Convergent phylogenetic and functional responses to altered fire regimes in mesic savanna grasslands of North America and South Africa

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Summary

- The importance of fire in the creation and maintenance of mesic grassland communities is well recognized. Improved understanding of how grasses – the dominant clade in these important ecosystems – will respond to alterations in fire regimes is needed in the face of anthropogenically driven climate and land-use change.
- Here, we examined how grass communities shift in response to experimentally manipulated fire regimes at multiple levels of community diversity – taxonomic, phylogenetic and functional – in C_4-dominanted mesic savanna grassland sites with similar structure and physiognomy, yet disparate biogeographic histories.
- We found that the grass communities were similar in their phylogenetic response and aspects of their functional response to high fire frequency. Both sites exhibited phylogenetic clustering of highly abundant species in annually burned plots, driven by species of the Andropogoneae, and a narrow range of functional strategies associated with rapid post-fire regeneration in a high-light, nitrogen-limited environment.
- By examining multiple facets of diversity in a comparative context, we identified convergent phylogenetic and functional responses to altered fire regimes in two mesic savanna grasslands. Our results highlight the importance of a common filtering process associated with fire that is consistent across grasslands of disparate biogeographic histories and taxonomic representation.

Introduction

Fire plays a fundamental role in the evolution and maintenance of plant diversity and community structure in mesic (> 500 mm) grasslands and savannas (hereafter referred to as savanna grasslands, *sensu* Scholes & Walker, 1993) globally (Briggs *et al.*, 2005; Keeley & Rundel, 2005; Osborne, 2008; Scheiter *et al.*, 2012). While the response of woody species to shifts in fire frequency in these systems has been widely studied (Pausas *et al.*, 2004; Crisp *et al.*, 2011; Keeley *et al.*, 2011), there has been less focus on the herbaceous community, which is generally dominated by perennial C_4 grasses. These grasses are responsible for the physiognomic structure, promote fire due to their higher productivity, and play important functional and structural roles in savanna grassland communities (Knapp *et al.*, 1998; Sage & Monson, 1998).

Human-induced changes in fire regime, either through direct manipulation of the frequency and intensity of fires or indirect interactions with changes in climate, are occurring at a global scale in savanna grasslands, with important implications for patterns of biodiversity and ecosystem processes (Fuhlendorf & Engle, 2004; Bowman *et al.*, 2009). Understanding how savanna grassland plant communities will respond to inevitable alterations in disturbance at a global scale necessitates a comparative approach that examines multiple aspects of community structure and function (Lavorel & Garnier, 2002; Diaz *et al.*, 2004; Knapp *et al.*, 2004). Yet most studies that explore community-level responses of savanna grasslands to altered fire regimes are restricted to a single site and/or are focused only on taxonomic patterns in plant community response (Smith *et al.*, 2013; Kirkman *et al.*, 2014; Koerner *et al.*, 2014). The analysis of other aspects of plant community structure, such as phylogenetic and functional diversity, could yield additional insights into the causes and consequences of observed community responses (Webb *et al.*, 2002; Fukami *et al.*, 2005; Cavender-Bares *et al.*, 2009; Cadotte *et al.*, 2013).

Phylogenetic approaches to understanding the processes that drive patterns of plant community assembly have been

implemented in many studies of community ecology (Webb *et al.*, 2002; Emerson & Gillespie, 2008; Cavender-Bares *et al.*, 2009). These generally rely on relatedness being a proxy for functional similarity. Functional trait approaches are also increasingly being used to understand the drivers of community assembly (McGill *et al.*, 2006; Violle *et al.*, 2007), and both functional and phylogenetic approaches have been utilized in studies of plant community responses to altered disturbance regimes, including fire (Verdu & Pausas, 2007; Helmus *et al.*, 2010; Silva & Batalha, 2010; Cavender-Bares & Reich, 2012).

Although fire has been shown to have varying effects on taxonomic richness in savanna grasslands (Koerner *et al.*, 2014), a prominent hypothesis for phylogenetic and functional responses to fire has been that frequent or intense fires will result in phylogenetic and functional clustering of species due to the environmental filtering effects of fire (Verdu & Pausas, 2007; Pausas & Verdú, 2008), which by removing accumulated biomass from the previous season, can increase light availability but decrease nitrogen availability due to volatilization (Knapp & Seastedt, 1986; Ojima *et al.*, 1994). This should favor species capable of rapid growth but also with lower nitrogen demand. Thus, in savanna grasslands, which historically experienced frequent and intense fires (Knapp *et al.*, 1998; van Wilgen *et al.*, 2003), those grass species that respond positively to fire are expected to exhibit response traits related to rapid post-fire regeneration, such as basal tillers and bud banks (Everson *et al.*, 1988; Bond *et al.*, 2003), low leaf nitrogen content indicative of higher nitrogen use efficiency in a nitrogen-limited environment (Wedin & Tilman, 1990; Ojima *et al.*, 1994), and/or higher stomatal density, size, stomatal pore index and specific leaf area, all of which have been correlated with higher maximum photosynthetic capacity (Reich *et al.*, 1999; Sack *et al.*, 2003; Franks & Beerling, 2009). However, several studies have reported contrary results (Silva & Batalha, 2010; Cavender-Bares & Reich, 2012). Silva & Batalha (2010) found that species in higher fire frequency plots in the cerrado of Brazil were phylogenetically over-dispersed, apparently due to the acquisition of fire-resistant traits in multiple distantly related lineages that moved into savannas from adjacent tropical forests (Simon *et al.*, 2009). This suggests that historical contingencies may influence how plant communities respond to altered fire regimes. Only by adopting a comparative approach and studying multiple aspects of community structure will we be able to distinguish responses to altered fire regimes that are generalizable from those that are historically contingent.

In the present study, we measured changes in taxonomic, phylogenetic and functional diversity of grasses in response to different fire frequencies. We took advantage of existing long-term fire manipulation experiments at mesic savanna grassland sites in South Africa (Ukulinga Research Farm) and North America (Konza Prairie Biological Station, Kansas, USA) to test the following hypotheses: (1) grass communities in Ukulinga and Konza will diverge in their taxonomic response (species diversity and evenness) to increased fire frequency due to broad differences in their grass diversity and phylogenetic representation; (2) despite the lack of species overlap between Ukulinga and Konza, grass

communities will converge in their phylogenetic and functional responses to increased fire frequency; that is, only one or a few clades exhibiting similar functional syndromes would be dominant under high fire frequency and, thus, grass communities would be under-dispersed both phylogenetically and functionally relative to a null expectation; (3) conversely, grass communities at Ukulinga and Konza will diverge in their response to the absence of fire owing to decreased selective filtering for a specific suite of fire-adapted traits; that is, we expect a broader set of clades/trait syndromes in unburned sites and variation in clade/trait representation between Ukulinga and Konza based on differences in their species pools; and finally, (4) we expect that grass communities burned at an intermediate fire frequency also to exhibit phylogenetic and functional clustering, but to a lesser degree than with high fire frequency.

Materials and Methods

Study area and experimental design

The study was conducted at two grassland sites, Konza Prairie Biological Station in northeastern Kansas, USA (39°05′ N, 96°35′W) and the Ukulinga Research Farm of the University of Kwazulu-Natal outside of Pietermaritzburg in southeastern South Africa (29°40′S, 30°24′E). Both sites are considered mesic savanna grasslands (820 and 790 mean annual precipitation, respectively) dominated by native perennial C_4 grass species, with precipitation and the primary growing season concentrated in the summer months. These sites are similar in their physiognomic structure, growing season climate, historical fire regimes and general edaphic characteristics (Knapp *et al.*, 2004; Kirkman *et al.*, 2014). Site descriptions are further detailed in Kirkman *et al.* (2014).

Experimental burn plots were established at Ukulinga in 1950 with a randomized block (three replicates) split-plot design that includes 251-m^2 subplot burning treatments of 1-, 2-, 3-yr burned and unburned plots. Grazing has been absent on the site for over 60 yr. Konza includes fully replicated watershed-level fire treatments in place since 1977. Each watershed is *c.* 60 ha in area and replicate watersheds are burned at 1-, 2-, 4-, 10- and 20-yr intervals. Watersheds used in this study had not been grazed for over 30 yr.

Comparable experimental designs and vegetation sampling were used at both Ukulinga and Konza for the annual and intermediate (3-yr at Ukulinga, 4-yr at Konza) burn sites and the unburned controls. At both sites, the replicated blocks of fire treatment captured a gradient in soil depth from relatively shallow to greater than a meter deep ($N = 3$/fire frequency/site). Four 2×2-m subplots were established in 2005 at both sites across the three fire treatments. Two of the four subplots were used for a nitrogen addition experiment. The two control plots were used in this study ($N = 6$ subplots/fire frequency/site). From 2005 through 2010, canopy cover was estimated (to the nearest %) during the early and late growing seasons and the maximum cover value for each species across the season was used to calculate annual relative cover for each species in the subplots. For this

study, subplots within each block or watershed were combined yielding a total of three replicates/fire frequency/site.

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 %N, %C, C : N, and ^{13}C were collected during the 2010–2012 field seasons as close to peak flowering as possible for all species (peak flowering ranged from June to September for Konza and December to March for Ukulinga). 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. Specimens were collected for functional trait analysis adjacent to survey plots across all blocks within each treatment to capture as much intraspecific diversity as possible. Species present in 5% or greater mean abundance in the burned (annual and intermediate) and unburned treatments were collected. 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. 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).

Four fully expanded green leaves in full sun were collected per individual plant and immediately placed in plastic bags with a wet paper towel in a cooler. Two leaves were rehydrated for 24–48 h following collection. Subsequently, leaf area and weight were recorded and the leaves were dried at 60°C for at least 48 h. Specific leaf area (leaf area divided by dry mass) and leaf dry matter content (dry mass divided by wet mass) were calculated for each leaf. The dried leaves were also ground for foliar C, N, C : N and ^{13}C isotope analysis. Organic carbon and nitrogen isotope samples were analyzed using a Costech ESC 4010 Elemental Combustion System (Costech Anlaytcial Technologies, Valencia, CA, USA) interfaced with a Thermo Finnigan Delta Plus Advantage isotope mass spectrometer (Thermo Finnigan-MAT, Bremen, Germany) at Yale University's Earth System Center for Stable Isotopic Studies. Ultimately, five of the 10 leaves per species and treatment were subjected to leaf tissue analysis.

The other two leaves were preserved in 70% ethanol solution for analysis of stomatal size, density and pore index. Dental putty (President Plus-light body; Coltene/Whaledent Ltd., Burgess Hill, West Sussex, UK) impressions were taken from the abaxial surface of the mid-section of five individuals and two preserved leaves per individual ($N = 10$) from each species and treatment. Nail polish peels produced from the impressions were transferred onto microscope slides and imaged using a Zeiss SteREO Discovery.V12 Stereoscope and AxioCam HRc at ×200 magnification. Along each peel, six stomata were measured for length and stomata were counted in two fields of view located on either side of the midrib to calculate stomatal density. Stomatal pore index, an index of total stomatal pore area per leaf area, was quantified as stomatal density × the square of the mean guard cell length (Sack et al., 2003).

Phylogenetic inference

We estimated phylogenetic relationships for species at the Ukulinga and Konza sites together using the nuclear ribosomal internal transcribed spacer (ITS) region, and matK, ndhF and rbcL chloroplast markers. These four markers were retrieved from Genbank and supplemented by sequences obtained from specimens collected at each site. We sequenced only the three chloroplast markers for our specimens, which matched 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. PCR products were sequenced using Applied Biosystems Big Dye Chemistry & 3730 xL DNA analyzers (Applied Biosystems, Carlsbad, CA, USA) at the Keck Biotechnology Resource Laboratory (Yale University). Five newly sequenced species and a total of twenty-seven new sequences were deposited in Genbank; voucher specimen information and Genbank accession numbers are listed in Supporting Information Table S1. The four markers were aligned using MUSCLE v3.7 (Edgar, 2004) and then edited manually. Individual alignment files were concatenated using Phyutility (Smith & Dunn, 2008). Our aligned sequence matrix consisted of 8606 base pairs. Models of nucleotide substitution and optimal partitioning strategies were chosen simultaneously under the Bayesian Information Criterion (BIC) using heuristic search algorithms in PartitionFinder (Lanfear et al., 2012). The noncoding nuclear gene region ITS was treated as its own partition. For the coding genes rbcL, matK and ndhF, all five combinations of codon partitions were considered as candidate partitions. Alternative nucleotide substitution models considered were those available in BEAST v1.6.2 (Drummond & Rambaut, 2007). The best-fit partition strategy according to BIC was a GTR+I+G model for all partitions. Phylogenetic relationships were reconstructed using Bayesian methods in BEAST v1.6.2 (Drummond & Rambaut, 2007). Mixed partition analyses were performed for each of the sampled genes and on the concatenated matrices using the optimal partition strategies identified by PartitionFinder (Lanfear et al., 2012). Trees were unlinked by gene region and analyses were conducted under a model of uncorrelated rates and a log-normal distribution. The tree was time-calibrated using normally distributed calibrations for the following four clades in the tree: BEP-PACMAD split (mean = 49.8, SD = 3.0); Andropogoneae (mean = 18.0, SD = 3.7); Chloridoideae (mean = 34.7, SD = 3.75); and Aristideae (mean = 8.6, SD = 10.5) following the BEAST dating analysis of Christin et al. (2014) based on macrofossil evidence. 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). Effective sample sizes for all model parameter estimates were examined to ensure adequate mixing of the chain, with ESS values above 200 indicating appropriate sampling. 1000 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.

Community taxonomic diversity analyses

All analyses were conducted separately for data from Konza and Ukulinga, focusing only on the grasses in these communities. Changes in plant community structure across fire treatments were assessed using multiple diversity metrics (Magurran, 2004), including species richness (S), Shannon–Wiener's diversity index (H′), evenness (J′), and the Berger-Parker dominance index (D). ANOVAs and Tukey's post hoc HSD tests were conducted to identify significant differences between fire treatments in measures of taxonomic diversity. Turnover in the identity and abundance of species in response to fire frequency was assessed using: permutational multivariate analysis of variance, which is a nonparametric analog of MANOVA (Anderson, 2001; McArdle & Anderson, 2001); and post hoc similarity percentage analysis (SIMPER). All analyses were conducted in the vegan package (Oksanen et al., 2013) of the R statistical library v3.0.0. Using the adonis function in R, the permutational MANOVA 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 fire treatments were assessed using F-tests based on sequential sums of squares of the permutation of the raw data. When significant differences between treatments were found, SIMPER was used to identify the grass species contributing the most to the differences observed between fire treatments. Individual grass species responses to fire frequency were tested using linear mixed-effects models implemented in the lme4 package in R (Bates et al., 2013). Each species was fit with a separate model with year treated as a repeated measure and fire as a fixed effect. If fire was found to be a significant effect, pair-wise comparisons of fire treatments were analyzed using Tukey's HSD to correct for inflated P-values as a result of multiple comparisons.

Community phylogenetic and functional trait analyses

Phylogenetic community structure was analyzed using abundance-weighted standardized effect sizes of mean pair-wise phylogenetic distances of species within each block and treatment. This measure is equivalent to −1 times the net relatedness index (NRI) of Webb et al. (2002) which is, similarly, the standardized mean pair-wise phylogenetic distance between species in a given community. High values indicate greater than expected phylogenetic dispersion (i.e. individuals within a block or treatment are more distantly related to each other than expected by chance) while low values indicate lower than expected phylogenetic dispersion. To assess significance, a null model was generated where taxon labels were shuffled across the site-level tree while maintaining the species diversity and abundance structure within each sample. 9999 permutations of the null model were generated and a two-tailed test was implemented to test for significant over or under phylogenetic dispersion of the community in question. Analyses were conducted separately for each fire treatment by block and by site. All community phylogenetic analyses were conducted in the picante package in R (Kembel et al.,

2010). Tests of node-based phylogenetic clustering of discrete responses to increased fire frequency were conducted using the clade significance statistic of J. C. Oliver, E. J. Edwards & M. J. Donoghue (unpublished) which builds upon the nodesig test in PhyloCom (Webb et al., 2008). In this metric, the clade density state is calculated for each node and clades are identified within which (1) descendant lineages are characterized by similar densities and (2) 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 fire frequency. 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).

As a complement to our measure of phylogenetic dispersion, we also measured the functional dispersion for each block and fire treatment. Functional dispersion is a multivariate measure of the mean functional distance of individual species to the centroid of a community, weighted by the species abundance (Laliberté & Legendre, 2010). We calculated standardized effect sizes for the functional dispersion measure of each block and treatment by generating a null distribution analogous to the null generated for our phylogenetic dispersion analyses. While the pair-wise distances generated for phylogenetic analyses were derived from the branch length distances in the phylogeny and the tips were shuffled for the null, Gower's dissimilarity metric was calculated for all species trait values at each site for the trait dispersion analyses (Gower, 1971). The analogous null model was then generated by shuffling the species identities in the trait distance matrix, thereby maintaining species richness and abundance values in the analyses. Functional dispersion was calculated separately for each fire treatment by block and by site. All functional trait analyses were conducted in the FD package (Laliberté & Shipley, 2011) in R.

In order to assess functional shifts in response to fire frequency, community aggregated trait values were calculated for each block and trait. The Community Weighted Mean (CWM) of a trait value is the mean value present in the community weighted by the relative abundance of the focal species in that community. The use of CWMs aligns with the biomass ratio hypothesis wherein the dominant species in the community and their traits are sufficient to describe how a community will respond to environmental change (Grime, 1998). To incorporate intraspecific trait variation, different trait means were calculated for burned (annual and intermediate) and unburned treatments for species that were present in 5% or greater abundance across treatments. ANOVAs were conducted on CWMs of traits with fire treatment as a fixed effect to test for significant shifts in individual traits associated with fire regime. A principal components analysis (PCA) of all CWM trait means of each block and treatment was conducted to examine shifts in multivariate axes of variation – that is, functional syndromes – associated with shifts in fire frequency. To assess individual species trait-based responses to fire, we conducted a phylogenetically corrected PCA in the phytools package of R (Revell, 2009).

Results

Taxonomic diversity patterns

The grass communities at Ukulinga and Konza differed in their taxonomic response to fire frequency. At Ukulinga, grass species richness, diversity and evenness were significantly higher with annual burning and lowest in the absence of burning. By contrast, grass richness and evenness was similar across treatments and diversity was highest in plots of intermediate fire frequency at Konza. Although dominance tended to be higher with intermediate burning at Ukulinga, there were no significant differences among the fire treatments in dominance at either site (Table 1).

The permutational MANOVA results indicated that fire frequency significantly changed species composition at both Konza and Ukulinga ($F = 3.62$, $R^2 = 0.34$, $P = 0.01$ and $F = 8.74$, $R^2 = 0.56$, $P = 0.004$, respectively). Plots within a particular fire treatment were more similar to each other than plots among fire treatments. In view of these results, the species responsible for shifts across fire treatment were identified using SIMPER analysis (Table S2). At Konza, the divergence between burned and unburned plots was driven primarily by increases in the abundance of *Schizachyrium scoparium* and *Sorghastrum nutans*, and decreases of *Poa pratensis* and *Andropogon gerardii* in response to increased fire frequency. While abundance varied, *Andropogon gerardii* maintained dominance across all treatments. At Ukulinga, there was a greater turnover of species across the fire treatments (Fig. 1, Table S2). *Themeda triandra*, *Diheteropogon amplectens* and *Heteropogon contortus* increased in abundance and *Aristida junciformis* decreased in abundance in response to increased fire frequency.

Phylogenetic diversity patterns

The site-level pool of grass species was larger at Ukulinga than Konza, with 22 vs 12 species encountered during sampling, respectively. For Ukulinga, all of the species were C_4, whereas at Konza 9 were C_4 and three were C_3 (*Poa pratensis*, *Koeleria pyramidata*, *Panicum oligosanthes*). There was significant clade overlap between the two sites, except for species of the Aristidoideae and Tristachyideae only represented at Ukulinga and species of Zoysieae and Pooideae only represented at Konza. Both

Andropogoneae and Paniceae were more diverse at Ukulinga (Fig. 2).

Annually burned communities at Konza and Ukulinga exhibited patterns of phylogenetic clustering or under-dispersion (i.e. negative values), and these patterns did not differ appreciably between the block and site levels (Table 2). The clustering response was largely driven by the dominance of Andropogoneae species with annual burning and their positive responses to fire (Fig. 2). In annually burned plots, patterns of phylogenetic clustering were stronger and highly significant at Konza, due to the dominance of only a few species, in comparison to Ukulinga with co-dominance by a number of species and variation among blocks in species representation and response to fire (Fig. 1, upper two panels; Table 2). By contrast, unburned grass communities exhibited phylogenetic over-dispersion (positive values) at both sites, but at each site there was the co-occurrence of different sets of distantly related species. At Ukulinga, *Arisitida junciformis* of Aristioideae, *Cymbopogon nardus* of Andropogoneae and *Eragrostis curvula* of Eragrostideae were co-dominant. By contrast, *Andropogon gerardii* and *Sorghastrum nutans* of Andropogoneae, *Poa pratensis* of Pooideae and *Sporobolus heterolepis* of Zoysieae were co-dominant at Konza (Figs 1, 2). Patterns of over-dispersion in unburned plots were significant and more pronounced at Ukulinga than Konza (Table 2). With intermediate fire frequency, the sites exhibited different patterns of phylogenetic clustering. At Konza, grass species were significantly under-dispersed, as a consequence of Andropogoneae species dominating. However, grass species were over-dispersed at Ukulinga (similar to the response observed for the unburned grass community) due to other clades becoming co-dominant with Andropogoneae species in the intermediate fire treatment (Fig. 1, middle panels).

The node-based test for the clustering of species' responses (positive or negative) to fire was significant for Andropogoneae at Ukulinga, yet insignificant at Konza ($P = 0.103$). The lack of significance at Konza was due to the negative shift in *A. gerardii* despite its continued dominance in annually burned plots, and the overall lower taxonomic diversity (3 vs 7 species). In addition, there was a significantly clustered negative response in a subclade of Paniceae at Ukulinga, but this was for two species that were not abundant (< 5%) in the community, and therefore not contributing significantly to the community response (Fig. 2).

Table 1 Mean (standard error) plot-level (4 m^2) grass richness, diversity, evenness and dominance by fire treatment in mesic savanna grasslands of Ukulinga Research Farm, South Africa and Konza Prairie Biological Station, North America

Site	Fire frequency	Richness (S′)	Evenness (J′)	Diversity (H′)	Dominance (D)
Konza	Annual	7.33 (0.33)[a]	0.60 (0.04)[a]	1.19 (0.08)[a]	0.45 (0.05)[a]
	Intermediate	10.00 (0.00)[a]	0.62 (0.03)[a]	1.44 (0.06)[b]	0.44 (0.02)[a]
	Unburned	8.00 (0.58)[a]	0.65 (0.04)[a]	1.34 (0.06)[ab]	0.40 (0.02)[a]
Ukulinga	Annual	15.67 (0.67)[a]	0.73 (0.01)[a]	2.01 (0.06)[a]	0.45 (0.04)[a]
	Intermediate	11.00 (1.73)[b]	0.53 (0.08)[b]	1.27 (0.27)[b]	0.63 (0.10)[a]
	Unburned	7.00 (1.00)[b]	0.55 (0.08)[b]	1.04 (0.10)[b]	0.54 (0.07)[a]

Mean values were calculated across block ($N = 3$) for each fire treatment. Significance ($P \leq 0.05$) between fire treatments is indicated by letters.
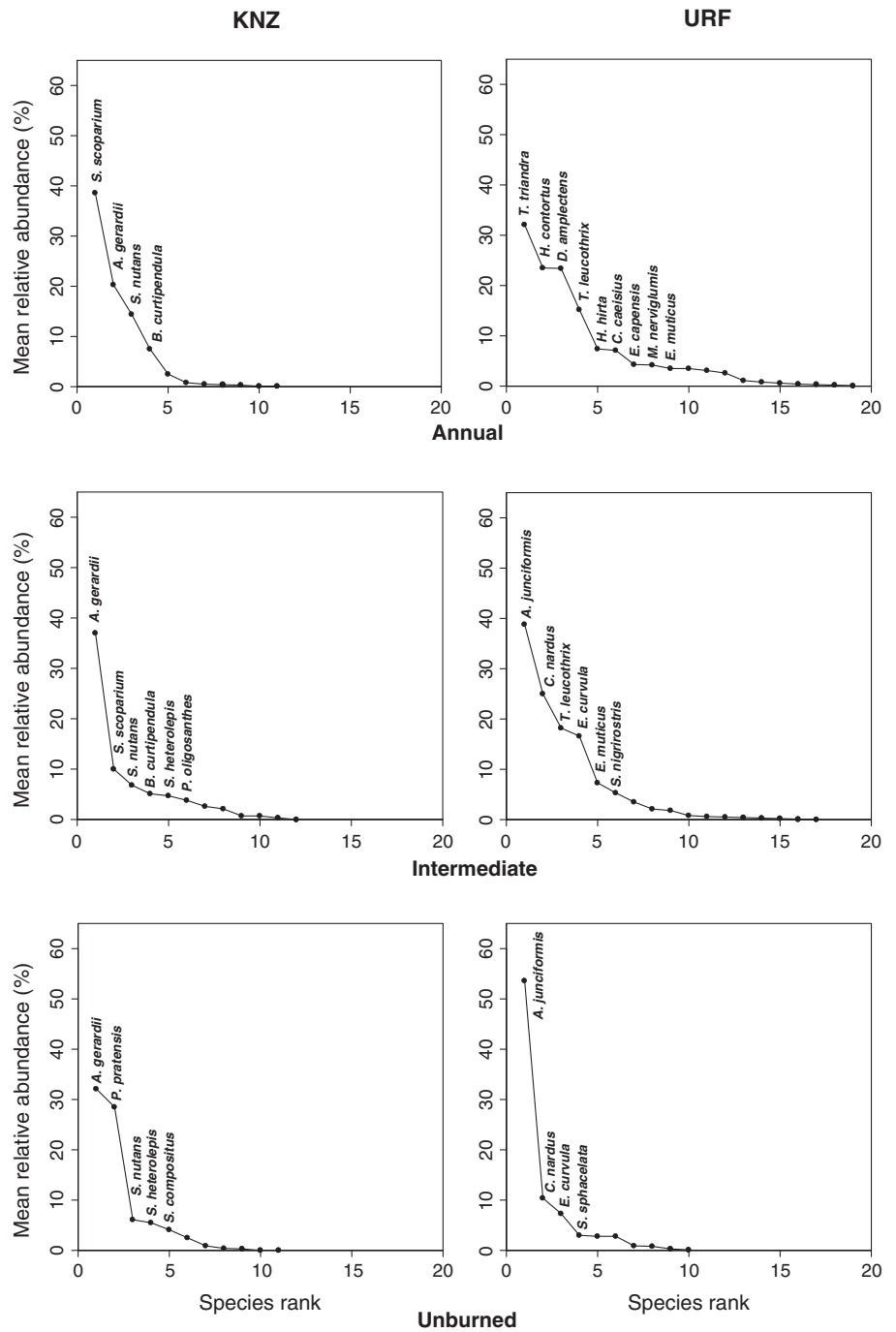
Fig. 1 Rank abundance curves of grass species in mesic grassland sites of North America (KNZ) and South Africa (URF) subjected to fire frequency treatments. Relative abundance values for each fire treatment are mean values across the three blocks at each site averaged across the 2006–2010 field seasons. Those species that consist of the top 90% of grass cover at each site are labeled on each panel. See Supporting Information Table S1 for complete names of species.

Functional diversity patterns

At Konza, annually and intermediate burned plots exhibited under-dispersion of functional traits, while unburned plots exhibited over-dispersion. Patterns of dispersion were only significant relative to the null model in annually burned plots and all results were consistent at the block and site levels (Table 2). At Ukulinga, annually burned plots exhibited significant functional clustering or under-dispersion. Intermediate burn plots were clustered at the block scale, yet slightly over-dispersed at the site scale (Table 2). Incongruence in the functional dispersion results

across scales was due to the turnover across blocks of functionally distinct species in intermediate burn plots (Fig. 3). Unburned plots exhibited both under and over-dispersion, but values were closer to zero and nonsignificant.

Overall, Ukulinga and Konza had comparable trait means for all traits measured (Table 3). At Konza, annually burned plots had significantly higher SLA, lower leaf N and C, higher leaf C : N, higher LDMC and lower stomatal density. Nonsignificant trends in trait means were greater stomatal size and higher stomatal pore index in burned plots. At Ukulinga, significant trends in community weighted trait means were higher SLA and lower leaf

KNZ   URF

|	Intermed	Annual		Intermed	Annual	
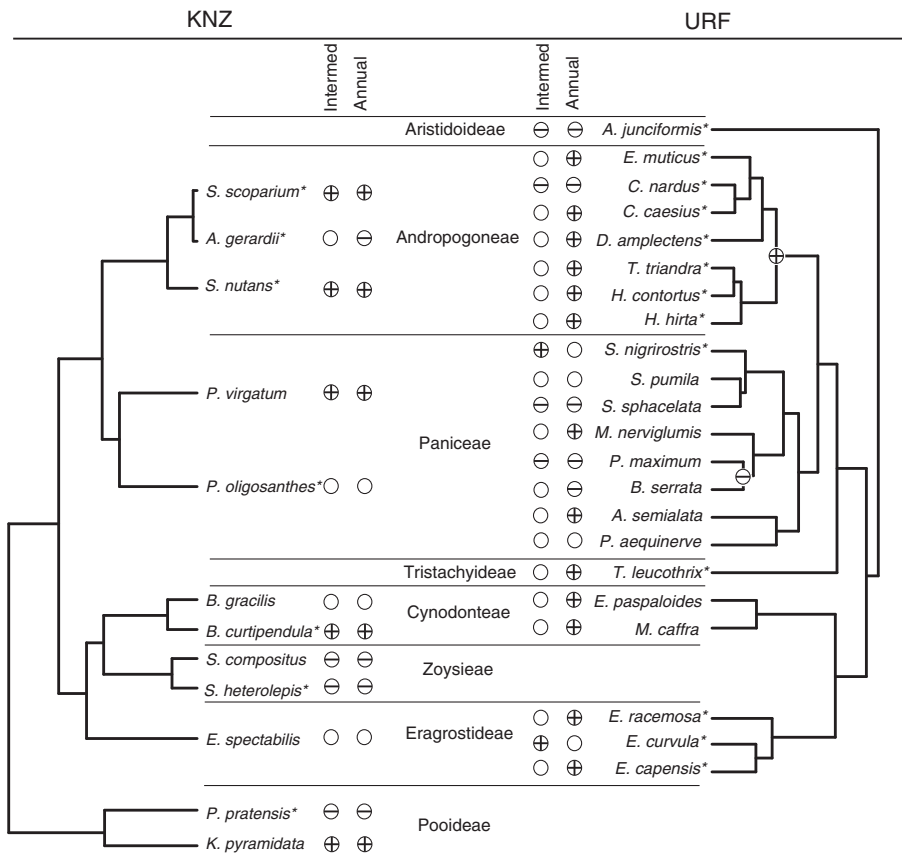
Fig. 2 Responses of grass species to increased fire frequency mapped onto the phylogeny of grass species at Konza Prairie Biological Station, North America (KNZ) and Ukulinga Research Farm, South Africa (URF). Circles with '+' and '−' signs indicate a significant deviation in relative abundance of a grass species with annual and intermediate burning when compared to the unburned treatment (GLMMs and Tukey's post hoc HSD, $P \leq 0.05$) (Open circles, no change; circles with +, increase; circles with -, decrease.). Species denoted with an asterisk are those that are in the top 90% of relative abundance for any of the fire treatments. Clades exhibiting relative responses that are significantly over or under-dispersed (according to J. C. Oliver *et al.* (unpublished)'s node based statistic, I, see text for details) are highlighted at the appropriate node with a positive (over-dispersed) or negative (under-dispersed) sign. At Ukulinga, the Andropogoneae clade exhibit a positive clustered response ($P = 0.0061$), while the Paniceae clade containing *Panicum maximum* and *Brachiaria serrata* exhibited a clustered negative response ($P = 0.041$). See Supporting Information Table S1 for species complete names.

Table 2 Patterns of phylogenetic and functional dispersion for each fire treatment

Site	Treatment	Block	Num taxa	SES. mpd	Significance	SES. Fdisp	Significance
Konza	Annual	1	10	−3.73	***	−2.22	**
		2	9	−3.16	**	−1.95	*
		3	11	−2.89	**	−1.90	*
		Combined	11	−3.46	**	−2.09	*
	Intermed.	1	10	−2.82	***	−1.39	NS
		2	9	−2.26	*	−1.24	NS
		3	11	−2.75	***	−1.51	NS
		Combined	11	−2.74	**	−1.42	NS
	Unburned	1	9	0.84	NS	1.25	NS
		2	7	1.11	NS	1.46	NS
		3	8	0.60	NS	1.37	NS
		Combined	10	0.90	NS	1.37	NS
Ukulinga	Annual	1	17	−1.70	NS	−2.31	**
		2	15	−0.67	NS	−2.46	**
		3	15	−2.64	**	−1.71	***
		Combined	17	−2.13	**	−2.45	**
	Intermed.	1	8	0.97	NS	−1.25	NS
		2	11	1.59	**	−1.81	*
		3	14	1.72	**	−0.94	NS
		Combined	14	1.85	**	0.12	NS
	Unburned	1	5	1.46	**	−0.51	NS
		2	7	1.9	**	−0.27	NS
		3	8	1.84	**	0.20	NS
		Combined	9	1.56	**	−0.28	NS

Sites: Ukulinga, Ukulinga Research Farm, South Africa; Konza, Konza Prairie Biological Station, North America. SES.mpd, mean standard effect sizes of phylogenetic distance and significance based on two-tailed test; SES.Fdisp, mean standardized effect sizes of functional dispersion by fire treatment. Significance was assessed by comparing the observed MPD or functional dispersion values to those generated from a null model (see text for details). Significance values: *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$; NS, not significant.
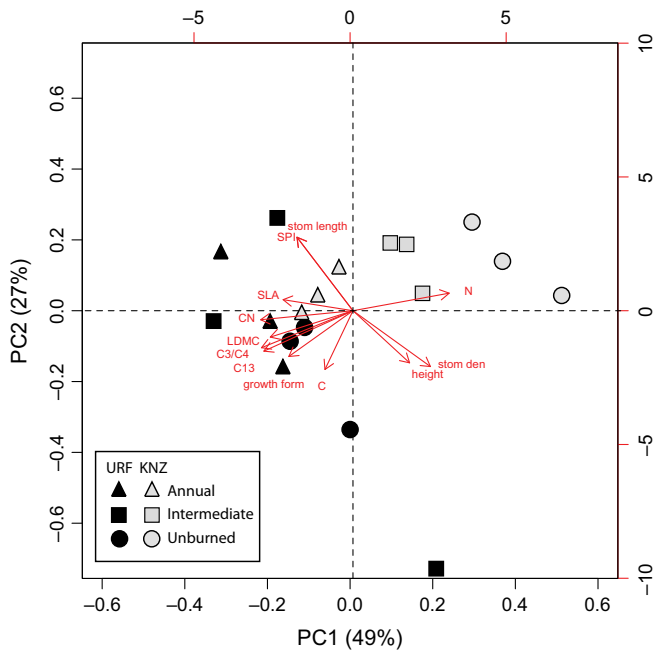
Fig. 3 A principal components analysis (PCA) of the community weighted trait means (based on $N = 3$ blocks per treatment) by fire treatment. Site abbreviations: KNZ, Konza Prairie Biological Research Station, North America; URF, Ukulinga Research Farm, South Africa. Trait abbreviations: height, maximum culm height; SLA, specific leaf area; LDMC, leaf dry matter content; stom length, stomatal length; stom density, stomatal density; SPI, stomatal pore index; growth form, caespitose/rhizomatous growth form; N, percent leaf nitrogen by mass; C, percent leaf carbon by mass; C : N, percent leaf percent carbon to nitrogen ratio; C13, stable carbon isotope ratio; C3/C4, photosynthetic pathway.

N in annually burned plots. Other trends in trait means, albeit not significant, were lower LDMC, larger stomata and higher stomatal pore index (Table 3). While there was overall similarity in community functional response across both sites, Konza exhibited a more pronounced functional response to altered fire regimes. The PCA of community weighted trait means by block and fire treatment further demonstrate the collective functional strategies of communities in response to different fire regimes. The first two principal components captured over 70% of the variance in the community trait means. Leaf nitrogen content, SLA, LDMC, stomatal density and culm height loaded most heavily on the first axis, while SPI and stomatal size loaded most heavily on the second axis (Fig. 3). The unburned communities exhibited strategies of higher leaf nitrogen, lower specific leaf area and slightly higher stomatal density, while most intermediate and annually burned communities had higher leaf nitrogen content, higher specific leaf area, larger stomates and higher stomatal pore index.

The PCA of species trait values in each fire treatment showed different patterns of clustering in functional trait space. Within the annually burned communities, the most abundant species, most of which belong to the Andropogoneae, clustered in PC space and exhibited a similar functional strategy (Fig. 4a). Traits related to leaf nutrient content and stomatal characteristics loaded most heavily on the first PC axis across all treatments and

Table 3 Effects of fire frequency treatments on community weighted traits means (standard errors in parentheses) for mesic grasslands at the Konza Biological Station, North America and the Ukulinga Research Farm, South Africa

Site	Treatment	Height	SLA	LDMC	Stom. length	Stom. density	SPI	Leaf %N	Leaf %C	Leaf C : N	C13
										Trait	
Konza	Annual	118.5 (5.2)[a]	133.6 (23.2)[a]	0.503 (0.012)[a]	30.0 (0.77)[a]	93.2 (2.8)[a]	8.10 (0.21)[a]	1.06 (0.02)[a]	43.1 (0.11)[a]	43.0 (1.65)[a]	−12.9 (0.13)[a]
	Intermed.	126.8 (1.7)[a]	126.1 (2.2)[a]	0.434 (0.009)[b]	31.8 (0.65)[a]	90.3 (2.4)[a]	8.58 (0.17)[a]	1.20 (0.01)[b]	43.5 (0.04)[b]	37.8 (0.25)[b]	−13.7 (0.35)[b]
	Unburned	121.1 (4.5)[a]	113.0 (3.3)[b]	0.413 (0.006)[b]	28.8 (1.15)[a]	107.1 (3.6)[b]	7.77 (0.31)[a]	1.31 (0.02)[c]	43.5 (0.09)[b]	34.7 (0.44)[c]	−17.9 (0.40)[b]
Ukulinga	Annual	97.3 (4.5)[a]	160.0 (31.5)[a]	0.497 (0.013)[a]	31.7 (1.7)[a]	91.3 (5.7)[a]	8.57 (0.44)[a]	1.05 (0.01)[a]	43.95 (0.06)[a]	43.4 (0.13)[ab]	−12.16 (0.03)[a]
	Intermed.	124.8 (28.4)[a]	130.4 (74.6)[b]	0.471 (0.019)[a]	30.3 (2.9)[a]	93.2 (1.3)[a]	8.19 (0.78)[a]	1.00 (0.08)[a]	44.06 (0.29)[a]	47.1 (4.48)[a]	−12.32 (0.27)[a]
	Unburned	110.4 (9.8)[a]	123.2 (57.8)[b]	0.522 (0.009)[a]	29.6 (0.8)[a]	93.3 (3.4)[a]	7.99 (0.22)[a]	1.35 (0.04)[b]	43.56 (0.14)[a]	33.0 (0.79)[b]	−12.65 (0.17)[a]

Community weighted trait means were calculated by averaging mean trait values for each species in a plot weighted by each species abundance. Subplots ($n = 2$ per block, 4 m^2) were averaged, and then the mean value and standard error calculated across blocks ($N = 3$) for each fire treatment. Treatment differences were assessed using general linear mixed models, and if significant differences were found, pair-wise comparisons were made using Tukey's post hoc HSD to control for multiple comparisons. Significant differences ($P \leq 0.05$) between treatments are indicated by letters. Trait abbreviations and units: Height (cm); SLA, specific leaf area (cm^2 g^{-1}); LDMC, leaf dry matter content; Stom. length, stomatal length (μm); Stom. density, stomatal density (# mm^{-2}); SPI, stomatal pore index (10^{-2}); Leaf %N, percent leaf nitrogen by mass; Leaf %C, percent leaf carbon by mass; Leaf C : N, percent leaf carbon to nitrogen ratio by mass; C13, stable carbon isotope ratio.
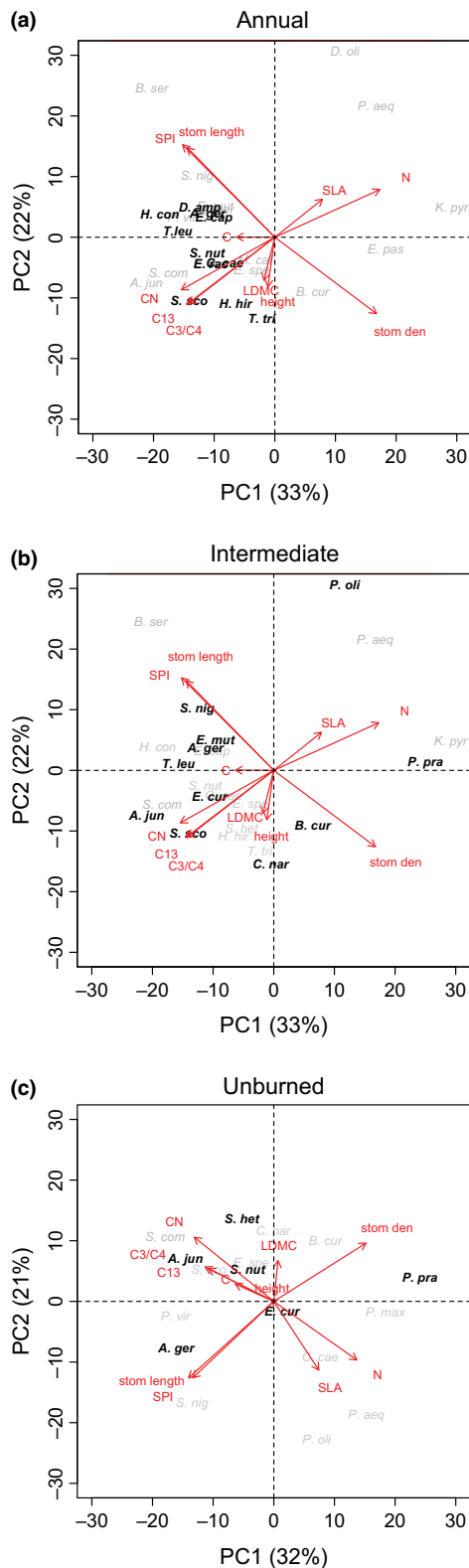
Fig. 4 A phylogenetically-corrected principal components analysis (PCA) of each species functional traits values in: (a) the annually-burned communities, (b) intermediate burn communities, and (c) the unburned communities. Means for species that constituted > 5% of the mean relative cover across the burn treatments were taken from the annual or intermediate burn plots for the burned communities, and the unburned plots for the unburned communities. In each panel, the bold-face species are those that comprise the top 90% of the mean relative abundance in each treatment. See Supporting Information Table S1 for species complete names. Trait abbreviations: height, maximum culm height; SLA, specific leaf area; LDMC, leaf dry matter content; stom length, stomatal length; stom den, stomatal density; SPI, stomatal pore index; N, percent leaf nitrogen by mass; C, percent leaf carbon by mass; C : N, percent leaf percent carbon to nitrogen ratio; C13, stable carbon isotope ratio; C3/C4, photosynthetic pathway.

Conversely, species that constituted the bulk of the abundance in the intermediate and unburned treatments exhibit a much wider, more diverse range of functional strategies and lack of clustering (Fig. 4b,c).

Discussion

Understanding how grassland communities will respond to inevitable alterations in fire regimes is essential to protecting the biodiversity and functional properties of these important ecosystems. Comparative studies such as ours, which integrate multiple facets of diversity, will help us identify general patterns in community assembly in response to altered disturbance regimes. We found that the grasses of mesic (> 500 mm precipitation) savanna grasslands in North America and South Africa differ in their taxonomic response to altered fire regimes, yet were similar in aspects of their phylogenetic and functional responses. We demonstrate that examination of these different facets of diversity improves understanding of the commonalities and contingencies of grass community response to fire across regions with different biogeographic and evolutionary histories.

As expected, we found divergence in the taxonomic response to fire, which was due to differences in patterns of diversity at the two sites. Ukulinga harbored greater grass species richness (Table 2) and the bulk of this diversity was within the Andropogoneae. This clade has much higher diversity in southern Africa than in North America (Hartley, 1958). The Andropogoneae generally responded positively to increased fire frequency (Fig. 2), and species from this clade were dominant in annually burned plots at both Ukulinga and Konza. In combination with the site-level patterns of taxonomic diversity, this resulted in increased richness and diversity at Ukulinga in response to increased fire frequency, while Konza exhibited no significant change in diversity across fire treatments and highest richness in intermediately burned plots. *Andropogon gerardii* was a notable exception to Andropogoneae species increasing in abundance in response to increased fire frequency. While there is evidence from other studies that this species responds positively to fire (Knapp, 1985; Knapp *et al.*, 1998), our analysis indicates a significant decrease in abundance in long-term annually burned plots (Fig. 2). It should also be noted that while *A. gerardii* maintains

represented *c.* 30% of the variance, whereas stomatal traits and culm height loaded most heavily on the second PC axis (22% of variance). Species that dominated in the annually burned communities exhibited lower leaf nitrogen, lower stomatal density, larger stomates and higher stomatal pore index values (Fig. 4a).

relatively high abundance in annually burned plots, it co-dominates with *Schizachyrium scoparium*, which is present at much lower abundance in intermediate and unburned plots.

Despite the divergent response in measures of taxonomic diversity, the two sites converged in their phylogenetic response to increased fire frequency. Annually burned plots exhibited phylogenetic clustering at both sites, supporting the hypothesis that fire is a strong environmental filter for grass species/clades and can be an important driver of patterns of dominance and community assembly in mesic savanna grasslands. Plots burned at intermediate frequency were also clustered at Konza, but not at Ukulinga, where there was greater turnover of species between the annual and infrequently burned plots (Fig. 1), suggesting that fire frequency plays an important role in structuring grass communities as well.

Importantly, by using a node-based clustering metric (J. C. Oliver *et al.*, unpublished), as opposed to taxa recognized at particular hierarchical ranks (subfamily, tribe, genus), we were able to attribute this pattern of phylogenetic clustering across sites to species of the Andropogoneae (Fig. 2). Species diversification within Andropogoneae was concentrated during the late Miocene spread of fire and now dominates in high fire frequency (mesic) grasslands around the world (Hartley, 1958; Bond *et al.*, 2003; Keeley & Rundel, 2005; Visser *et al.*, 2011). It is most dominant and diverse in subtropical and tropical savannas, a biome largely maintained by fire (Staver *et al.*, 2011; Visser *et al.*, 2014), and exhibits a suite of functional traits that are highly advantageous in regularly burned grasslands (Everson *et al.*, 1988; Morgan & Lunt, 1999; Bond *et al.*, 2003; Spasojevic *et al.*, 2010). Many Andropogoneae are heavily reliant on fire to persist, and are lost from the community with fire suppression (Everson *et al.*, 1988; Morgan & Lunt, 1999; Uys *et al.*, 2004).

We also found similarities in community-level functional responses to high frequency fire regimes broadly indicative of a resource acquisitive strategy that is advantageous in high light and nitrogen-limited post-burn environments. Annually burned communities converged on having higher specific leaf area, greater stomatal size, lower leaf nitrogen and higher stomatal pore index than unburned communities (Table 3, Fig. 3), all traits that confer higher net assimilation and photosynthetic rates (Reich *et al.*, 1999; Sack *et al.*, 2003; Franks & Beerling, 2009). These traits likely contribute to the ability of individuals to grow quickly and become dominant after fires. While more pronounced at Konza, the lower leaf N in annually burned communities, which was reflected in both interspecific and intraspecific responses to increased fire frequency (Fig. 4), is indicative of greater nitrogen use efficiency and lower N mineralization rates. These traits are related to higher photosynthetic rates and productivity for given amounts of soil and leaf nitrogen (Wedin & Tilman, 1990), and also contribute to a lower rate of decomposition, which is important for the retention of the litter necessary to fuel fires (Knapp & Seastedt, 1986). Overall, community-level trait shifts in response to fire frequency were more significant in Konza. This seems to be driven primarily by the dominance of the C$_3$ species, *Poa pratensis*, in the absence of fire (Figs 1, 4c). Much research has been focused on the functional differences

associated with photosynthetic type in grasses (Ehleringer & Monson, 1993; Ripley *et al.*, 2008; Edwards *et al.*, 2010; Taylor *et al.*, 2012), especially the advantages of C$_4$ photosynthesis in high light, high temperature and/or arid environments, some of which are associated with post-fire environments in these mesic grasslands (Ripley *et al.*, 2010). Yet, despite this strong driver of patterns at Konza, we demonstrate by combining phylogenetic and functional approaches that there are also important functional differences within C$_4$ lineages that drive patterns of dominance and community assembly. For example, we found that species within Paniceae had higher leaf N content than species of Andropogoneae (Fig. 4). While Paniceae exhibited mixed responses to increased fire frequency in our study, likely due to their low abundance, they have been found to be less diverse and less abundant in high fire frequency environments (Visser *et al.*, 2011; Koerner *et al.*, 2014). *Aristida junciformis*, another C$_4$ species that dominated in unburned plots at Ukulinga, had lower leaf N, higher LDMC and low SLA, functional attributes found in other Aristidoid species as well (E. J. Forrestel, unpublished data).

Our phylogenetically corrected principal components analyses show that the species that dominate in annually burned communities – mostly within Andropogoneae – are clustered in functional trait space at both sites (Fig. 4a). Based on community weighted trait means, we also found that the annually burned plots were significantly functionally clustered or under-dispersed at both sites (Table 2). Conversely, the diversity of species and clades present in the unburned plots, both within and among sites, exhibit a wider range of functional strategies than in burned communities (Table 2, Fig. 4c). Phylogenetic over-dispersion in the unburned plots at both sites corroborates these findings (Table 2). Overall, as we hypothesized, the functional clustering in annually burned plots and the over-dispersion in unburned plots at both sites indicates a strong role of fire as an environmental filter. The absence of the fire filter in the unburned plots resulted in different sets of species/clades, with different functional strategies, dominating at each site (i.e. Aristidoideae in Ukulinga and Pooideae in Konza, see Figs 2, 3c).

It is well-recognized that both fire and grazing affect the community structure and function of savanna grasslands (McNaughton, 1985; Fuhlendorf & Engle, 2004; Koerner *et al.*, 2014). Thus, it is important to consider our results in light of that fact that grazing by large mammals was absent at both sites. In a separate study of the effects of native ungulate grazer removal from historically grazed sites in North American and South African savanna grasslands, we found that while Andropogoneae species did differ in their ability to tolerate grazing, they maintained their dominance in high fire frequency environments in the presence and the absence of grazers (E. J. Forrestel, M. J. Donogue & M. D. Smith, unpublished). This suggests that while there would be an influence of grazing on community structure at the sites used in the present study, our basic findings regarding the influence of fire would still hold.

By utilizing long-term experimental manipulations of fire frequency, our study demonstrates that the response of the grass community to alterations in fire frequency in two mesic

grasslands located in South Africa and North America are convergent in many aspects of their function and structure, and the differences between them can largely be explained by biogeographic contingencies (i.e. different patterns of taxonomic diversity and representation of lineages). The convergent responses were largely driven by the strong filter that fire exerts on the grass community at both sites, with savanna grasslands exposed to high fire frequency being dominated by species of Andropogoneae that exhibit a suite of traits that confer an advantage in post-fire environments and also maintain fuel loads necessary to promote fire. Our study demonstrates that phylogenetic and functional comparative approaches, combined with the measurement of multiple facets of community diversity, can enhance our ability to find general patterns and explain contingencies in the response of communities to environmental change.

Acknowledgements

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Supporting Information

Additional supporting information may be found in the online version of this article.

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

Table S2 Analysis of similarity percentage results for each site

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