

Fire ecology of C₃ and C₄ grasses depends on evolutionary history and frequency of burning but not photosynthetic type

BRAD RIPLEY,^{1,6} VERNON VISSER,² PASCAL-ANTOINE CHRISTIN,³ SALLY ARCHIBALD,^{4,5} TARRYN MARTIN,¹
 AND COLIN OSBORNE³

¹Botany Department, Rhodes University, P.O. Box 94, Grahamstown 6140 South Africa

²Centre for Invasion Biology (CIB), Department of Botany and Zoology, Stellenbosch University, Matieland 7602 South Africa

³Department of Animal and Plant Sciences, University of Sheffield, Sheffield S10 2TN United Kingdom

⁴Natural Resources and the Environment, Council for Scientific and Industrial Research, Pretoria 0001 South Africa

⁵School of Animal, Plant and Environmental Sciences, University of the Witwatersrand, Johannesburg, Private Bag X3, WITS 2050 South Africa

Abstract. Grasses using the C₄ photosynthetic pathway dominate frequently burned savannas, where the pathway is hypothesized to be adaptive. However, independent C₄ lineages also sort among different fire environments. Adaptations to fire may thus depend on evolutionary history, which could be as important as the possession of the C₄ photosynthetic pathway for life in these environments. Here, using a comparative pot experiment and controlled burn, we examined C₃ and C₄ grasses belonging to four lineages from the same regional flora, and asked the following questions: Do lineages differ in their responses to fire, are responses consistent between photosynthetic types, and are responses related to fire frequency in natural habitats? We found that in the C₄ Andropogoneae lineage, frost killed a large proportion of aboveground biomass and produced a large dry fuel load, which meant that only a small fraction of the living tissue was lost in the fire. C₃ species from the Paniceae and Danthonioideae lineages generated smaller fuel loads and lost more living biomass, while species from the C₄ lineage *Aristida* generated the smallest fuel loads and lost the most living tissue. Regrowth after the fire was more rapid and complete in the C₄ Andropogoneae and C₃ Paniceae, but incomplete and slower in the C₃ Danthonioideae and C₄ *Aristida*. Rapid recovery was associated with high photosynthetic rates, high specific leaf area, delayed flowering, and frequent fires in natural habitats. Results demonstrated that phylogenetic lineage was more important than photosynthetic type in determining the fire response of these grasses and that fire responses were related to the frequency that natural habitats burned.

Key words: adaptation; C₃ photosynthesis; C₄ photosynthesis; Eastern Cape, South Africa; evolution; fire; flammability; grasslands; Poaceae; resprouting; savannas.

INTRODUCTION

Savannas and grasslands account for 86% of the world's fires (Mouillot and Field 2005), and are frequently dominated by grasses using the C₄ rather than C₃ photosynthetic pathway (Robinson et al. 1979, Tix and Charvat 2005, Ratnam et al. 2011). The dominance of C₄ grasses in fire-prone environments has been attributed to their high productivity, and their superior water and nutrient use efficiency in postfire environments, that are characterized by high light, high surface temperatures, and low soil fertility (Knapp 1985, Long 1999, Forrester et al. 2014). These characteristics of C₄ species result from a physiological CO₂-concentrating mechanism that almost eliminates photorespiration, elevates photosynthesis under high light

conditions, and allows photosynthetic rates to be maintained at lower stomatal conductance than in their C₃ competitors (Long 1999, Sage 2004, Keeley and Rundel 2005). The high productivity of C₄ grasses contributes to their ability to regrow quickly after fire, while the seasonal die back of their aboveground biomass generates the fuel load necessary for frequent surface fires. These fires exclude fire-intolerant species and reduce woody plant cover in savanna and other grassy ecosystems (Bond et al. 2003). Equally important is that many C₄ grasses are shade intolerant and require frequent fire or grazing to prevent the accumulation of dead biomass and to maintain open habitats (Ellis et al. 1980, Everson et al. 1988, Foster et al. 2004).

For grasses, which generally lose all their aboveground biomass in a fire, key fire tolerance traits relate to the ability to regrow lost biomass quickly after a fire event. Fire frequency and intensity increase with rainfall (van Wilgen 2004, Govender et al. 2006), such that in mesic regions fires can occur annually, but are generally rare in arid systems. Fire frequency rather than

Manuscript received 8 August 2014; revised 9 January 2015; accepted 9 March 2015. Corresponding Editor: J. Cavender-Bares.

⁶ E-mail: b.ripley@ru.ac.za

intensity, has a significant effect on the regrowth and survival of grasses (Trollope and Tainton 1986, Trollope et al. 2002, Uys et al. 2004, Peláez et al. 2013) and is likely to be the stronger selective force in determining fire-associated traits. As grasses are the fuels in these systems, fire tolerance and flammability traits are likely to be somewhat related, with fast regrowth rapidly producing fuel and allowing for frequent fires.

However, traits associated with flammability and fire tolerance are only indirectly linked to C₄ photosynthesis and are likely to be associated with particular phylogenetic lineages of grasses. C₄ grass lineages are known to sort differently along environmental gradients and to differ in traits that are unrelated to photosynthesis (Edwards and Still 2008, Edwards and Smith 2010, Taylor et al. 2010, 2011, Liu et al. 2012, Visser et al. 2012, 2014). A phylogenetic association with fire frequency is especially evident in analyses of grass distributions in southern Africa. High species richness of the lineages Andropogoneae (Everson and Tainton 1984, Trollope 1984, Bond 2008, Visser et al. 2012, Forrester et al. 2014) and Paniceae (Bond et al. 2003, Visser et al. 2012) occurs in frequently burned environments, while the number of Chloridoideae and Aristidoideae species increase in the absence of fire (Uys et al. 2004, Visser et al. 2012). Species of Andropogoneae are typically intolerant of shading and accumulate tannins, which discourage herbivory and retard decomposition. The latter helps to ensure the accumulation of a flammable fuel load and frequent fires (Everson et al. 1988, Ellis 1990). In contrast, the Chloridoideae are generally tolerant of grazing but not fire, while the Aristidoideae are tolerant of drought but not disturbance (Visser et al. 2012).

Demonstrating the phylogenetic basis to grass fire responses is an important step towards better understanding how the evolution of key traits could allow lineages to dominate particular fire environments, or to increase their competitive advantage by altering fire regimes (Bond and Midgley 1995). These traits may evolve directly in response to fire, or may arise in response to other environmental factors, but confer advantages in burned environments (Keeley et al. 2011). In fact, the successful evolution of flammable plants requires additional traits that are not necessarily associated with flammability, but that confer success in a postfire environment (Bond and Midgley 1995).

In an investigation of the fire response of C₃ and C₄ subspecies of *Alloteropsis semialata* as a model system, Ripley et al. (2010) demonstrated that individual plant canopies of the C₄ subspecies contained a greater proportion of dead biomass prior to the natural winter fire season. This contributed to a higher flammable fuel-load and meant the loss of less living biomass during fire. The C₄ subspecies also had a larger belowground biomass, which supported new leaf production after the fire and allowed for a more rapid and complete recovery of aboveground biomass relative to unburned controls

(Ripley et al. 2010). However, whether these differences are associated generally with C₃ and C₄ photosynthetic types in grasses, or reflect ecological differences between the *Alloteropsis* taxa, remain unknown.

Here we experimentally investigate the relationships between fire ecology, photosynthetic pathway, and evolutionary history using four grass lineages found in the Eastern Cape of South Africa. Lineages included C₄ species from the genus *Aristida* and those from the tribe Andropogoneae, which share a biochemical subtype (NADP-ME), but have distinct forms of Kranz anatomy (Hattersley and Watson 1992), reflecting the independent origins of their C₄ syndrome. The two C₃ groups were the subfamily Danthonioideae and C₃ species from the tribe Paniceae. Andropogoneae, Paniceae, and Danthonioideae shared a last common ancestor 37 Mya (Christin et al. 2008, Vicentini et al. 2008), prior to the divergence between the Paniceae and their C₄ sister clade Andropogoneae. The *Aristida* and other lineages share a common ancestor at the crown node of the PACMAD clade (Panicoideae, Arundinoideae, Chloridoideae, Micrairoideae, Aristidoideae, and Danthonioideae; Grass Phylogeny Working Group II 2012). Comparisons between lineages, each represented by three to four species, tested the hypothesis that lineages differ in their responses to fire, and that the response is not consistent between lineages with the same photosynthetic type. Instead we hypothesized that fire responses would be more clearly related to the fire frequency occurring in the natural habitats of each species. Using potted plants, we assessed both flammability (fire-promoting characteristics) prior to burning, and tolerance traits (postfire recovery and changes in biomass allocation) after burning grasses during the winter fire season.

METHODS

Plant collection and growth conditions

Thirteen species were randomly selected from species present at the study region, from three grass subfamilies, representing two independent origins of C₄ photosynthesis as follows: three C₃ species from the subfamily Panicoideae, tribe Paniceae (*Panicum aequinerve* Nees, *Panicum ecklonii* Nees, *Alloteropsis semialata* (R.Br.) ssp. *eckloniana* Nees); three C₄ (NADP-ME) species from the subfamily Panicoideae, tribe Andropogoneae (*Heteropogon contortus* (L.) Roem. and Schult., *Hyparrhenia hirta* (L.) Stapf, *Themeda triandra* Forssk.); four C₃ species from the Danthonioideae (*Karrochloa curva* Nees, *Merxmuellera disticha* Nees, *Pentaschistis pallida* Thunb., *Pentaschistis curvifolia* Schrad.); and three C₄ (NADP-ME) Aristidoideae species from the genus *Aristida* (*Aristida congesta* Roem. and Schult., *Aristida diffusa* Trin. and Rupr., *Aristida junciformis* Trin.). *Panicum ecklonii* has been reported as having both C₃ and C₄ forms (Botha et al. 1988, Gibbs Russell 1990); however, a $\delta^{13}\text{C}$ value of -28.8‰ confirmed that the



PLATE 1. Sixty replicate pots of each species being randomly assigned positions (see *Methods*) in a bare soil plot before being covered with a fuel load and burned. Picture credit: T. Martin.

material we sampled used the C₃ photosynthetic pathway.

Species were collected in grassland habitats near Grahamstown (33°19.8' S, 26°31.4' E; Dyer 1937), in the Eastern Cape of South Africa between 12 and 30 August 2007. These grasslands receive a mean annual rainfall of 545 mm with a bimodal distribution peaking from October–November and February–March, a mean annual temperature of 16.5°C, and a mean annual frost incidence of five days per year (Mucina and Rutherford 2006). Fire return times over the 2000–2006 period were 2.3 years for the vegetation surrounding Grahamstown (Tansey et al. 2007).

Plants of each species were divided into five-tiller clumps and potted into 10-L pots containing topsoil collected in Grahamstown. Sixty replicate pots of each species were prepared in this way, and weeded and watered regularly. As rainfall at the study site is bimodal, watering that simulates winter rainfall while grasses are curing is typical of the natural situation. Potted plants were grown for 10 months under outdoor conditions as characterized by daily mean temperatures of 5–18°C in July (austral winter) and 15–26°C in February (austral summer). Measurements of ambient temperatures at canopy height recorded for two months prior to the burn are reported in Ripley et al. (2010), and plants were subjected to three frosts during this period.

Experimental design

The plants were burned in a controlled fire on 5 August 2008. This coincided with the end of winter fire season in Grahamstown. Immediately prior to the burn,

plants were placed in a 42-m² plot of bare soil, such that each square meter contained one replicate of each of the thirteen species. Species were randomly assigned to two rows of four individuals placed down the center of each block, with two rows of two or three individuals placed one on either side of them. The plants were carefully removed from their pots, with minimal root disturbance, and packed closely together while ensuring that the soil surface of all the plants was at the same level. Additional soil was carefully added to gaps between individuals to produce a continuous flat soil surface from which the plants emerged (see Plate 1).

The plants were covered with a fuel load of hay (444 g/m² and moisture content of ~10%), which extended two meters beyond the plot to reduce edge effects. The plot was ignited along one of its sides when the wind speed and direction were appropriate to produce an even burn across the plot with a fire line intensity of 1900 kW/m (Ripley et al. 2010), which is typical of grassland fires in Africa (Trollope 1983).

After the fire, each plant was carefully returned to a pot and care was taken to remove ash from the soil surface. The plants were then allowed to resprout in a clear, naturally lit polytunnel where they were weeded and watered regularly during a 60-day regrowth period. For the duration of the recovery experiment in the polytunnel, a weather station (Vantage Pro, Davis Instruments, Hayward, California, USA) recorded an average relative humidity of 63%, and an average day and night temperature of 15.4° ± 0.4°C and 11.3° ± 0.5°C, respectively (Ripley et al. 2010).

Plant recovery and resource reallocation in response to burning were measured by subjecting the potted plants to three treatments. Treatments included control plants that were not burned, light-recovered plants that were burned and allowed to recover under ambient conditions, and dark-recovered plants that were burned and allowed to recover in the dark. To be consistent with the burned treatments, the control plants were carefully removed and returned to their pots. The conditions for the dark-recovered plants were created by covering the plants with an inverted pot that was painted silver and ventilated with five 15-mm holes covered with black cloth. This dark-recovery treatment allowed us to estimate the contribution made by the remobilization of resources stored belowground.

Six plants of each species per treatment were harvested on four occasions, each ~20 days apart. The first harvest was on 5 August, prior to the burn when only control plants were harvested. The subsequent harvests of control, light-recovered, and dark-recovered plants following the burn were on 25 August, 14 September, and 6 October. Immediately after the burn, an examination of each burned plant showed that the fire had removed all aboveground biomass.

Harvests

Plants were removed from their pots and the soil was carefully washed from the roots over a fine plastic mesh. Each plant was oven dried at 70°C to constant mass and divided into the following components: roots, corms, leaf sheaths, leaves, inflorescences, and dead material. The corm was defined as the basal part of the tiller from the beginning of the photosynthetic tissue, marked by a white to green color change, to the point where the roots are attached. The leaf sheath was defined as the portion of photosynthetic tissue above the corm and up to the ligule. The leaf and sheath masses were combined as photosynthetic aboveground biomass. The reproductive aboveground biomass consisted of the inflorescence, which included culms and spikes containing the spikelets and individual flowers. The attached dead leaves, leaf sheaths, and inflorescences made up the dead aboveground biomass.

Immediately after harvesting, the total area of all leaves on each plant was measured using digital images and the computer program WinDIAS (Delta-T Devices, Cambridge, UK). The moisture content of the canopy (living and dead) was calculated as the percentage water per unit dry aboveground biomass. The moisture content of either leaves or portions of leaves that remained green after the winter frosts, was calculated as the percentage of water per unit leaf fresh mass.

Recovery rates were calculated as the slope of the linear recovery of aboveground biomass over time. These regressions were fitted to combined replicates for each grass species. *A. contortus* had low survival after fire with only one replicate surviving to day 60 of the

experiment. Hence, this species was excluded from the analyses of recovery rate.

Fire frequency data

Southern Africa was divided into 20 km² grid cells. The MODIS MOD14 Thermal Anomalies product for the period 2000–2012 (Friedl et al. 2002, Justice et al. 2002, Giglio et al. 2003) was then used to calculate the numbers of active fire hot spots that occurred within each of these grid cells. Species distributions were obtained from the SIBIS data portal (*available online*)⁷, and were used to identify grid cells in which each of the 13 individual species occurred. If there were duplicate records for a particular grid cell, they were removed. Data from all the grid cells containing a particular species were then used to calculate the mean number of times that a species burned during the 2000–2012 period. The MOD14 active fire product is strongly correlated with burned area (Roy et al. 2008) and therefore is a good index of fire return period, as a greater number of active fires equates to a shorter fire return period.

Photosynthetic rates

Photosynthetic rates measured on plants grown under natural conditions in a common garden near our experimental site were extracted from the supplementary information data of Taylor et al. (2014: Supplement 4). Data collected during March 2010 were selected, as this had been a good rainfall month in summer, and photosynthetic rates were likely to represent the well-watered postfire recovery conditions in our experiment. Taylor et al. (2014) included all the same species in their experiment with the exception of *P. pallida*, which was excluded from our analysis of regrowth and photosynthetic rate.

Phylogenetic analyses

The markers *ndhF* and/or *trnK-matK* were retrieved from GenBank for 12 of the species and were newly sequenced for *Panicum aequinerve* from extracted genomic DNA with the method and primers described previously (Grass Phylogeny Working Group II 2012, Taylor et al. 2012). These new sequences were aligned and used for phylogenetic inference as implemented in BEAST (Drummond and Rambaut 2007). The phylogenetic tree was inferred under a general time-reversible substitution model with a gamma-shape parameter and a proportion of invariants (GTR+G+I). *Flagellaria indica* (Flagellariaceae) was used to root the tree, following previous results (Givnish et al. 2010).

Statistics

General linear models (GLMs) were used to compare the living and dead aboveground biomass, belowground biomass, and aboveground biomass moisture content

⁷ <http://sibis.sanbi.org/faces/DataSources.jsp?l=1>

between the four lineages prior to the experimental burn. Analyses were designed with species nested in lineage, thereby accounting for species variation within lineage and allowing for differences among lineages to be determined. Similar nested analyses were performed to compare the recovery of photosynthetic biomass, canopy area, specific leaf area (SLA), aboveground biomass, total biomass, and reproductive biomass between lineages on three harvest dates subsequent to the experimental burn. This design tested main effects and the interaction of species nested in lineage, harvest date, and treatment. These analyses compared data from control and light-recovered plants with the exception of the aboveground biomass measure, where data from light-recovered and dark-recovered plants were compared to assess the contribution of belowground biomass to recovery. In order to assess whether rates of recovery differed between lineages independently of plant size, these GLMs were repeated on burned plants, including as covariates the average total biomass for each species from control plants harvested at day zero. This design tested main effects, and the interaction of species nested in lineage and harvest date. The day zero values could not be included in the analyses of aboveground characteristics, as the fire removed all aboveground biomass and GLM methods cannot function in the absence of variation. Day zero values were included in analyses of total biomass. All analyses were performed with Statistica Version 8 (StatSoft, 2007). The raw data were transformed where required, and statistical differences between means were determined by Fisher post-hoc tests where appropriate.

The relationships between recovery rates, the average fire frequency for each species, average photosynthetic rates and the average SLA at the end of the experiment were tested using a linear model approach in the R package stats on log-transformed data (R Development Core Team 2013). These relationships were further investigated accounting for the phylogenetic differences between species with a phylogenetic linear model, using the R package CAPER (Orme et al. 2012).

RESULTS

Prefire characteristics

The C_4 Andropogoneae accumulated at least twice as much aboveground biomass as any of the other lineages, and as 71% of this biomass was dead, it constituted the largest fire fuel load. The canopies of the C_3 Paniceae and Danthonioideae lineages were 50% smaller but contained similar proportions of dead biomass (69–71%), while the *Aristida* canopy was four times smaller and contained only 59% dead biomass (Fig. 1a; Table 1). This, combined with a relatively small total plant biomass, meant that the *Aristida* lineage lost 26% of its living biomass in the fire, whereas other lineages lost between 12 and 19% of living biomass.

Flammability of the fuel load is influenced by the moisture content of the canopy, which was lower in the

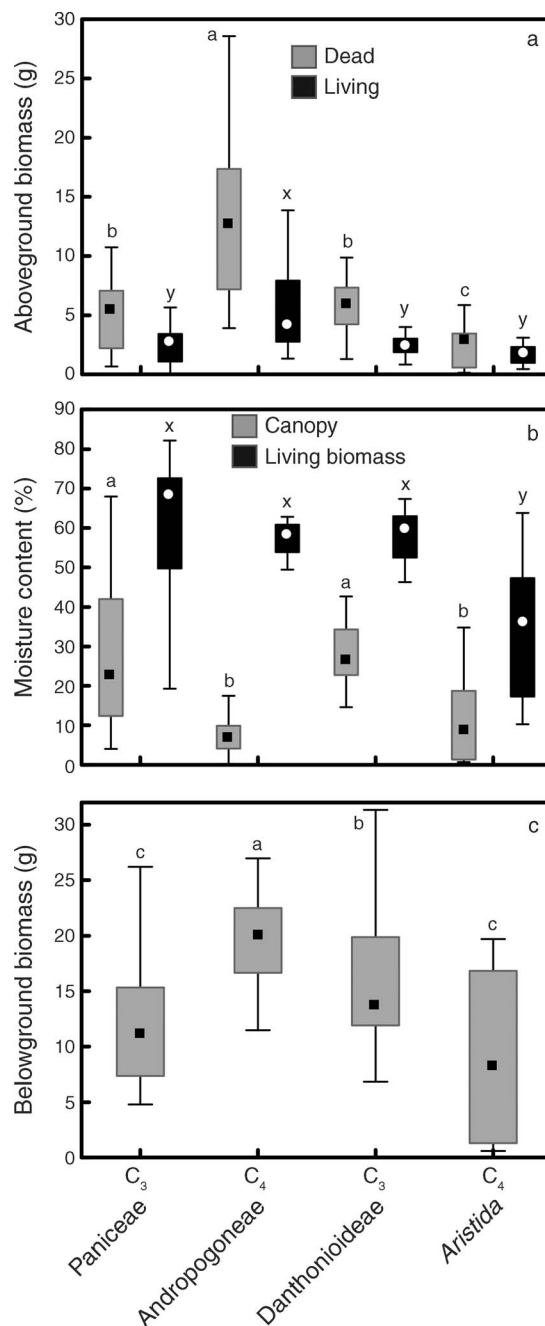


FIG. 1. Box-and-whisker plots of (a) living and dead aboveground biomass, (b) moisture content across living and dead aboveground biomass (i.e., canopy) and of living aboveground biomass alone, and (c) belowground biomass compared between lineages for C_3 and C_4 grasses in the Eastern Cape of South Africa. Medians are represented by the square or circle symbols in each box; the lower and upper ends of each box represent 25% and 75% quartiles; the whiskers represent non-outliers. Sample size is $n = 3$ or 4 species per lineage; $n = 6$ replicates per species. Values assigned the same letter represent homogeneous groups at $P > 0.05$ for Fisher LSD post hoc tests conducted independently on living and dead biomass data, and between moisture content of canopy and living aboveground biomass data.

TABLE 1. General linear models with species nested in lineage comparing (1) differences in plant characteristics measured prior to the experimental burn, and (2 and 3) recovery responses measured subsequent to fire, for C₃ and C₄ grasses in the Eastern Cape of South Africa.

Parameter	1. Plant traits pre-burn		2. Recovery responses of burned compared to control plants				3. Recovery responses of burned lineages, based on pre-burn plant size	
	Lineage(spp.)		Lineage(spp.) × d × trt		Lineage × d × trt		Lineage(spp.) × d	
	df	F	df	F	df	F	df	F
Living aboveground biomass	3, 65	16.7***						
Dead aboveground biomass	3, 65	49.5***						
Belowground biomass	3, 65	25.3***						
Green leaf or part of leaf moisture content	3, 65	36.2***						
Canopy moisture content	3, 65	26.3***						
Photosynthetic biomass			45, 390	3.3***	17, 390	6.4***	15, 149	2.0*
Canopy area			45, 390	2.0***	17, 390	4.9***	15, 149	2.2**
SLA			45, 390	4.5***	17, 390	6.4***		n.s.
Reproductive biomass			45, 390	4.7***	17, 390	20.6***	15, 149	13.9***
Total biomass			45, 390	1.6**	17, 390	2.3***	15, 149	14.1***
Aboveground biomass			45, 390	2.1***	17, 390	12.0***	15, 149	1.9*

Notes: Sample sizes are $n = 3$ or 4 species per lineage; $n = 6$ replicates per species. The second analysis compared regrowth of control and burned plants between lineages, with the exception of aboveground biomass recovery response which compared burned plants recovered under ambient conditions to plants recovered in the dark. The third analysis compared burned plants recovery while accounting for plant total biomass of control plants immediately prior to the experimental burn.

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; n.s., not significant.

C₄ Andropogoneae and *Aristida* lineages than in the C₃ Danthonioideae and Paniceae lineages (Fig. 1b). Patterns in moisture content did not parallel those for proportions of dead canopy biomass because the moisture content of leaves or portions of leaves that remained green subsequent to winter frost varied among lineages (Fig. 1b). The moisture content of living *Aristida* biomass was only 27%, while that of the other three lineages ranged between 50% and 58% (Fig. 1b).

After frosts and prior to the experimental burn, at least 80% of the living plant material of the C₄ Andropogoneae species was belowground. This potential storage reserve for allocation to regrowth after fire was ~30% smaller in the C₃ Danthonioideae lineage, while C₃ Paniceae and C₄ *Aristida* lineages accumulated ~50% less belowground biomass than the C₄ Andropogoneae lineage (Fig. 1c).

Resprouting after fire

After fire, the photosynthetic biomass (leaves + leaf sheaths) recovered fastest and most completely in the C₄ Andropogoneae and C₃ Paniceae species and reached values statistically indistinguishable to those of control plants by day 40 (Fig. 2a–d; Table 1). In contrast, the recovery of C₃ Danthonioideae and C₄ *Aristida* lineages was much slower and never recovered to control plant values (Fig. 2a–d; Table 1). These patterns were unaltered if the regrowth of photosynthetic tissues was expressed relative to the average total biomass of control plants at day zero, demonstrating that the patterns did not arise from differences in plant size (Appendix A: Fig. A1). Patterns were distinct between lineages (Table 1) and were not obscured by the variation between

individual species' responses (Appendix A: Figs. A2 and A3).

Patterns in the recovery of canopy area were similar to those of photosynthetic biomass, and the larger canopies of the C₃ Paniceae and C₄ Andropogoneae lineages recovered within 20–40 days (Fig. 2e–h). Furthermore, fire stimulated the regrowth of the C₄ Andropogoneae canopy, meaning that burned plants produced larger canopies than control plants by the end of the experiment. In contrast, the smaller C₃ Danthonioideae and C₄ *Aristida* canopies did not recover completely within the duration of the experiment (Fig. 2e–h; Table 1).

The rapid recovery of C₃ Paniceae canopy was possible because the regrowth after the fire produced leaves of high SLA. Fire increased the SLA of newly produced leaves in all lineages, which subsequently declined over the duration of the experiment (Fig. 2i–j, Table 1). The recovery and stimulation of canopy growth observed for the C₄ Andropogoneae lineage was less dependent on higher SLA, and results from high rates of biomass production.

In contrast to photosynthetic biomass, fire decreased reproductive biomass or delayed its production. This response was strongest in the C₄ Andropogoneae, but was evident in all the lineages with the exception of C₃ Paniceae, where reproductive output was low but similar in control and burned plants (Fig. 3a–d, Table 1).

Total biomass of the C₄ Andropogoneae and C₃ Paniceae was little affected by the fire and increased over the duration of the experiment, although this effect was larger in the Andropogoneae than the Paniceae lineages. In contrast, fire decreased the total biomass of the

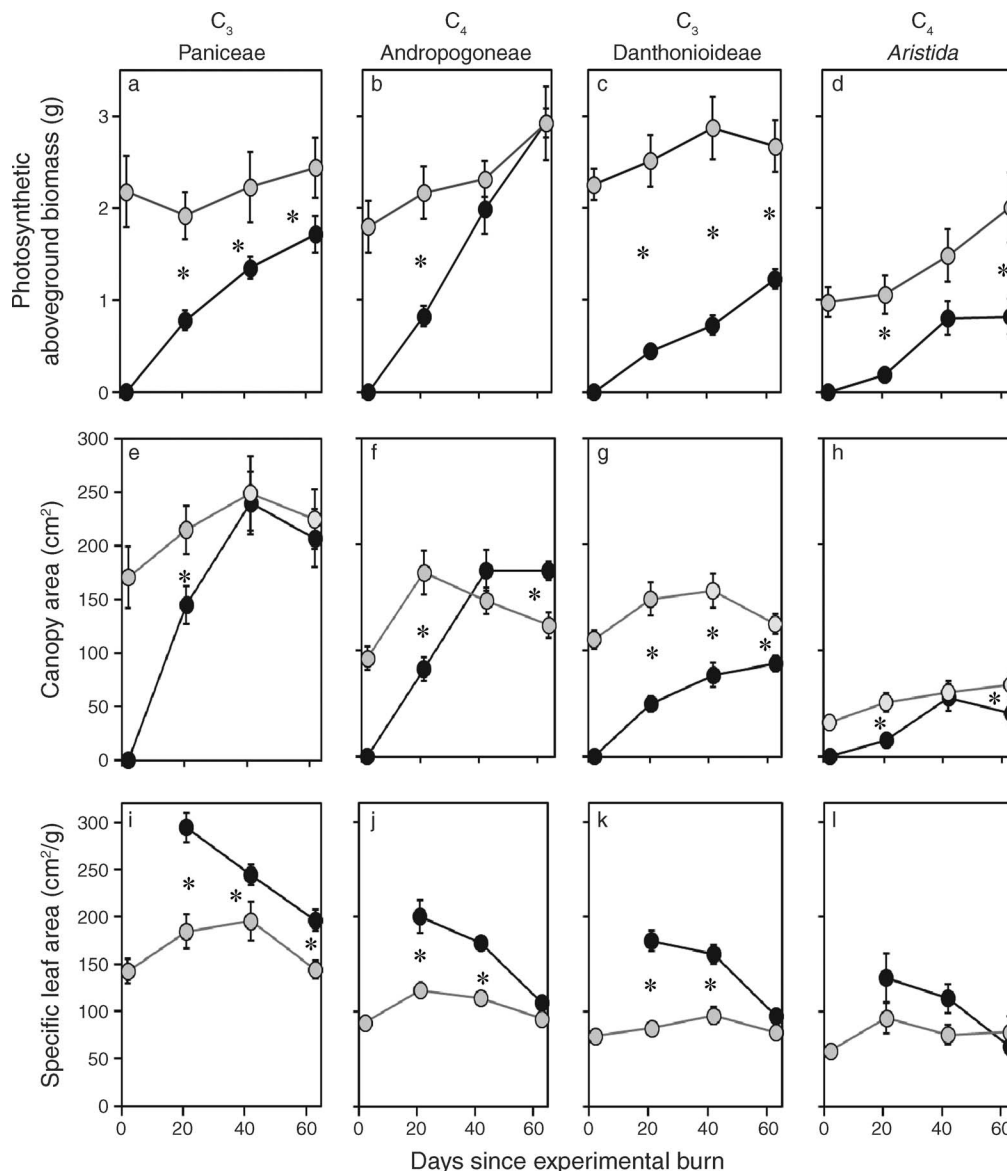


FIG. 2. Recovery of (a–d) photosynthetic biomass, (e–h) canopy area, and (i–l) changes to specific leaf area of burned plants recovered in the light (black symbols) or control plants (gray symbols) compared between lineages. Burned plants recovered in the light were plants burned and recovered under conditions of natural sunlight. Burned plants recovered in the dark were plants recovered with inverted pots placed over them to exclude any incident sunlight. Values are means and vertical bars are standard errors. Sample size is $n = 3$ or 4 species per lineage; $n = 6$ replicates per species. Asterisks indicate significant differences between means of the control and burned plants for a given time point at $P < 0.05$ for Fisher LSD post hoc tests.

Danthonioideae and *Aristida* lineages, which continued to decline over the duration of the experiment (Fig. 3e–h, Table 1).

The growth of burned plants in the dark indicates the remobilization of belowground resources to support the recovery of aboveground biomass, and differed between lineages (Fig. 3i–l, Table 1). Regrowth in the dark was largest in the C₃ Paniceae and was equivalent to 15% of the recovery in the light by day 60. In contrast, the equivalent comparison for other lineages accounted for

only 6% in C₄ Andropogoneae, 5% in C₃ Danthonioideae, and 3% in C₄ *Aristida* (Fig. 3i–l).

Regrowth rate of aboveground biomass was positively correlated to the average number of active fires experienced by each species in their natural habitats ($F_{1,10} = 9.0$, $P < 0.013$, $r^2 = 0.42$; Fig. 4a, b) and this correlation remained significant ($F_{1,10} = 5.7$, $P < 0.038$) when it was repeated accounting for phylogenetic structure. There was a strong phylogenetic signal for regrowth rate of aboveground biomass (Pagel's $\lambda = 0.99$;

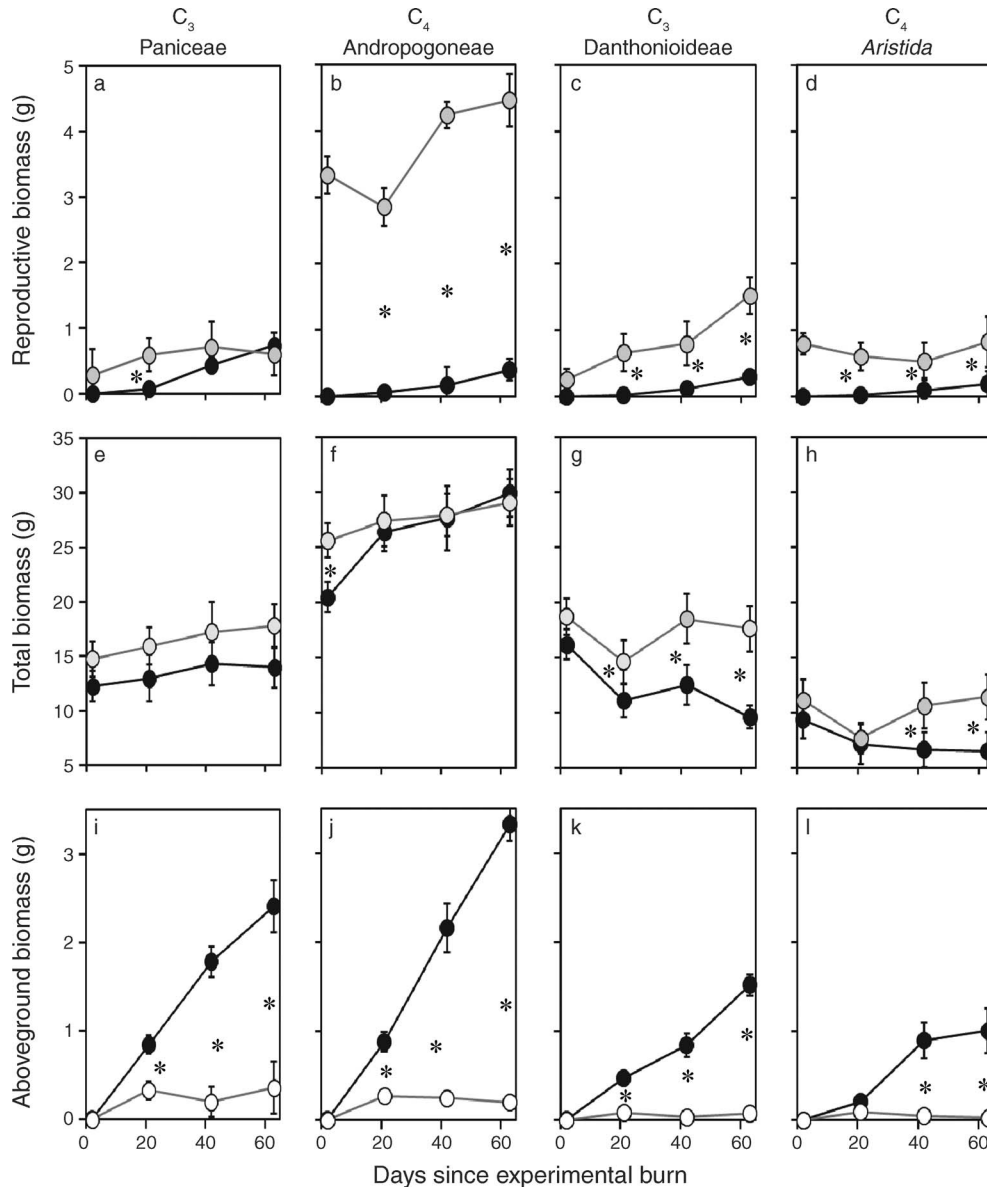


FIG. 3. Changes to (a–d) reproductive biomass, (e–h) total biomass, and (i–l) recovery of aboveground biomass compared between lineages. Reproductive and total biomass were compared between burned plants recovered in the light (black symbols) and control plants (gray symbols), and aboveground biomass between burned plants recovered in the light (black symbols) or in the dark (gray symbols). Burned plants recovered in the light were plants burned and recovered under conditions of natural sunlight. Burned plants recovered in the dark were plants recovered with inverted pots placed over them to exclude any incident sunlight. Values are means and vertical bars are standard errors. Sample size is $n = 3$ or 4 species per lineage; $n = 6$ replicates per species. Asterisks indicate significant differences between means of the control and burned plants for a given time point at $P < 0.05$ for Fisher LSD post hoc tests.

$P = 0.99$ for $\lambda = 1$; $P < 0.009$ for $\lambda = 0$), meaning that closely related species tended to have a similar regrowth rate.

The rates of regrowth were positively correlated to the SLA of leaves produced after fire and measured on day 60, when photosynthetic type was included as covariate in the GLM analysis ($F_{2,9} = 5.8$, $P < 0.02$, $r^2 = 0.56$), such that *C*₄ species achieved higher regrowth rates per unit SLA than *C*₃ species (Fig. 5a). The relationships

remained significant when the phylogenetic structure was accounted for but there was no phylogenetic signal for the relationship (Pagel's $\lambda = 0$; $P = 0.05$ for $\lambda = 1$; $P = 1$ for $\lambda = 0$). Regrowth rates were also correlated to photosynthetic rates ($F_{1,9} = 5.4$, $P < 0.044$, $r^2 = 0.31$) measured for the same species in a common garden (Fig. 5b). However, the correlation to photosynthetic rate did not remain significant when phylogenetic structure was accounted for.

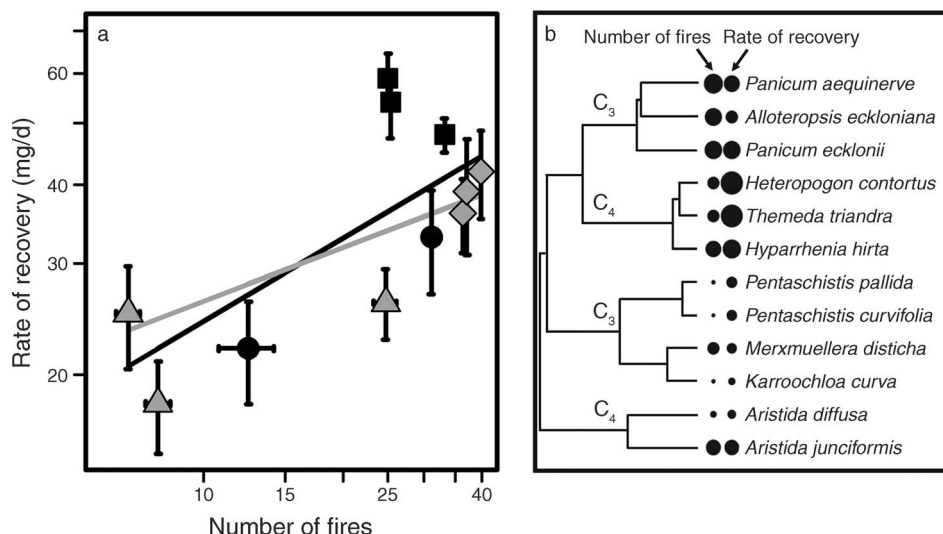


FIG. 4. (a) Average rate of aboveground biomass recovery for each species related to average number of fires recorded in each 20-km² grid where there were location data for each species. Note the log-log scale. Lineages are indicated as Paniceae (diamonds), Andropogoneae (squares), Danthonioideae (triangles), and *Aristida* (circles), and photosynthetic types as C₄ (black symbols) and C₃ (gray symbols). Vertical and horizontal bars are standard errors. Linear regressions were fit to recovery rates that were uncorrected (black line) or corrected (gray line) for phylogenetic relationships. (b) Species-level phylogeny with the average number of fires and recovery rates indicated by the size of solid circles.

The fuel load, consisting of dead and a smaller proportion of living aboveground biomass, prior to the experimental burn was positively correlated to the rates of regrowth after fire (Fig. 6), although this was only significant at the 92% confidence level ($F_{1,10} = 4.06$, $P < 0.072$, $r^2 = 0.22$). The relationship was strongly influenced by data for *P. aequinerve* and r^2 increased

to 0.63 when this species was omitted from the analysis. The relationship did not remain significant when phylogenetic structure was accounted for.

DISCUSSION

Our results are consistent with the hypothesis that ecological responses to fire in grasses evolve as adapta-

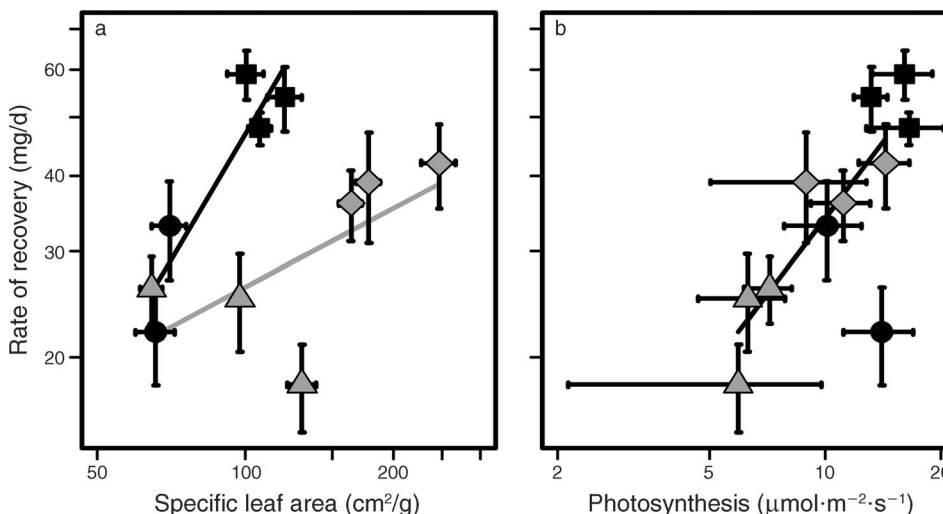


FIG. 5. (a) Average rate of aboveground biomass recovery related to specific leaf area (SLA) measured at day 60 of the recovery after the experimental burn, or (b) photosynthetic rate measured in a separate field plot experiment (Taylor et al. 2014). Note the log-log scale. Lineages are indicated as Paniceae (diamonds), Andropogoneae (squares), Danthonioideae (triangles), and *Aristida* (circles), and photosynthetic types as C₄ (black symbols) and C₃ (gray symbols). Vertical and horizontal bars are standard errors. Linear regressions were fit separately to recovery responses of SLA for C₃ (gray symbols) and C₄ species (black symbols). A linear regression (solid line) was fitted to the relationship between recovery and photosynthetic rate.

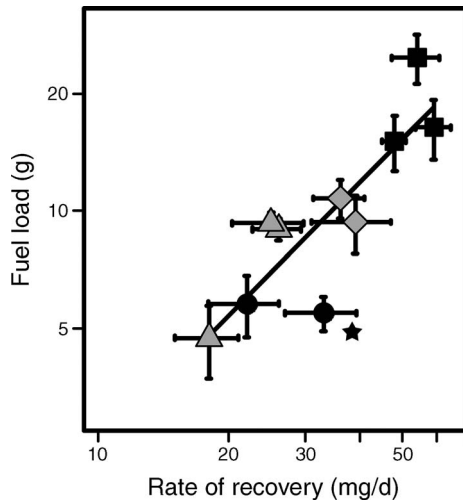


FIG. 6. Fuel load (living and dead aboveground biomass) prior to the experimental burn related to the rate of biomass recovery after the experimental burn. Note the x-axis log scale. Lineages are indicated as Paniceae (diamonds), Andropogoneae (squares), Danthonioideae (triangles), and *Aristida* (circles), and photosynthetic types as C_4 (black symbols) and C_3 (gray symbols). Vertical and horizontal bars are standard errors, and the fitted solid line is a linear regression. The star (★) symbol indicates data for *P. aquinerve*.

tions to frequent burning, but are contingent upon physiological and structural traits inherited from ancestors. Fire responses therefore varied among major grass lineages and were largely independent of photosynthetic physiology. Crucially, we also found some evidence of a correlation between fuel load and rapid regrowth after fire. Since fuel load has a strong positive effect on the flammability of dry grass fuel beds, this finding represents the first quantitative evidence of a mechanistic linkage between high flammability and ecological success in frequently burned savanna grasslands.

The response of species from the Andropogoneae and *Aristida* lineages were distinct, despite sharing the C_4 NADP-ME photosynthetic pathway. Clear differences were also apparent between C_3 species from the Paniceae and Danthonioideae lineages, while similarities in the regrowth responses of species from the C_4 Andropogoneae and C_3 Paniceae lineages suggest fire tolerance in the Panicoideae subfamily, to which both clades belong. The phylogenetic basis for the correlation of regrowth and fire in natural habitats shows that a history of frequent burning has selected for lineages with fast regrowth, either demonstrating that grasses can evolve in response to fire or that intolerant species are filtered out of frequently burned habitats. Equally important is that other traits are only indirectly associated with fire. This is important for understanding how particular lineages such as the Panicoideae have acquired the collection of traits that allow them to dominate frequently burned environments.

The Andropogoneae lineage produced high fuel loads by the end of the growing season, which tends to promote fire. Previous work has shown that leaf traits such as the concentration of tannins, may also aid the accumulation of a fuel load by discouraging herbivory and retarding decomposition (Ellis 1990). Furthermore, the small proportion of living aboveground biomass and the end of season reallocation of leaf resources including nitrogen to belowground storage (Blair et al. 1998, Thornton and Bausenwein 2000), would minimize the costs of losing biomass to fire. In our experiment, such responses are likely to have been induced by winter conditions that increased leaf death (Osborne et al. 2008, Martin 2009, Chamaille-James and Bond 2010). However, large areas of subtropical grassy ecosystems are not subject to frosts, and here it is likely that end-of-season drought is the causal mechanism for leaf mortality and the generation of a dry fuel load (Taylor et al. 2011).

Fire stimulated the recovery of leaf canopies in species from the Andropogoneae, and was associated with an increase in leaf area at the expense of delayed reproductive effort. It is likely that flowering was delayed rather than suppressed, as fire stimulates grass flowering and seed production in many species (Howe 1994, Ellsworth and Kauffmann 2010); these responses may well have been evident if our experiment had been continued to the end of the growing season. In combination with the high nitrogen use efficiency of growth in the high light, nutrient limited environment after fires, these recovery characteristics offer some explanation for the high abundance of C_4 Andropogoneae species in frequently burned ecosystems (Visser et al. 2012, Forrester et al. 2014). Here, species do not only tolerate fire, but may be dependent on it to remove shading from the accumulation of dead biomass or from woody plants (Everson et al. 1988, Sage et al. 1999), and are eliminated from field experiments where fire is absent (Everson et al. 1988, Uys et al. 2004, Fynn et al. 2005, Furley et al. 2008, Fynn et al. 2011).

Unlike the Andropogoneae, the C_4 species from the genus *Aristida* promoted fire less than the other lineages and were intolerant of burning. They did not accumulate large fuel loads, but lost large proportions of living biomass in the fire and were unable to recover photosynthetic biomass and canopy area subsequent to burning. Presumably these traits would compromise *Aristida* species in frequently burned environments; this is supported by observations from long-term burning experiments where *Aristida* species are excluded if the fire-return interval is shorter than three years (Uys et al. 2004, Fynn et al. 2005). Regional distribution patterns show that the number of species from this lineage increase in regions where fire is infrequent and biomass accumulation is limited by drought (Visser et al. 2012).

C_3 species from the Danthonioideae and the Paniceae lineages produced similar fuel loads and proportions of dead biomass prior to the fire. After burning, however, the C_3 Paniceae species recovery was rapid and

complete, whereas the Danthonioideae recovery was not. The Paniceae canopy recovery was associated with the reallocation of belowground resources and leaves of high SLA. This contrasted with findings from previous work on species from the C₃ Pooideae lineage, where spring growth was supported with N mobilized from tillers overwintering aboveground (Bausenwein et al. 2001), a resource that would have been lost if plants had burned. Unfortunately, the ecological significance of these characteristics is difficult to interpret, as there are few data available for long-term fire responses or climate correlations for these C₃ lineages.

Unlike the contrasting responses measured within the C₃ and C₄ lineages, there were similarities in the fire response for the C₃ Paniceae and C₄ Andropogoneae lineages, both of which recovered from the burn. This ability to recover from fire would also confer advantages in environments where herbivory is prevalent and removes aboveground biomass (Fornoni 2011). Regrowth after fire was supported by high photosynthetic rates and by leaves with high SLA. However, while we found evidence of correlated evolution of regrowth and SLA, this was not the case for photosynthetic rate, and high rates are unlikely to have resulted directly from selection for rapid regrowth in frequently burned habitats, and are presumably driven by some other environmental factor. This finding supports the notion that the adaptation to frequently burned habitats requires additional traits that are unrelated to flammability, and that confer success in postfire environments (Bond and Midgley 1995). For example, plants that evolve flammability can only succeed in frequently burned environments if they can take advantage of the post fire environments and have the traits that allow them to rapidly colonize available space through regrowth or recruitment. However, species that tolerate fire, such as the Paniceae, did not necessarily require flammability traits and had prefire characteristics that were not dissimilar to those of the fire-intolerant Danthonioideae. Such traits might explain why the Paniceae are able to coexist in environments with frequent fires that are fueled by the dead biomass of Andropogoneae lineages.

Fire acts as a stringent ecological filter on community structure and, in frequently burned mesic grasslands, leads to phylogenetic clustering of highly abundant grass species (e.g., the C₄ Andropogoneae; Edwards et al. 2010, Forrester et al. 2014). It has been hypothesized that the expansion of grasslands into mesic and fire-prone environments in the Miocene involved the acquisition of traits leading to fuel load accumulation and fire tolerance in multiple lineages of C₃ and C₄ grasses (Strömberg 2005, Osborne 2008, Edwards et al. 2010). Our results, by showing the contingency of grass fire ecology on traits inherited from ancestors, suggest that this expansion into fire-prone environments would have been more likely in some lineages than others. The possession of C₄ photosynthesis may also confer some

additional advantages in postfire high light environments related to high productivity, water and nutrient use efficiency, and the flexible allocation of resources (Ghannoum et al. 2005, Osborne et al. 2008, Ripley et al. 2008, Forrester et al. 2014). However, there is accumulating evidence that many of the traits we associate with C₄ photosynthesis also depend strongly on characters inherited from C₃ ancestors (Christin and Osborne 2014). Other selective pressures such as drought may have been more important in sorting other lineages (Taub 2000) and in conferring characters such as drought tolerance in the Aristidoideae lineage. Here too there is a strong effect of evolutionary history on the traits of modern species (Christin and Osborne 2014).

Fire can favor species adapted to fire-prone environments, increasing fitness by killing less flammable neighboring individuals and opening recruitment possibilities (Bond and Midgley 1995). Crown fire has been demonstrated as the driver of trait divergence in intraspecific comparisons of *Ulex parviflorus* from environments of differing fire regimes (Pausas et al. 2012). Similarly, the simultaneous emergence of independent fire traits in *Banksia* and *Eucalyptus* in the early Paleocene (Crisp et al. 2011, He et al. 2011) suggest that fire was also the selective agent here (Pausas and Schilke 2012). Our study presents evidence that surface fires drive trait divergence in grassy ecosystems by selecting for species that can regrow rapidly after fire.

In conclusion, our experiments provide the first quantitative evidence that the fire ecology of savanna grasses depends on adaptations acquired in fire-prone environments, while interacting with the background of traits inherited from ancestors. Comparative evidence suggests that rapid regrowth after fire is an adaptation to frequent burning, and is driven by high rates of photosynthesis and high SLA rather than the substantial remobilization of belowground stores. The C₄ photosynthetic pathway was at best only indirectly related to fire tolerance per se. However, in grass lineages adapted to fire, it may give a competitive advantage in regrowth. Finally, we find intriguing support for the hypothesis that high flammability is linked to ecological success in fire-prone savanna grasslands. This work provides novel examples of how ecological behavior depends crucially upon historical processes, and how the evolution of plant traits may change aspects of the abiotic environment.

ACKNOWLEDGMENTS

We gratefully acknowledge funding from the following sources: the South African National Research Foundation and the Rhodes University Research Committee (awarded to B. Ripley and T. Martin).

LITERATURE CITED

- Bausenwein, U., P. Millard, and J. A. Raven. 2001. Remobilized old-leaf nitrogen predominates for spring growth in two temperate grasses. *New Phytologist* 152:283–290.
- Blair, J. M., T. R. Seastedt, C. W. Rice, and R. A. Ramundo. 1998. Terrestrial nutrient cycling in tallgrass prairie. Pages 222–243 in A. K. Knapp, J. M. Briggs, and D. C. Hartnett,

- editors. Grassland dynamics: long term ecological research in tallgrass prairie. Oxford University Press, New York, New York, USA.
- Bond, W. J. 2008. What limits trees in C₄ grasslands and savannas? *Annual Review of Ecology, Evolution, and Systematics* 39:641–659.
- Bond, W. J., G. F. Midgley, and F. I. Woodward. 2003. The importance of low atmospheric CO₂ and fire in promoting the spread of grasslands and savannas. *Global Change Biology* 9:973–982.
- Bond, W. J., and J. J. Midgley. 1995. Kill thy neighbour: an individualistic argument for the evolution of flammability. *Oikos* 73:79–85.
- Botha, C. E. J., S. R. Russell, and B. P. Phillipson. 1988. *Panicum ecklonii* Nees, a new record of C₄ photosynthetic variant. *South African Journal of Botany* 54:89–93.
- Chamaille-James, S., and W. J. Bond. 2010. Will global change improve grazing quality of grasslands? A call for a deeper understanding of the effects of shifts from C₄ to C₃ grasses for large herbivores. *Oikos* 119:1857–1861.
- Christin, P. A., G. Besnard, E. Samaritani, M. R. Duvall, T. R. Hodkinson, V. Savolainen, and N. Salamin. 2008. Oligocene CO₂ decline promoted C₄ photosynthesis in grasses. *Current Biology* 18:37–43.
- Christin, P.-A., and C. P. Osborne. 2014. The evolutionary ecology of C₄ plants. *New Phytologist* 204:765–781.
- Crisp, M. D., G. E. Burrows, L. G. Cook, A. H. Thornhill, and D. M. J. S. Bowman. 2011. Flammable biomes dominated by eucalypts originated at the Cretaceous–Palaeogene boundary. *Nature Communications* 2:193.
- Drummond, A. J., and A. Rambaut. 2007. BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evolutionary Biology* 7:214.
- Dyer, R. A. 1937. The vegetation of the divisions of Albany and Bathurst. *Memoirs of the Botanical Survey of South Africa* 17:133–138.
- Edwards, E. J., C. P. Osborne, C. A. E. Strömberg, and S. A. Smith. 2010. The origins of C₄ grasslands: integrating evolutionary and ecosystem science. *Science* 328:587–591.
- Edwards, E. J., and S. A. Smith. 2010. Phylogenetic analyses reveal the shady history of C₄ grasses. *Proceedings of the National Academy of Sciences USA* 107:2532–2537.
- Edwards, E. J., and C. J. Still. 2008. Climate, phylogeny, and the ecological distribution of C₄ grasses. *Ecology Letters* 11: 266–276.
- Ellis, R. P. 1990. Tannin-like substances in grass leaves. *Memoirs of the Botanical Survey of South Africa* 59:1–80.
- Ellis, R. P., J. C. Vogel, and A. Flus. 1980. Photosynthetic pathways and the geographical distribution of grasses in South West Africa/Namibia. *South African Journal of Science* 76:307–314.
- Ellsworth, L. M., and J. B. Kauffmann. 2010. Native bunchgrass response to prescribed fire in ungrazed mountain big sagebrush ecosystems. *Fire Ecology* 6:86–96.
- Everson, C. S., T. M. Everson, and N. M. Tainton. 1988. Effects of intensity and height of shading on the tiller initiation of six grass species from the Highland sourveld of Natal. *South African Journal of Botany* 54:315–318.
- Everson, C. S., and N. M. Tainton. 1984. The effect of thirty years and plant size explain the response of annual species to grazing exclusion in a Mediterranean semi-arid rangeland. *Journal of Ecology* 92:297–309.
- Fornoni, J. 2011. Ecological and evolutionary implications of plant tolerance to herbivory. *Functional Ecology* 25:399–407.
- Forrestel, E. J., M. J. Donoghue, and M. D. Smith. 2014. Convergent phylogenetic and functional responses to altered fire regimes in mesic savanna grasslands of North America and South Africa. *New Phytologist* 203:1000–1011.
- Foster, B. L., T. L. Dyckson, C. A. Murphy, I. S. Karel, and V. H. Smith. 2004. Propagule pools mediate community assembly and diversity-ecosystem regulation along a grassland productivity gradient. *Journal of Ecology* 92:435–449.
- Friedl, M. A., D. K. McIver, J. C. Hodges, X. Y. Zhang, D. Muchoney, A. H. Strahler, and C. Schaaf. 2002. Global land cover mapping from MODIS: algorithms and early results. *Remote Sensing of Environment* 83:287–302.
- Furley, P. A., R. M. Rees, C. M. Ryan, and G. Saiz. 2008. Savanna burning and the assessment of long-term fire experiments with particular reference to Zimbabwe. *Progress in Physical Geography* 32:611–634.
- Fynn, R. W. S., C. D. Morris, and T. J. Edwards. 2005. Long-term compositional responses of a South African mesic grassland to burning and mowing. *Applied Vegetation Science* 8:5–12.
- Fynn, R., C. Morris, D. Ward, and K. Kirkman. 2011. Trait-environment relations for dominant grasses in South African mesic grassland support a general leaf economic model. *Journal of Vegetation Science* 22:528–540.
- Ghannoum, O., J. R. Evans, W. S. Chow, T. J. Andrews, J. P. Conroy, and S. von Caemmerer. 2005. Faster Rubisco is the key to superior nitrogen-use efficiency in NADP-malic enzyme relative to NAD-malic enzyme C₄ grasses. *Plant Physiology* 137:638–650.
- Gibbs Russell, G. E., L. Watson, M. Koekemoer, L. Smook, N. P. Barker, H. M. Anderson, and M. J. Dallwitz. 1990. Grasses of southern Africa. *Memoirs of the Botanical Survey of South Africa* 58:1–437.
- Giglio, L., J. Descloitres, C. O. Justice, and Y. J. Kaufman. 2003. An enhanced contextual fire detection algorithm for MODIS. *Remote Sensing of Environment* 87:273–282.
- Grass Phylogeny Working Group II. 2012. New grass phylogeny resolves deep evolutionary relationships and discovers C₄ origins. *New Phytologist* 193:304–312.
- Hattersley, M. D., and L. Watson. 1992. Diversification of photosynthesis. Pages 38–116 in G. P. Chapman, editor. *Grass evolution and domestication*. Cambridge University Press, Cambridge, UK.
- He, T., B. B. Lamont, and K. S. Downes. 2011. Banksia born to burn. *New Phytologist* 191:184–196.
- Howe, H. F. 1994. Managing species diversity in tallgrass prairie: assumptions and implications. *Conservation Biology* 8:691–704.
- Justice, C. O., L. Giglio, S. Korontzi, J. Owens, J. T. Morissette, D. Roy, and Y. Kaufman. 2002. The MODIS fire products. *Remote Sensing of Environment* 83:244–262.
- Keeley, J. E., J. G. Pausas, P. W. Rundel, J. William, W. J. Bond, and R. A. Bradstock. 2011. Fire as an evolutionary pressure shaping plant traits. *Trends in Plant Science* 8:406–411.
- Keeley, J. E., and P. W. Rundel. 2005. Fire and the Miocene expansion of C₄ grasslands. *Ecology Letters* 8:1–8.
- Knapp, A. K. 1985. Effect of fire and drought on the ecophysiology of *Andropogon gerardii* and *Panicum virgatum* in a tallgrass prairie. *Ecology* 66:1309–1320.
- Liu, H., E. J. Edwards, R. P. Freckleton, and C. P. Osborne. 2012. Phylogenetic niche conservatism in C₄ grasses. *Oecologia* 170:835–845.
- Martin, T. 2009. Photosynthetic and evolutionary determinants of the response of selected C₃ and C₄ (NADP-ME) grasses to fire. Thesis. Rhodes University, Grahamstown, South Africa.
- Mouillot, F., and C. B. Field. 2005. Fire history and the global carbon budget: a 1 × 1 fire history reconstruction for the 20th century. *Global Change Biology* 11:398–420.
- Mucina, L., and M. C. Rutherford. 2006. The vegetation of South Africa, Lesotho and Swaziland. South African National Biodiversity Institute, Pretoria, South Africa.
- Orme, C. D. L., R. Freckleton, G. Thomas, T. Petzoldt, S. Fritz, N. Isaac, and W. Pearse. 2012. Caper: comparative analyses of phylogenetics and evolution in R. Package version 0.5.2. <http://cran.r-project.org/web/packages/caper/index.html>

- Osborne, C. P. 2008. Atmosphere, ecology and evolution: What drove the Miocene expansion of C₄ grasslands? *Journal of Ecology* 96:35–45.
- Osborne, C. P., E. J. Wythe, D. G. Ibrahim, M. E. Gilbert, and B. S. Ripley. 2008. Low temperature effects on leaf physiology and survivorship in the C₃ and C₄ subspecies of *Alloteropsis semialata*. *Journal of Experimental Botany* 59: 1743–1754.
- Pausas, J. G., G. Alessio, B. Moreira, and G. Corcobado. 2012. Fires enhance flammability in *Ulex parviflorus*. *New Phytologist* 193:18–23.
- Pausas, J., and D. Schilck. 2012. Fire and plant evolution. *New Phytologist* 193:301–303.
- Peláez, D. V., J. A. Romina, R. E. Omar, E. E. Bontti, A. A. Tomas, and F. R. Blazquez. 2013. Response of grass species to different fire frequencies in semi-arid rangelands of central Argentina. *Rangeland Journal* 35:385–392.
- R Development Core Team. 2013. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. www.R-project.org/
- Ratnam, J., W. J. Bond, R. J. Fensham, W. A. Hoffmann, S. Archibald, C. E. R. Lehmann, M. T. Anderson, S. I. Higgins, and M. Sankaran. 2011. When is a “forest” a savanna, and why does it matter? *Global Ecology and Biogeography* 20: 653–660.
- Ripley, B. S., T. I. Abraham, and C. P. Osborne. 2008. Consequences of C₄ photosynthesis for the partitioning of growth: a test using C₃ and C₄ subspecies of *Alloteropsis semialata* under nitrogen limitation. *Journal of Experimental Botany* 59:1705–1714.
- Ripley, B., G. Donald, C. P. Osborne, T. Abraham, and T. Martin. 2010. Experimental investigation of fire ecology in the C₃ and C₄ subspecies of *Alloteropsis semialata*. *Journal of Ecology* 98:1196–1203.
- Robinson, E. R., G. E. Gibbs Russell, W. S. W. Trollope, and B. H. Downing. 1979. Short-term treatments and ecological interaction in the herb layer of False Thornveld of the Eastern Province. *Proceedings of the Grassland Society of South Africa* 14:79–83.
- Roy, D. P., L. Boschetti, C. O. Justice, and J. Ju. 2008. The collection 5 MODIS burned area product—global evaluation by comparison with the MODIS active fire product. *Remote Sensing of Environment* 112:3690–3707.
- Sage, R. F. 2004. The evolution of C₄ photosynthesis. *New Phytologist* 161:341–370.
- Sage, R. F., M. Li, and R. K. Monson. 1999. The taxonomic distribution of C₄ photosynthesis. Pages 551–584 in R. F. Sage and R. K. Monson, editors. *C₄ plant biology*. Academic Press, San Diego, California, USA.
- Strömberg, C. A. E. 2005. Decoupled taxonomic radiation and ecological expansion of open-habitat grasses in the Cenozoic of North America. *Proceedings of the National Academy of Sciences USA* 102:11980–11984.
- Tansey, K., J.-M. Grégoire, P. Defourny, R. Leigh, J.-F. Pekel, E. van Bogaert, and E. Bartholomé. 2007. A new, global, multi-annual (2000–2007) burnt area product at 1 km resolution. *Geophysical Research Letters* 35:L01401.
- Taub, D. R. 2000. Climate and the US distribution of C₄ grass subfamilies and decarboxylation variants of C₄ photosynthesis. *American Journal of Botany* 87:1211–1215.
- Taylor, S. H., P. J. Franks, S. P. Hulme, E. Spriggs, P. A. Christin, E. J. Edwards, F. I. Woodward, and C. P. Osborne. 2012. Photosynthetic pathway and ecological adaptation explain stomatal trait diversity amongst grasses. *New Phytologist* 193:387–396.
- Taylor, S. H., S. P. Hulme, M. Rees, B. S. Ripley, F. I. Woodward, and C. P. Osborne. 2010. Ecophysiological traits in C₃ and C₄ grasses: a phylogenetically controlled screening experiment. *New Phytologist* 185:780–791.
- Taylor, S. H., B. S. Ripley, T. Martin, L. De Wet, F. I. Woodward, and C. P. Osborne. 2014. Physiological advantages of C₄ grasses in the field: a comparative experiment demonstrating the importance of drought. *Global Change Biology* 20:1992–2003.
- Taylor, S. H., B. S. Ripley, F. I. Woodward, and C. P. Osborne. 2011. Drought limitation of photosynthesis differs between C₃ and C₄ grass species in a comparative experiment. *Plant, Cell and Environment* 34:65–75.
- Thornton, B., and U. Bausenwein. 2000. Seasonal protease activity in storage tissue of the deciduous grass *Molinia caerulea*. *New Phytologist* 146:75–81.
- Tix, D., and I. Charvat. 2005. Aboveground biomass removal by burning and raking increases diversity in a reconstructed prairie. *Restoration Ecology* 13:20–28.
- Trollope, W. S. W. 1983. Control of bush encroachment with fire in the arid savannas of southeastern Africa. Dissertation. University of KwaZulu-Natal, Pietermaritzburg, South Africa.
- Trollope, W. S. W. 1984. Fire behaviour. Pages 199–218 in P. D. E. V. Booysen and N. M. Tainton, editors. *Ecological effects of fire in South African ecosystems*. Springer-Verlag, Berlin, Germany.
- Trollope, W. S. W., and N. M. Tainton. 1986. Effect of fire intensity on the grass and bush components of the eastern Cape thornveld. *Journal of the Grassland Society of Southern Africa* 3:37–42.
- Trollope, W. S. W., L. A. Trollope, and D. C. Hartnett. 2002. Fire behaviour a key factor in the fire ecology of African grasslands and savannas. Pages 1–15 in D. X. Viegas, editor. *Forest fire research and wildland fire safety*. Millpress, Rotterdam, The Netherlands.
- Uys, R. G., W. J. Bond, and T. M. Everson. 2004. The effects of different fire regimes on plant diversity in southern African grasslands. *Biological Conservation* 118:489–499.
- Van Wilgen, B. W., N. Govender, H. C. Biggs, D. Ntsala, and X. N. Funda. 2004. Manipulating savanna fire regimes to meet conservation objectives: lessons from a large African national park. *Conservation Biology* 18:1533–1540.
- Vicentini, A., J. C. Barber, S. S. Aliscioni, L. M. Giussani, and E. A. Kellogg. 2008. The age of the grasses and clusters of origins of C₄ photosynthesis. *Global Change Biology* 14: 2963–2977.
- Visser, V., W. D. Clayton, D. A. Simpson, R. P. Freckleton, and C. P. Osborne. 2014. Mechanisms driving an unusual latitudinal diversity gradient for grasses. *Global Ecology and Biogeography* 23:61–75.
- Visser, V., F. I. Woodward, R. P. Freckleton, and C. P. Osborne. 2012. Environmental factors determining the phylogenetic structure of C₄ grass communities. *Journal of Biogeography* 39:232–246.

SUPPLEMENTAL MATERIAL

Ecological Archives

The Appendix is available online: <http://dx.doi.org/10.1890/14-1495.1.sm>