1978; Hartnett, Hickman & Walter 1996; Collins & Calabrese 2011; Koerner *et al.* 2014), and this response is generally driven by the decrease of palatable C_4 dominant grasses in savanna grasslands (Milchunas, Sala & Lauenroth 1988; Knapp *et al.* 1999; Eby *et al.* 2014). Yet Koerner *et al.* (2014) found a muted and transient taxonomic response of the herbaceous community in savanna grasslands to large-grazer exclusion in South Africa as compared to North America, which they speculated to be attributable to functional differences between the dominant grasses. There is also purported to be a conserved set of functional strategies associated with adaptation to grazing pressure (Coughenour 1985; Milchunas, Sala & Lauenroth 1988; Augustine & McNaughton 1998; Adler *et al.* 2005; Diaz *et al.* 2007), which include smaller stature (i.e. shorter height, smaller leaves), the presence of silica and/or grazer-deterring

secondary compounds and greater structural investment (high leaf dry matter content, low specific leaf area, higher leaf per cent carbon:nitrogen). Together these lead to less palatable, accessible and digestible leaf tissue. Grazing and drought have been hypothesized to be convergent selection pressures and traits associated with climate and grazing could therefore interact, such that those individuals or species with a more arid climatic niche may be able to increase in abundance in the presence of grazing (Coughenour 1985; Milchunas, Sala & Lauenroth 1988; Quiroga *et al.* 2010). These traits are generally associated with an avoidance strategy. An alternative functional strategy is low investment in structural defence and high investment in rapid tissue regeneration (Augustine & McNaughton 1998; Strauss & Agrawal 1999). Some of these traits have been found in species that respond positively to higher fire frequency in mesic grasslands (Forrestel, Donoghue & Smith 2014), which suggests an interaction between functional responses to grazing and fire. Despite general convergence in functional strategies, both Diaz *et al.* (2007) and Adler *et al.* (2005) found that functional syndromes associated with grazing differed between regions with different grazing and biogeographic histories, highlighting the importance of a comparative approach to understanding the generalities and historical contingencies associated with responses to disturbance.

Here, we measured the taxonomic, phylogenetic and functional response of the grass community to grazing and fire manipulations at mesic savanna grassland sites in North America (Konza Prairie Biological Research Station, Kansas, USA) and South Africa (Kruger National Park) that have experienced different evolutionary histories of grazing (Axelrod 1985; Bond, Midgley & Woodward 2003). To assess the impacts of loss of native large herbivores on grass communities and their interaction with fire regime, we utilized identical 7-year experiments with permanent exclosures to simulate large herbivore loss. These exclosures were established across comparable fire regimes (annual, 3–4 year and unburned) at both sites. Specifically, we tested the hypothesis that despite taxonomic differences between the regions, species that decrease with release from grazing would exhibit convergent functional strategies due to the strong selective pressure that grazing has historically placed on grasses. As both of these sites have evolved in concert with fire and large herbivore grazing, we expect that some species/clades have evolved conserved herbivore tolerance and/or avoidance strategies. This would especially be true in South Africa where there has been a consistent, longer history of grazing (Bond, Midgley & Woodward 2003), and there is a more diverse assemblage of extant native large herbivores. Furthermore, we expect that those species/clades that are resistant to grazing will occupy a more arid climatic niche. Lastly, as dominance generally increases in the absence of grazing by large mammals (Collins 1987; Belsky 1992; Blair, Smith & Collins 2008; Eby *et al.* 2014; Koerner *et al.* 2014), we hypothesize that functional differences between the dominant species at both sites will drive differences in the taxonomic response to grazing release.

Materials and methods

STUDY SITES

Large herbivore manipulations were established in long-term fire treatments at the Konza Prairie Biological Station (Konza), north-eastern Kansas, USA, and the Kruger National Park (Kruger), north-eastern SA (see Buis *et al.* 2009 and Koerner *et al.* 2014 for detailed site descriptions). These sites are similar in their physiognomic structure, growing season climate and historical fire regimes, and they have similar ANPP responses to grazing (Knapp *et al.* 2012; Koerner *et al.* 2014).

At Konza, to address the role of native grazers and fire-grazing interactions, bison were reintroduced in 1987 to a 1000 ha fenced area that includes 10 replicate watersheds burned in the spring (mid-April) at 1-, 2-, 4- and 20-year intervals. The overall grazing intensity is considered moderate, with ca. 295 bison present year round (Towne 1999), averaging 129 kg of herbivore/hectare.

At Kruger, the experimental burn plots (EBPs) were established in 1954 to determine the effects of fire seasonality and frequency on forage production. Similar to Konza, experimental burns occur in the spring (August) at 1-, 2-, 3- and 6-year intervals, along with unburned controls, each applied to ~7 ha plots (Biggs *et al.* 2004). Our research focused on the Satara, Marheya and N'wanetsi blocks of the knobthorn–marula EBPs in south-central Kruger, where precipitation, soil type, and the mix of herbaceous and woody plants are similar to Konza (Koerner & Collins 2013; Koerner *et al.* 2014). Herbivore abundance and grazing intensity in this area are considered moderate for regional savanna grasslands (du Toit 2003), 12 herbivore species (e.g. blue wildebeest, plains zebra, impala) commonly graze on the EBPs, averaging 104 kg of herbivore/hectare (Burkepile *et al.* 2013).

EXPERIMENTAL DESIGN

Comparable experimental designs and vegetation sampling were used at both Konza and Kruger for the large herbivore manipulation in the annual and intermediate (4 years at Konza, 3 years at Kruger) burn sites and the unburned controls. To manipulate the presence of large herbivores, replicate 38.5 m^2 (7 m diameter circular) herbivore exclosures were established prior to the growing season in 2005/06 in all burn treatments in grazed areas at both Kruger and Konza. Three blocks of seven exclosures were established with co-located paired plots open to grazing in each of the three fire treatments ($n = 21$ exclosures/treatment/site).

From 2005 through 2012, the herbaceous plant community was surveyed in permanent 2 × 2 m plots located within each of the fenced and paired plots during the early and late growing seasons. The 4-m^2 plot was divided into four 1-m^2 subplots, and in each subplot, we estimated per cent aerial cover (to nearest 1%) for each species rooted therein. Maximum cover values for each species across the season were used to calculate relative cover for each species. For more details on study sites and experimental design, see Koerner *et al.* (2014).

PHYLOGENETIC INFERENCE

We estimated phylogenetic relationships for species at the Konza and Kruger sites together using the nuclear ribosomal internal transcribed spacer (ITS) region and *mat*K, *ndh*F, and *rbc*L chloroplast markers. These four markers were obtained from GenBank and supplemented by sequences from specimens collected at each site. We sequenced only the three chloroplast markers for our specimens to match the gene regions used by the Grass Phylogeny Working Group II (Grass Phylogeny Working Group II 2011). Total genomic DNA was isolated from dried plant tissue and amplified following the protocol and using the primers specified in GPWG (2011). Thirty-six new sequences from 18 species were deposited in GenBank; voucher specimen information and GenBank accession numbers are listed in Table S1. Sequences were aligned using MUSCLE V 3.7 (Edgar 2004) and concatenated using PHYUTILITY (Smith & Dunn 2008). Models of nucleotide substitution and optimal partitioning strategies were chosen using PARTITIONFINDER (Lanfear *et al.* 2012), and phylogenetic relationships were inferred using Bayesian methods in BEAST v.1.6.2 (Drummond & Rambaut 2007). The MCMC chain was run for 20 000 000 generations and sampled every 10 000 generations. Convergence of the chain was assessed by visualizations of the state likelihoods using TRACER v1.5 (Drummond & Rambaut 2007). A total of 2 000 000 generations were discarded as burn-in, and the remaining trees were combined to generate a maximum clade credibility tree that was used for all analyses. See Appendix S2 for further details on the phylogenetic inference methods.

FUNCTIONAL TRAIT COLLECTION

At both sites, data on maximum height, growth habit, photosynthetic pathway, specific leaf area, leaf dry matter content, stomatal pore length, stomatal density, stomatal pore index, foliar per cent nitrogen (%N), per cent carbon (%C), per cent carbon to nitrogen ratio (C:N) and 13C were collected during the 2010–2012 field seasons as close to peak flowering as possible for all species (peak flowering ranged from June–September for Konza and December–March for Kruger). A minimum of 10 individuals per species were measured across the fire treatments in which they represented > 5% of the relative abundance for all grass species. Thus, those that were abundant in both burned and unburned plots were collected across both treatments to control for intraspecific trait differences due to treatment effect. A subset of the dominant species were collected across grazing treatments to confirm that there were no significant trait differences between species due to grazing within fire treatment. Phenology, height and collection location were recorded for each species. Standard methods were used for the collection and processing of all leaf traits (Garnier *et al.* 2001; Cornelissen *et al.* 2003). For a detailed description of functional trait measurements, see Appendix S3.

To quantify the precipitation niche of each species across their entire ranges, geo-referenced herbarium specimens point records were downloaded from the Global Biodiversity Information Facility (http://www.gbif.org, accessed on 8 April 2014). Curated records were used to extract mean annual precipitation (MAP) data from the WorldClim data base (Hijmans *et al.* 2005) at 30 arc degree resolution. To remove outliers, the mid-90% of the range of each species set of points was used to calculate the mean and range of MAP for each species. For a detailed description of methods used to curate the GBIF records see Appendix S4.

STATISTICAL ANALYSES

All analyses were conducted separately for data from Konza and Kruger, focusing only on the grasses in these communities, which constitute the majority of the cover at both sites (Fig. S4). Changes in plant community structure across treatments were assessed for taxonomic, phylogenetic and functional diversity metrics, including species

richness (S), Shannon–Wiener's diversity index (H′), evenness (J′), the Berger–Parker dominance index (D; Magurran 2004), mean phylogenetic distance (MPD; Webb 2000) and functional dispersion (FDis; Laliberté & Legendre 2010). Significant changes in relative abundance for each species across treatments were also assessed for all grasses in > 2% abundance in any treatment. To test for differences in these measures, we used nested, repeated-measure linear mixed-effects models with fire as whole plot treatment, grazing nested within fire and year as the repeated measure. When treatment effects or interactions were significant, Tukey's *post hoc* HSD tests with alpha set to 0.05 were used to test for differences among treatments. Turnover in the identity and abundance of species in response to grazing release and fire frequency was assessed using permutational analysis of variance (PERMANOVA) and *post hoc* similarity percentage analysis (SIMPER) conducted in the vegan package (Oksanen *et al.* 2013) of R v 3.0.3. The PERMANOVA was conducted with community matrices generated using the Euclidean distance metric and 9999 permutations of the raw data among treatment groups. Significance tests of differences between treatments were assessed using *F*-tests based on sequential sums of squares of the permutations of the raw data. When significant differences between treatments were found, SIMPER was used to identify the grass species contributing most to differences among treatments.

Individual species responses to treatments were classified as significantly positive or negative, or exhibiting no change based on the mixed model results and differences in mean abundance between the grazing release and grazed treatment plots across each fire regime. Tests of node-based phylogenetic clustering of species' discrete responses to grazing release were conducted using the clade significance statistic of J. C. Oliver, E. J. Edwards & M. J. Donoghue (unpubl. data) which builds upon the nodesig test in PhyloCom (Webb, Ackerly & Kembel 2008). In this metric, the clade density state is calculated for each node, and clades are identified within which (i) descendant lineages are characterized by similar densities, and (ii) the clade density differs substantially from its sister clade. This was used to identify nodes where descendant taxa exhibited significant positive or negative shifts in abundance in response to increased grazing release. Significance was assessed using a null model where tip states were shuffled and a one-tailed test ($P < 0.05$) of significant clustering was conducted independently for each state (positive or negative shift in abundance). Species from both sites were combined in this analysis.

To assess individual species trait-based responses to grazing and fire, we conducted a phylogenetically corrected principal components analysis in the phytools package of R (Revell 2009). While there were intraspecific differences in a few traits across fire treatments, these differences did not significantly alter the overall results, so species mean values across all treatments were used.

Using time-lag analysis (Collins, Micheli & Hartt 2000; Collins & Smith 2006), we determined the effects of grazing and fire on the rate of grass community taxonomic, phylogenetic and functional change over time (2006–2012). For each measure of community change, we generated a species × distance matrix for each plot within a treatment, a Euclidean distance matrix for taxonomic change, a mean phylogenetic distance matrix for phylogenetic change and a Gower's functional dissimilarity matrix for functional change. For each time series, linear regressions were calculated for the square root of the time-lag versus each distance/dissimilarity measure. Significant positive or negative slopes indicate directional change in community structure. Higher rates of change (steeper slopes) indicate greater sensitivity of a system to a particular disturbance over the study period (Collins, Micheli & Hartt 2000). Linear mixed models were used to

assess significant differences between the regression slopes for each treatment. All analyses were conducted in the basic statistical package and the vegan (Oksanen *et al.* 2013) and FD (Laliberté & Shipley 2011) packages of R v. 3.0.3.

Results

COMMUNITY DIVERSITY PATTERNS

The grass communities were more diverse at Konza than Kruger at both the site (35 vs. 26 species) and plot level (10.21 ± 3.27 vs. 4.95 ± 1.91 species) based on 2006–2012 survey data. At both sites, the effect of large herbivore exclusion on the taxonomic, phylogenetic and functional structure of the grass community varied significantly with fire frequency over time (Table 1). Over the 7-year study period at Konza, the grazing removal treatment resulted in a decrease in grass richness and diversity and an increase in grass dominance. These effects were most pronounced in the annual and intermediate burn treatments (Fig. S6). Mean plot-level grass richness declined by ~4 species or ~40%, mean diversity decreased by ~33% and mean abundance increased by ~45% with the grazing removal treatment. *Andropogon gerardii* was the most dominant species in both the grazed and grazing removal treatments despite significant shifts in relative abundance and grass community diversity (Fig. S4).

At Kruger, fire and grazing had significant effects on taxonomic richness and diversity (Table 1). However, these effects were transient and resulted in no directional trend over the 7-year study period for the grazing exclusion treatment (Fig. S6). In contrast to Konza, there was little effect of grazing exclusion in any of the burn treatments at Kruger, where *Bothriochloa radicans* maintained its dominance and relative abundance across grazing treatments in the annual and intermediate burn plots, and *Panicum coloratum* was dominant in the unburned plots (Fig. S4).

In line with the responses observed for taxonomic richness and diversity, grazing release significantly changed grass species composition at Konza, but not Kruger (PERMANOVA $F = 35.919$, $R^2 = 0.2114$, $P \leq 0.0001$; $F = 0.812$, $R^2 = 0.197$, $P = 0.464$, respectively). In contrast, fire frequency significantly altered grass species composition at both sites ($F = 6.225$, $R^2 = 0.0366$, $P = 0.0017$; $F = 29.419$, $R^2 = 0.197$, $P \leq 0.0001$, respectively), and there was a significant fire and grazing treatment interaction at Konza ($F = 5.746$, $R^2 = 0.034$, $P = 0.0025$). At Konza, the divergence in grass composition between grazed and grazing release plots across all fire treatments was driven primarily by the increases in *Andropogon gerardii* and *Sporobolus compositus* and a decrease in *Bromus japonicus* in response to large-grazer removal (Table S5). These same species were primarily responsible for the divergence across fire regimes, where there was an increase in *A. gerardii* and decreases in *S. compositus* and *B. japonicus* in response to higher fire frequency. At Kruger, the divergence between fire regimes was driven by an increase in *Bothriochloa radicans* and decreases in *Urochloa mosambicensis* and

Table 1. Effects of fire (F), the removal of grazing (G) and their interaction (F*G) over the 7-year study period (Y = year) on total richness (S), evenness (J′), diversity (H'), Berger-Parker dominance (D), mean phylogenetic distance (MPD) and functional dispersion (FDis) of the grass community in mesic savanna grasslands in North America (Konza) and South Africa (Kruger). Statistical results are *F* values from mixed model repeated measures anovas conducted for each site and community metric separately. Significant results (*P* < 0.05) are bolded

Site	Treatment	d.f.	S	J'	$e^{H'}$	D	MPD	FDisp
Konza	F	2	**16.43*****	**29.13*****	**40.45*****	2.94	**101.45*****	**220.45*****
	G	1	**426.03*****	**247.65*****	**57.31*****	**251.00*****	**226.35*****	**155.75*****
	F*G	2	**53.84*****	**56.06*****	**31.05*****	**102.03*****	**54.78*****	**37.21*****
	Y	6	**6.24****	2.94	1.40	**5.00****	**11.46*****	**16.56*****
	Y*F	12	0.25	1.31	2.63	0.98	0.90	2.82
	Y*G	6	**67.95*****	**55.72*****	**24.00*****	**53.57*****	**39.52*****	**61.32*****
	Y*F*G	12	2.48	**6.12****	**6.11****	**16.01*****	**9.56*****	**6.306****
Kruger	F	2	**12.50*****	**107.15*****	**112.13*****	**67.40*****	**19.03*****	0.69
	G	1	**45.59*****	**22.19*****	0.44	**5.69***	**16.45*****	**8.01****
	F*G	2	**9.43****	2.28	0.03	2.80	0.72	**11.37*****
	Y	6	3.47	1.65	0.01	0.00	**5.28***	1.31
	Y*F	12	**8.35****	**7.16****	1.24	3.48	3.01	0.43
	Y*G	6	**4.02***	3.02	2.08	2.70	**5.78***	**4.92***
	Y*F*G	12	0.77	1.61	1.95	1.08	1.00	1.45

Significance values of reported *F* values: *$P \leq 0.05$; **$P \leq 0.01$; ***$P \leq 0.001$.

Panicum coloratum in response to higher fire frequency (Table S5).

PHYLOGENETIC RESPONSES TO GRAZING

There was significant clade overlap between sites except for species of Pooideae, which were only represented at Konza. Konza had 13 C_3 species, including 11 species in the Pooideae, *Panicum oligosanthes* and *Panicum acuminatum*, while Kruger harboured only C_4 species. In contrast, both Paniceae and Eragrostideae were more diverse at Kruger. Species at both sites exhibited consistent, albeit not always significant, responses to grazing release across fire frequency treatments. Generally, there were more significant species responses to exclusion of grazing in the annual and intermediate fire treatments (Fig. 1). The Paniceae was an exception; two South African species exhibited positive responses to grazing exclusion only within the unburned treatment, where they had higher abundance (Fig. 1).

The node-based test for significant responses to grazing release across fire treatments located significant decreases in abundance within a subclade of the Pooideae present only at Konza (*P* = 0.050) and within two nested subclades of the Cynodonteae (*P* ≤ 0.001, *P* = 0.014). The Cynodonteae subclades, while more diverse at Konza, were represented by negatively responding species at both sites. A significant clustering of positive responses was found in a subclade within the Andropogoneae (*P* = 0.005), which included two dominant species from the Konza site. Yet, there were also negative and neutral responders within Andropogoneae (Fig. 1).
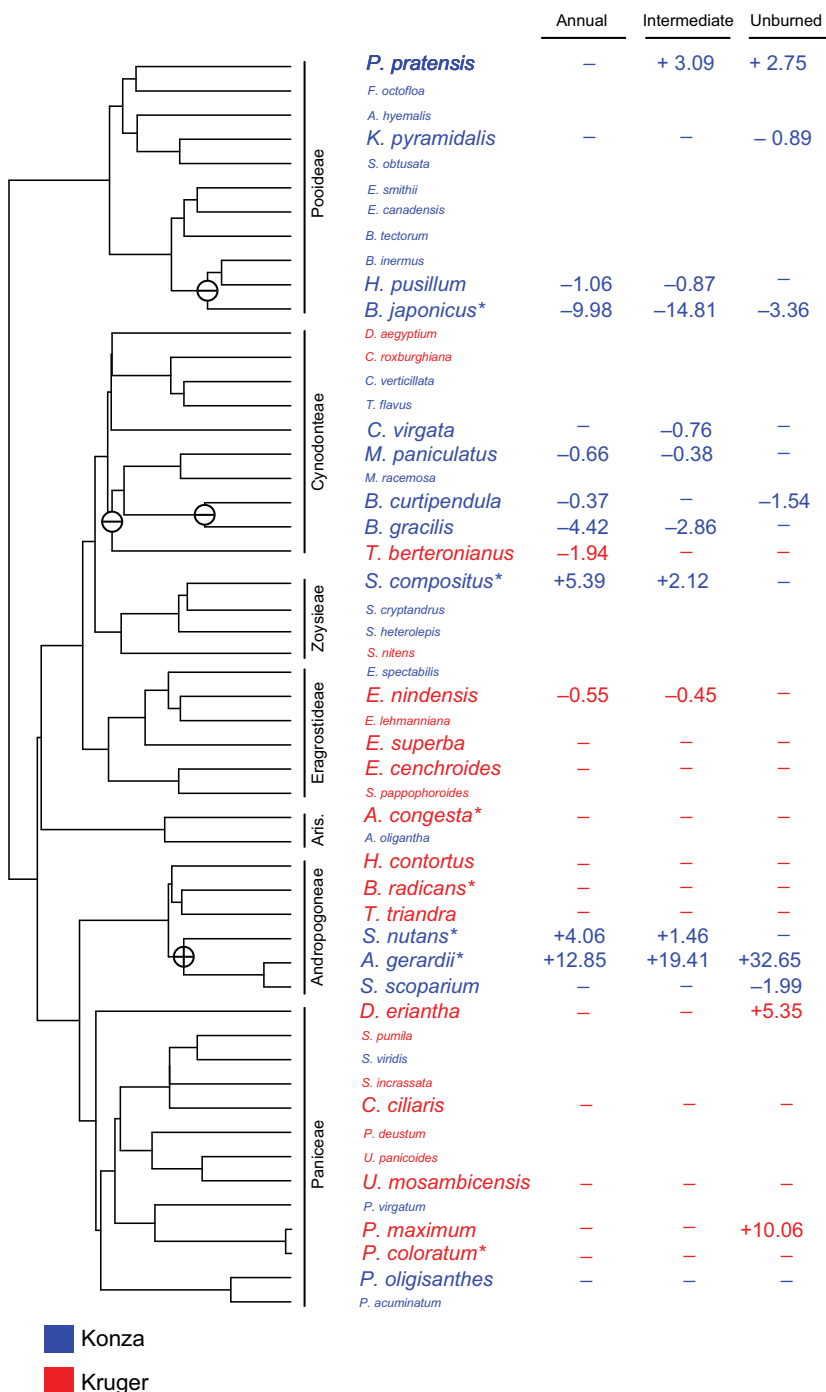
Over the 7-year study period, mean phylogenetic distance (MPD) decreased significantly at Konza in the annual and intermediate burn treatments. At Kruger, there was a significant effect of grazing release on MPD, but this was driven by year-to-year variation with no significant directional change (Table 1, Fig. S4).

FUNCTIONAL TRAIT RESPONSES TO GRAZING

The principal components analysis of species mean trait values demonstrated that species with similar grazing responses generally shared similar functional strategies (Fig. 2a). Height, leaf size, stomatal pore index, traits related to leaf nutrient status (leaf %N, leaf C:N) and precipitation (mean and range of mean annual precipitation) loaded most heavily on the first PC axis, whereas stomatal size and density, specific leaf area and leaf dry matter content loaded most heavily on the second PC axis (Fig. 2a). Species that increased in abundance in response to grazing release were generally taller, had larger leaves, larger and lower density of stomata, higher stomatal pore index and lower leaf %N. Conversely, those species that did not exhibit significant responses or decreased in abundance in response to grazing release were shorter, had smaller leaves, smaller and dense stomata and lower leaf dry matter content. Exceptions were *Schizyachrium scoparium*, which exhibited a decrease, albeit not large in magnitude, in mean abundance in response to grazing exclusion, yet shared functional attributes with other Andropogoneae species at Konza that positively responded to grazing release. *Poa pratensis*, a Pooideae species at Konza, increased in abundance in response to grazing release in the absence of fire, but shared traits with species exhibiting a decrease in response (Fig. 2a).

Three out of the four most abundant (dominant) species at Konza responded positively to grazing release (*Andropogon gerardii*, *Sorghastrum nutans*, *Sporobolus compositus*), whereas *B. japonicus* responded negatively. The four dominants at Kruger all lacked a significant response to grazing release (*Aristida congesta*, *Bothriochloa radicans*, *Panicum coloratum*, *Urochloa mosambicensis*; see Figs 1 and 2a). Overall, the grazing-resistant species, or those that decreased or did not shift their in abundance with grazing release (*A. congesta*, *B. radicans*, *P. coloratum*, *B. japonicus* and

	Annual	Intermediate	Unburned
P. pratensis	–	+ 3.09	+ 2.75
F. octofloa			
A. hyemalis			
K. pyramidalis	–	–	– 0.89
S. obtusata			
E. smithii			
E. canadensis			
B. tectorum			
B. inermus			
H. pusillum	–1.06	–0.87	–
*B. japonicus**	–9.98	–14.81	–3.36
D. aegyptium			
C. roxburghiana			
C. verticillata			
T. flavus			
C. virgata	–	–0.76	–
M. paniculatus	–0.66	–0.38	–
M. racemosa			
B. curtipendula	–0.37	–	–1.54
B. gracilis	–4.42	–2.86	–
T. berteronianus	–1.94	–	–
*S. compositus**	+5.39	+2.12	–
S. cryptandrus			
S. heterolepis			
S. nitens			
E. spectabilis			
E. nindensis	–0.55	–0.45	–
E. lehmanniana			
E. superba	–	–	–
E. cenchroides	–	–	–
S. pappophoroides			
*A. congesta**	–	–	–
A. oligantha			
H. contortus	–	–	–
*B. radicans**	–	–	–
T. triandra	–	–	–
*S. nutans**	+4.06	+1.46	–
*A. gerardii**	+12.85	+19.41	+32.65
S. scoparium	–	–	–1.99
D. eriantha	–	–	+5.35
S. pumila			
S. viridis			
S. incrassata			
C. ciliaris	–	–	–
P. deustum			
U. panicoides			
U. mosambicensis	–	–	–
P. virgatum			
P. maximum	–	–	+10.06
*P. coloratum**	–	–	–
P. oligisanthes	–	–	–
P. acuminatum			

Clades: Pooideae, Cynodonteae, Zoysieae, Eragrostideae, Aris., Andropogoneae, Paniceae.

■ Konza
■ Kruger

Fig. 1. Phylogeny (Bayesian maximum clade credibility tree) of all grass species in the study plots from Konza Prairie Biological Station, Kansas, North America (in blue) and Kruger National Park, South Africa (in red). Individual species responses to grazing across fire treatments are reported for all species that constituted over 2% relative mean abundance in any treatment (species in larger font and bolded). Species denoted by an asterisk are the four most abundant (dominant) species at each site. In cases where there were significant effects of grazing ($P \leq 0.05$), mean shifts in abundance in response to grazing release within each fire treatment are reported for the 2012 field season. Clades exhibiting relative responses that are significantly clustered on the phylogeny according to Oliver *et al.*'s node-based statistic (see text for details) are highlighted with a positive or negative sign (circles with –, decrease; circles with +, increase; $P \leq 0.05$).

U. mosambicensis), occupied a drier, narrower precipitation niche breadth than did those that increased in the absence of grazing (*A. gerardii*, *S. compositus* and *S. nutans*) (Fig. 2a,b).

Over the 7-year study period, functional dispersion (i.e. the volume of functional space occupied by the species in a community weighted by their abundance) decreased significantly at Konza in the annual and intermediate burn treatments in response to grazing release. While there was a significant effect of fire and grazing at Kruger on functional dispersion, there was no significant directional change (Table 1, Fig. S5).

RATES OF COMMUNITY CHANGE THROUGH TIME

At Konza, taxonomic rates of change over time (2006–2012) were significantly higher for the grazing release than the grazed treatments in annually burned plots, with similar trends for the 4-year and unburned treatments. There were no consistent significant differences between fire regimes and rates of change across the grazing treatments (Fig. 3a). Conversely, at Kruger, there were no significant differences in rates of taxonomic change between grazing treatments, yet significantly higher rates of change in the less frequently burned sites than the annually burned sites (Fig. 3d). At Konza, rates of
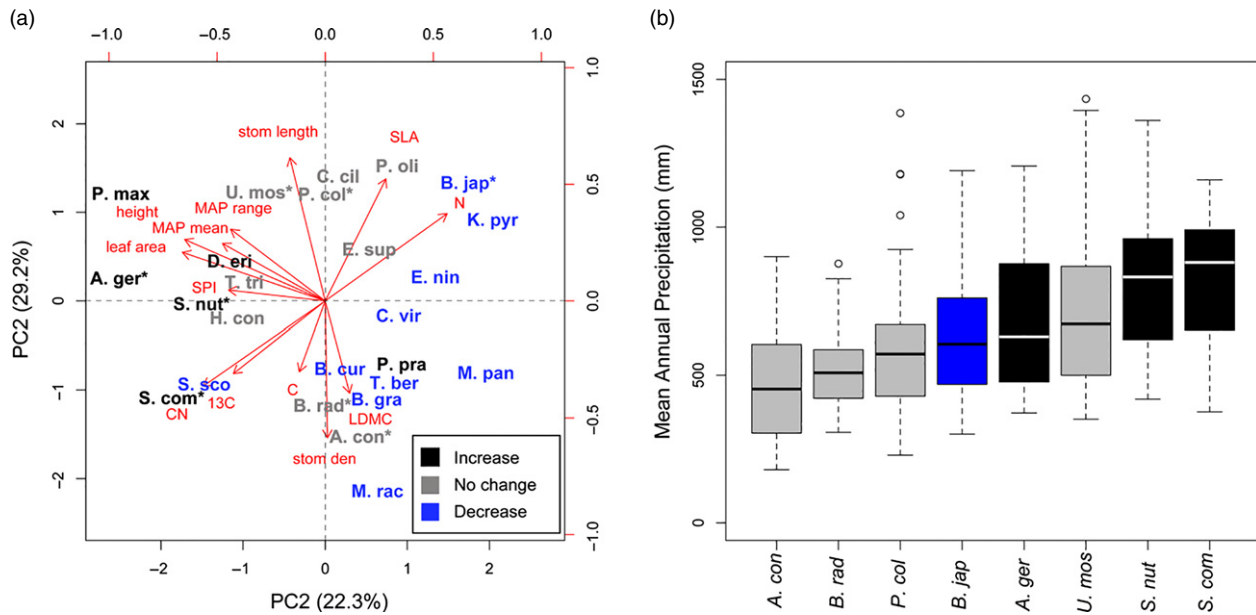
Fig. 2. Functional trait syndromes and precipitation niches of species at the Konza Prairie Biological Station (Kansas, US) and the Kruger National Park, South Africa. (a) Results from a phylogenetically corrected principal components analysis of functional trait syndromes of species present at > 5% abundance within any experimental treatment at both Konza and Kruger sites from the 2012 field season. Species denoted by an asterisk are the four most abundant species at each site. See supporting information Table S1 for species complete names. Trait abbreviations: SLA, specific leaf area; LDMC, leaf dry matter content; stom length, stomatal length; stom den, stomatal density; SPI, stomatal pore index; C, per cent leaf carbon by mass; N, per cent leaf nitrogen by mass; C:N, per cent leaf carbon to nitrogen ratio; 13C, stable carbon isotope ratio; MAP mean, average mean annual rainfall; MAP range, mid-90% range of mean annual precipitation. (b) A box plot of the mid-90% values of the mean annual precipitation niche of the four most dominant species at each site (eight species in total) derived from extracted Bioclim values with GBIF coordinates. Different colours indicate a significant ($P \leq 0.05$) increase (black) or decrease (blue), or no significant change (grey) in relative abundance in response to exclusion of grazing (the difference in mean abundance between the grazed and grazing release plots across all fire treatments).

phylogenetic and functional change were significantly lower in annually burned, grazed treatments as well as in both grazing treatments in unburned plots (Fig. 3b,c). At Kruger, there were no consistent effects of grazing or fire treatment on phylogenetic rates of change, and no significant differences between rates of functional change for the fire or grazing treatments (Fig. 3e,f).

Discussion

The goal of our study was to determine how the loss of large herbivores will influence different aspects of grass community structure – taxonomic, phylogenetic and functional – across a range of long-term fire manipulations at two mesic savanna grasslands in North America (Konza) and South Africa (Kruger). Although we showed in a previous study (Forrestel, Donoghue & Smith 2014) that grass communities at both sites are similar in their functional and phylogenetic responses to alterations in fire frequency in the absence of grazing, we found that Konza and Kruger exhibited divergent responses at the taxonomic, phylogenetic and functional levels to grazing release through time, with these differences consistent across fire regimes.

We found that species with similar responses to large herbivore exclusion generally shared similar functional strategies at both sites. Those species that decreased in response to grazer

loss (i.e. grazing-resistant species) were generally shorter, had smaller leaves, higher leaf dry matter content, lower stomatal pore index and smaller, more densely distributed stomata (Fig. 2a). Yet, these responses were not conserved within the Andropogoneae, which was the most abundant (dominant) grass clade at both sites (Fig. 1, Fig. S6). While this cosmopolitan clade has been found to exhibit largely consistent responses to alterations in fire regimes (Forrestel, Donoghue & Smith 2014), our study and others have found members of this clade to exhibit a wide range of strategies and responses to grazing pressure (Fig. 1, Table S8). This was in contrast with the Cynodonteae, a more dry-adapted clade, which exhibited a conserved negative response to the release of grazing pressure. Members of this clade have been found to be dominant in semi-arid grasslands (Hattersley 1992; Taub 2000; Visser *et al.* 2012). These species are able to persist in lower abundance in mesic grasslands, especially in the presence of grazing that reduces competition with the dominant palatable, C_4 grasses that are generally from more fire and/or mesic-adapted clades such as Andropogoneae and Paniceae (Visser *et al.* 2012; Forrestel, Donoghue & Smith 2014). We also found that those species that responded negatively or neutrally to grazing removal occupy a drier, narrower precipitation niche than those that responded positively (Fig. 2a,b), which supports the hypothesis that drought or aridity and grazing have both acted as important selective forces in the
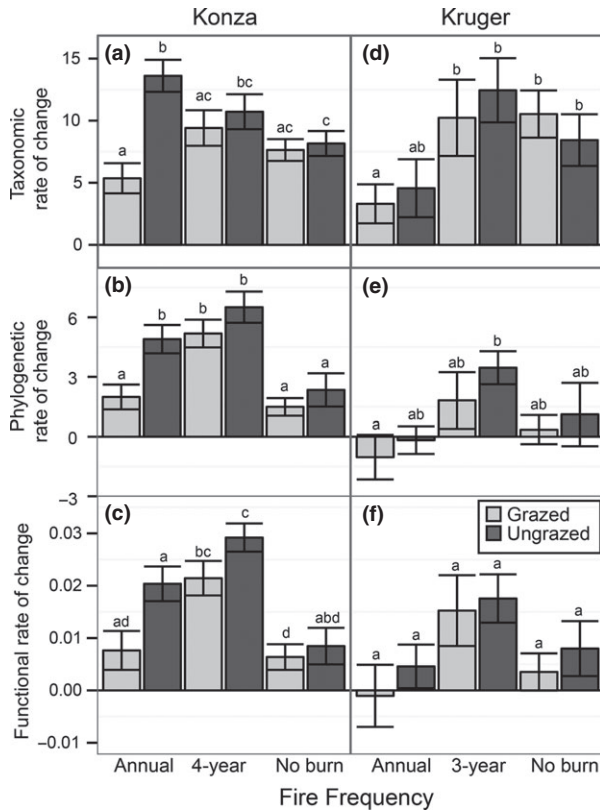
Fig. 3. Taxonomic (a,d), phylogenetic (b,e) and functional (c,f) rates of changes through time (2006–2012 field seasons) in grazed and grazing release plots using the time lag analysis of Collins, Micheli & Hartt 2000 (see text for details) at North American (Konza) and South African (Kruger) sites. The paired grazed and grazing release plots are nested within fire treatments. Significant differences between treatments are indicated by letters (ANOVAs and Tukey's HSD *post hoc* tests of regression slopes, an indication of directional change through time, for each plot; $P \leq 0.05$).

evolution of grasses and grasslands (Stebbins 1981; Coughenour 1985; Milchunas, Sala & Lauenroth 1988; Quiroga *et al.* 2010). This suggests that the interaction of climate and grazing will need to be considered when predicting the effects of changing grazing regimes on savanna ecosystem structure and function. The alteration or elimination of grazing regimes could result in the extirpation of drought-adapted species/clades from mesic grasslands, which has important implications for the resilience of savanna grasslands in the face of changing climates.

We found that the grass community at Konza decreased in diversity and increased in dominance in response to large-grazer exclusion in annual and intermediate burn treatments from 2006 to 2012. In contrast, Kruger did not exhibit a significant directional response to large-grazer exclusion in any fire treatment, yet there was a transient decrease in diversity and increase in dominance in the intermediate fire frequency and unburned treatments (Fig. S7). At Konza, the significant response to grazer exclusion was driven by the increase in abundance of the palatable grass, *Andropogon gerardii*, which despite favouring frequent burning (Knapp 1985), maintained its status as the most abundant (dominant) species across all

fire and grazing treatments (Fig. S5). The significant decrease in phylogenetic diversity and functional dispersion in response to large-grazer removal at Konza reflects the increase in dominance of *A. gerardii* and the corresponding decrease in diversity (Fig. S5). These patterns were also reflected in the community rates of change where we found significantly higher rates of taxonomic, phylogenetic and functional change in the grazing release relative to the grazed plots in the annual burn treatment at Konza (Fig. 3). This was driven primarily by the increase in dominance of the palatable *A. gerardii* and subsequent decrease in *Bromus japonicus*, which is both functionally and phylogenetically distinct (Figs 1 and 2a). At Kruger, community turnover and the identity of the dominant species was driven by the fire regime (Table S5), where *Bothriochloa radicans* was dominant in annual and intermediate fire frequencies, and *Panicum coloratum* and *Bothriochloa radicans* were co-dominant in the unburned treatment regardless of grazing regime (Fig. S4). Accordingly, there were no significant differences in community rates of change between grazed and grazing release plots in any fire treatment (Fig. 3).

The differences in grass community responses between Konza and Kruger to grazing exclusion appear to be driven by traits of the dominant grasses. Despite being members of the Andropogoneae, the dominant grasses at both sites, *A. gerardii* and *B. radicans*, exhibited very different responses to the loss of large herbivores (Fig. 1). While both species are favoured by frequent fires, *Andropogon gerardii* (Konza) is highly palatable, while *Bothriochloa radicans* (Kruger) is unpalatable and grazing resistant. Specifically, *B. radicans* plants are shorter and have smaller leaves, slightly higher LDMC than some other co-dominants, smaller stomates and lower SPI – traits associated with grazing resistance and drought tolerance (Sack *et al.* 2003; Adler *et al.* 2004; Diaz *et al.* 2007). Secondary chemicals that deter grazers have been found in several Andropogoneae genera (McNaughton 1978; Ellis 1990), including *Bothriochloa* (Pinder & Kerr 1980; Scrivanti, Anton & Zygadlo 2011). *Bothriochloa* species may also have an allelopathic effect, releasing chemicals into the soil that can retard nitrogen mineralization by microbial communities (Hussain, Ahmad & Ilahi 2010; Scrivanti, Anton & Zygadlo 2011). This allelopathic effect could contribute to the lack of community response to grazing release in South Africa, where *B. radicans* became established as a dominant species in historically grazed areas. Many of the species that contain volatile compounds are found in African savannas (Ellis 1990; Bond, Midgley & Woodward 2003), which suggests that a longer history of grazing, together with a greater diversity of herbivores could have promoted the evolution of different strategies for grazer avoidance and tolerance. While North America also harboured a diverse assemblage of large herbivores throughout much of its evolutionary history, the current extent and species composition of North American grasslands were assembled primarily in the post-glacial when there was a lower diversity of grazers (Axelrod 1985). By including functional and phylogenetic perspectives, we were able to expand on results of previous studies which only compared the taxonomic responses of

herbaceous plant communities to grazer manipulation. Similar to our study, Koerner *et al.* (2014) also found divergent taxonomic responses to grazing exclusion between Konza and Kruger when considering the entire herbaceous community (both grasses and forbs). Conversely, Eby *et al.* (2014) reported similar responses of the entire herbaceous plant communities at Konza and Kruger to exclusion of a single grazer. While one could potentially attribute this to differences in the grazers between the two studies (bison versus African buffalo versus multiple herbivores), it could also be attributed to the dominance of *Themeda triandra* at the Eby *et al.* (2014) sites. We found that this species shares traits with the more palatable Andropogoneae species such as *A. gerardii* (Fig. 2a), which further highlights the importance of integrating a functional perspective to gain a more mechanistic understanding of grassland responses to large herbivore loss. Taken together, these observations suggest that responses to the removal of grazers may be quite spatially variable in the more heterogeneous savanna grasslands at Kruger, which is situated in a region of much higher regional grass diversity than North America or Konza (Clayton *et al.* 2002). Overall, it appears that South African mesic savanna grasslands exhibit a broader range of functional strategies in response to grazing by a more diverse extant herbivore community with which they have coexisted over a longer time period (McNaughton 1978; Bond, Midgley & Woodward 2003). Although, in general, one would expect chemically or structurally defended species to become dominant, other variable factors, such as nutrient input and the heterogeneity of grazing patterns, may maintain substantial populations of palatable species in the presence of grazing, as observed in other mesic savanna grasslands, such as the Serengeti (McNaughton 1983; Augustine & McNaughton 1998). Soil fertility or salinity could also influence the distribution of grazing-resistant or tolerant species (Adler *et al.* 2004, 2005; Anderson, Ritchie & McNaughton 2007).

Fire and herbivory are both significant forces in the evolution and maintenance of mesic savanna grasslands globally. Thus, one might expect responses to fire and grazing to evolve in concert and elicit similar community phylogenetic and functional responses across the savanna grassland biome (Bond & Keeley 2005). Our study demonstrates the presence of clades, such as the arid-adapted Cynodonteae, that exhibit similar functional responses to the removal of grazers across sites of divergent biogeographic and evolutionary histories. This suggests that climatic tolerances, evolutionary and grazing histories contribute to contemporary responses to grazing regimes (Adler *et al.* 2004, 2005; Diaz *et al.* 2007). Yet despite conservatism within some subclades, Andropogoneae, which accounts for the greatest per cent of cover at both sites, exhibited a wide range of responses to the removal of grazers which contrasts with the conserved response of Andropogoneae to fire (Forrestel, Donoghue & Smith 2014). At Konza, dominance of the tall, palatable *A. gerardii* resulted in a rapid increase in its abundance and shift in community composition in response to large-grazer removal. In contrast, *B. radicans* exhibited intermediate functional strategies relative to fire and grazing pressures. By being of intermediate height with small

leaves, having higher LDMC and low leaf N content, and containing grazer-deterring secondary chemicals, *B. radicans* harboured traits of both grazing-resistant and fire-tolerant species. This enabled the species to maintain high relative abundance in the presence of fire regardless of grazing regime, which explains the lack of community response to large-grazer removal at Kruger. By studying phylogenetic and functional responses to large herbivore loss, we were able to gain an improved mechanistic understanding of the community response and attribute the divergent responses of Konza and Kruger to the contrasting functional strategies of the dominant species at each site. Overall, our results highlight the importance of understanding community patterns of dominance and heterogeneity in savanna grasslands, within the context of the biogeographic and evolutionary history of a region, to predict how communities will respond to the alteration or elimination of important disturbance regimes. Gaining a better understanding of the regional differences between C_4 dominated savanna grassland ecosystems will be highly relevant to maintaining the taxonomic, phylogenetic and functional diversity important in buffering responses to shifts in both disturbance and climate.

Acknowledgements

Data accessibility

Genetic data: GenBank accessions are listed in the online supporting information.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. List of taxa examined in this study, voucher numbers from personal collections and NCBI accession numbers for all genes and taxa.

Appendix S2. Functional trait collection methods.

Appendix S3. Details of phylogenetic inference.

Appendix S4. Cleaning of GBIF records.

Appendix S5. Rank abundance curves of grass species in mesic grassland sites of North America (Konza) and South Africa (Kruger) subjected to grazing release and fire frequency treatments.

Appendix S6. Analysis of similarity percentage (SIMPER) results for each site and treatment contrast with significant PERMANOVA results.

Appendix S7. Effects of grazing removal of large herbivores on taxonomic, phylogenetic and functional diversity of the grass communities in mesic savanna grasslands of North America (Konza) and South Africa (Kruger).

Appendix S8. Grazing studies compiled from other studies reporting either significant increases (I), decreases (D) or inconsistent (I/D) shifts in abundance for Andropogoneae species in response to active grazing.