THE INFLUENCE OF FIRE ON GRASS FUNCTIONAL TRAITS

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

The University of Sheffield
Faculty of Science
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Submission Date

March 2018

Abstract

Fire is a global disturbance that is increasingly recognised as having a central role in shaping plant traits and floral communities. Despite the progress in establishing fire as a selective filter and agent for woody plants, less research has focussed on the effects of fire on herbaceous species. This thesis addresses the influence of fire on functional traits relating to flammability, recruitment and regeneration in fire-prone grasses, which experience and fuel the most frequent fire regimes on Earth.

Enhanced flammability may be adaptive for recurrently-burnt grasses via the maintenance of an open canopy and an increase in spatio-temporal opportunities for recruitment and regeneration. However, grass flammability has been little explored. Using fire-prone South African grasses, we established the traits that influence grass flammability, and how these link to regrowth rate after fire. We found grasses are not homogenous fuels to fire but differ in functional traits that in turn demonstrably influence flammability.

If grass traits are shaped by fire, they should show patterns across gradients of fire behaviour. We determined how the traits of grass populations are influenced by fire frequency, and whether any trait variation seen is due to selection or plasticity. Our finding that the development of grasses is influenced by prior exposure to fire, independent of the current environmental conditions, suggests these plants possess a ‘memory’ for fire. We also explored how fire frequency and intensity influence grass traits at the biome scale, and found that these fire characteristics filter grasses for specific functional traits.

This work establishes fire as a force shaping the functional traits of fire-prone grasses, the effect of which can be seen at multiple biological scales.

Statement of contributions

The personal pronoun ‘we’ is used throughout this thesis as this work was developed, supported, or improved by input and advice from collaborators. Nevertheless, the vast majority of the ideas, data collection, modelling, analysis, and writing in this thesis are the candidate’s work. Chapters 1 (General Introduction) and 6 (General Discussion) were written by the candidate with editorial advice from Prof Colin Osborne and Dr Gavin Thomas. For all other chapters, major contributions are listed at the start of the chapter and any additional contributions in the acknowledgements at the end.

Declaration by the author

I declare that this thesis is an original report of my research, has been written by me and has not been submitted for any previous degree. The experimental work is almost entirely my own work; the collaborative contributions have been indicated clearly and acknowledged. Due references have been provided on all supporting literatures and resources.

I declare that this thesis was composed by myself, that the work contained herein is my own except where explicitly stated otherwise in the text, and that this work has not been submitted for any other degree or professional qualification.

Acknowledgements

This work was made possible by funding from a Natural Environment Research Council studentship. In addition, I was grateful to receive funding from the Department of Animal & Plant Sciences, the Ecological Society of America and the British Ecological Society to extend field seasons and attend academic conferences.

I feel incredibly lucky to have had two supportive, patient and encouraging supervisors during this PhD journey: Professor Colin Osborne and Dr Gavin Thomas. I thank Gavin for his methodological inputs and for providing a critical eye and a non-plant scientist perspective on things! Colin has always shown great confidence in me when I've not always felt it myself, and has always been on hand with excellent advice, whether it be work- or life-related. He is a great academic role model and a good friend.

The Botany Department at Rhodes University was a home away from home for many months during fieldwork. A huge thank you to all of the staff (Julie Coetzee, Barry Hartley, Sam Mzangwa, Tracey Nowell, Craig Peter, Nosipho Plaatjie, Riaan Strauss, William Tleki, & Susi Vetter) and students who made me feel me feel so welcome! Particular thanks go to Brad Ripley for his excellent supervision whilst in SA. His advice and enthusiasm greatly aided my field work, and he took me to see my first grassland wildfire! I'm grateful that I was invited into the Ripley household many times for great food, drink and conversation! Justin, Freyni, Max and Jack Du Toit – thanks for your fieldwork help and superb hospitality whilst in Middelburg. A particularly big shout out to Claire Adams, Sean Gallagher, Sheena Talma, Lavinia Perumal and Cara-Jayne Thorne who made my stays in Grahamstown so enjoyable, I owe you all many pints in the Rat!

The Department of Animal and Plant Science has been a wonderfully friendly, supportive and fun place to work over the past four years. There are many people who have made every day working in APS a pleasure, particularly Bethan Hindle, Millie Mockford, Helen Hicks, Luke Dunning, Jill Olofsson, Chris Bennett, Helen Eachus, Bex Atkinson, Catherine Preece, Remi Vergnon, Kirsty Burnet, Jason Griffiths, Bianca Santini, Tamora James, Emma Jardine, Jose Moreno, Danny Wood, Shaun Coutts and everyone else in the C214 and the Osborne-Christin lab groups. I thank Pascal-Antoine Christin, my project supporter, for his help and expertise. Outside of Sheffield, I've worked with a number of academics (Caroline Lehmann, Claire Belcher and Sally Archibald) who have contributed positively to my work and have been a source of inspiration.

I want to thank my friends outside of academia for much needed entertainment and non-science

chat (Lucy Dunning, Scott McKenzie, Chris Duffy, Emily Mitchell, Katie Bassett, Lucy Hillier and Freddie Ransom).

My parents and sisters have shown unfailing confidence in me throughout my studies. I’m so grateful for the sacrifices they’ve made for me over the years to give me a good education. I certainly wouldn’t be here today without their support. I hope I have made them proud! Ryan, Henry, Cerys, Phoebe and George – you’ve made ‘Crazy Auntie Kimmy’ laugh a lot over the last few years and helped her keep things in perspective.

I gained a whole new family during my PhD when I became a Simpson. Jackie, Bobby & Richard welcomed me into their life and into their home. Thanks to Ralph, the newest member of the Simpson clan. He is a most excellent lap-warmer and tester of paper quality (chewability), who has made thesis writing a lot more fun.

Finally, I want to thank James. The ways in which he has helped me over the past four years would fill a thesis if I wrote them all down. He’s a good egg ☺

This thesis is dedicated in loving memory to

Jackie Lesley Simpson (1957 – 2017)

Who taught me that:
One can never be *too* prepared,
Stubbornness can achieve a lot,
No detail is too small for ample consideration,
Perfection can be achieved (which may not be reflected in this thesis)
Knitting is excellent resilience training
Prosecco is always appropriate.

We miss you.

“All we have to decide is what to do with the time that is given us” –

Gandalf, The Fellowship of the ring

Chapter 1

Chapter 1

General Introduction

General introduction

1.0 FIRE AND PLANTS

The shared history of plants and fire goes back over 420 million years. Fires have been burning on Earth ever since there has been plant biomass to fuel them, with the earliest evidence of fire coming from the Silurian (Glasspool, Edwards & Axe, 2004; Bond, Woodward & Midgley, 2005). This association is widespread in space as well as in time, with 40% of Earth's terrestrial surface being covered in fire-prone ecosystems (Chapin et al., 2002). The plant-fire relationship is bidirectional: plants influence fire through their flammability (Bond & van Wilgen, 1996; Beckage, Platt & Gross, 2009; Archibald et al., 2017), and are influenced by fire via the adaptive and plastic responses of their traits and the structure of their ecological communities (Emerson & Gillespie, 2008; Keeley et al., 2011).

Fire can vary considerably in frequency, intensity, size and season (Gill, 1975; Belcher et al., 2010; Archibald et al., 2013; Keeley & Syphard, 2016), and a key determinant of a fire's characteristics is the flammability, or propensity to burn, of the vegetation that fuels it (Bond & van Wilgen, 1996; Beckage, Platt & Gross, 2009). Considerable variation in flammability exists across and between species (e.g. Scarff & Westoby, 2006; Murray, Hardstaff & Phillips, 2013; Grootemaat et al., 2015; Cornwell et al., 2015), and changes in plant community composition have repeatedly shown significant alterations in vegetation flammability and fire regime in time (Belcher et al. 2010) and space (Rossiter et al., 2003; Brooks et al., 2004).

On the other hand, fire represents a major disturbance to plants. It regularly kills mature plants with the mechanism of death predicted to be a result of heat-induced hydraulic failure (Balfour & Midgley, 2006; Midgley, Kruger & Skelton, 2011) or meristem death (Bond & van Wilgen, 1996). For those that can survive, a large proportion of above-ground biomass is typically damaged or removed entirely. Fire also has several indirect effects on plant fitness through changes to the local environment. The removal of dead and living biomass by fire can significantly alter light availability, humidity and temperature (Knapp & Seastedt, 1986). Soil properties, including the availability of nutrients such as nitrogen and phosphorus, may be altered by fire through the combustion of organic matter, the volatilisation of some nutrients and the mineralisation of others (DeBano et al., 1979; Grove, O'Connell & Dimmock, 1986). Finally, fire may also modify biotic interactions through the reduction of competition and the alteration of herbivory levels (Carlson et al., 1993; van de Vijver, 1999; Bowman et al., 2016).

In order to persist through recurrent fire, plants must possess functional traits (characteristics of plant morphology and physiology that determine performance and ecological role; Voille et al., 2007) that are adaptive in fire-prone environments (Webb et al., 2002; Emerson & Gillespie, 2008). Fire can therefore act as an 'environmental filter', selectively allowing species into fire-prone communities based on the possession of these adaptive traits, and producing communities that are functionally clustered (Bathalha et al., 2011; Cianciaruso et al., 2012; Hollingsworth et al., 2013; Shryock et al., 2014). Such traits may be a result of selection by fire, and therefore represent adaptations. Fire-sensitive species, on the other hand, may possess traits that aid escape from fire altogether, such as a preference for habitats that do not burn (e.g. rocky crags or semi-aquatic environments).

2.0 PLANT ADAPTATION TO FIRE

Plant species may increase their fitness in fire-prone environments through changes to their functional traits. The traits that are likely to be under selection in fire-prone environments relate to how a population may survive through recurrent fire ("persistence"), the ability to withstand damage from fire ("resistance") and the ability of plant biomass to ignite and sustain burning ("flammability"). The significant variation that exists in fire characteristics, such as frequency and intensity, means that no species is adapted to all fires but rather to a particular fire regime, and that different fire regimes can select for very different plant traits (Pausas & Bradstock, 2007; Verdu & Pausas, 2007; Silva & Batalha, 2010; Keeley et al., 2011; Forrestel et al., 2014).

2.1 Persistence traits

For plant populations to persist through recurrent fire, they must either resprout from surviving parental tissues ('resprouter' strategy) or recruit from the seedbank ('seeder' strategy; Bond & Midgley, 2001; Pausas et al., 2004). These two key mechanisms are not necessarily mutually exclusive, and a number of strategies exist (obligate seeder, obligate resprouter, facultative seeder; Pausas & Keeley 2014). Both modes of persistence are widespread, but the proportion of species possessing each strategy changes across gradients of fire behaviour, suggesting that the relative benefit of seeding or resprouting depends on the fire regime (Keeley, 1986; Bellingham & Sparrow, 2000; Knox & Morrison, 2005; Pausas & Keeley, 2014). For example, in highly frequent fire regimes, seeders are susceptible to 'immaturity risk' (Zedler, 1995) due to the time it takes for a species to reach maturity versus the likelihood of fire, and thus the seeder strategy becomes more viable as fire frequency decreases. In contrast, resprouters are predicted to dominate frequently burnt environments, due to their capacity to quickly recover biomass lost in the fire.

2.1.1 Resprouting

Resprouting species have the ability to generate new shoots from dormant buds after being scorched by fire. Resprouters can tolerate the removal of all (or the vast majority of) above-ground biomass, and then recover by utilising reserves (often below-ground) to regenerate the lost biomass. Depending upon the positioning of the resprouting buds, regrowth can occur from a number of locations on the scorched individual (roots, epicormics, lignotubers etc.)

Resprouting is adaptive in fire-prone ecosystems, as rapid recovery after fire confers a competitive advantage by recapturing space previously occupied by the mature plant. The possession of traits related to high photosynthetic capacity, such as high specific leaf area (SLA) and stomatal density, and large stomatal size, will aid in rapid post-fire regeneration (Reich et al., 1999). Having large below-ground carbohydrate reserves also facilitates robust resprouting after fire (Verdaguer & Ojeda, 2002; Paula & Pausas, 2011).

2.1.2 Recruitment

The persistence of seeder species in fire-prone environments is dependent on dormant seeds germinating after fire, replacing the mature plants that were destroyed in the fire. The seeds of these species must be resistant to the heat shock associated with fire, and there is growing evidence that fire has shaped the evolution of seed traits in fire-prone species, making them more resilient to heat pulses (Naveh, 1975; Gómez-González et al., 2011).

The timing of recruitment relative to fire occurrence is important for seedling success. The conditions after fire are ideal for seedlings, typically having high light availability, low competition and a flush of soil nutrients. Therefore, by synchronising recruitment to the post-fire environment, plant establishment after fire is enhanced. Indeed, many species of fire-prone ecosystems, particularly pyrophilic shrublands, display mass seedling recruitment following fire (e.g. Wright & Fensham, 2017). This synchrony may be achieved through fire-stimulated germination in which the heat-shock associated with fire or chemicals found within smoke act as germination cues (Keeley & Fotheringham, 2000). Heat-stimulated germination is largely associated with hard-seeded species (e.g members of the Cistaceae, Ericaceae and Fabaceae), and occurs when heat disrupts the hard seed case and breaks dormancy. For soft-seeded species of flammable ecosystems, many organic and inorganic combustion chemicals derived from smoke stimulate germination (van Staden et al., 2004; Flematti et al., 2011; Downes et al., 2014). Another mechanism used to coordinate recruitment and fire occurrence in species that do not have a soil-stored seed bank is fire-stimulated flowering, a trait common in monocots (Bytebier et al., 2011; Lamont & Downes, 2011; Pyke, 2017), and the fire-stimulated release of seeds from serotinous woody structures (Lamont et al., 1991).

2.2 Resistance traits

Some species that reside in fire-prone ecosystems rarely attain damage from fires due to their possession of ‘fire-resistant’ traits that protect vulnerable plant parts from being burnt in a typical fire (i.e. a “non-flammable” strategy *sensu* Pausas, Keeley & Schwilk, 2017). Such traits include thick, insulating bark (Vines, 1968; Niklas, 1999; Pausas, 2015a), the shedding of lower branches (reducing the connectivity of surface to crown fuels; He et al., 2012; Pausas, 2015b) and inflammable succulent or large foliage (Givnish, McDiarmid & Buck, 1986; Thomas, 1991; Dantas, Batalha & Pausas, 2013). Even fire-prone herbaceous species, in which the majority of above-ground biomass is removed by fires, can have fire-resistant structures like the tightly-packed leaf bases of savanna grasses which prevent excessive heat from reaching basal meristems.

2.3 Flammability

A plant trait becoming increasingly recognised as being shaped by fire is flammability. This complex trait consists of several interdependent components (Anderson, 1970): ignitability (the ease of ignition), combustibility (the intensity of combustion) and sustainability (the maintenance of burning over time). Plant physical and chemical traits influencing some or all components of flammability largely relate to the amount, density, surface area and moisture content of fuels (Scarff & Westoby, 2006; Murray, Hardstaff & Phillips, 2013; Grootemaat et al., 2015; Simpson et al., 2016). Other traits relating to the chemistry of biomass, such as the energy content or the presence of high levels of flammable compounds, may be important to the flammability of particular vegetation types (Ormeño et al., 2009; Pausas et al., 2016).

Whilst climate is an important determinant of fire regimes, the flammability of plants also has a significant effect (Krawchuck & Moritz, 2011; Swetnam & Betancourt, 1990). The influence of plant flammability can be seen in ecosystems that have dissimilar climates but that have converged on similar fire regimes due to comparable plant flammabilities, such as North American longleaf pine savanna and Australian eucalypt savanna (Archibald et al., 2017). Therefore plants may influence local fire regimes to engender favourable conditions through their flammability (Schwilk, 2003). The optimal fire regime for a species depends largely upon the vegetation type and mode of post-fire persistence, and thus species adopt alternative ‘flammability strategies’ (*sensu* Pausas, Keeley & Schwilk, 2017). Serotinous, obligate seeder species, such as members of the *Pinus* genus, require infrequent intense fire to complete their life cycle. Such species possess traits consistent with a ‘hot flammable’ strategy (Pausas, Keeley & Schwilk, 2017) and fuel high-temperature crown fires that are vital for the release of stored seeds from mature serotinous cones (Lamont et al., 1991; Keeley et al., 2011). In contrast, resprouting species, such as perennial grasses of tropical grasslands and savannas, may benefit from very frequent, low-intensity fires (Archibald et al., 2013) and follow a ‘fast flammability’ strategy (Pausas, Keeley & Schwilk, 2017). These shade-intolerant species require the regular removal of standing dead herbaceous and woody biomass (Everson, Everson & Tainton, 1988; Bond, 2008), which is aided by high plant flammability arising from biomass that burns rapidly. The low fire residence times and cool burn temperatures that characterise these fast-moving surface fires provide advantages to resprouting grass species because they do not expose basal meristems to excessive heat (Bradstock & Auld, 1995; Gagnon et al., 2010; Archibald et al., 2013).

For both resprouter and seeder species, the high temperatures produced by flammable individuals can reduce post-fire competition by increasing neighbour mortality (Bond & Midgley, 1995). This may benefit obligate seeders with low dispersal, in which recruited seedlings colonise gaps produced by their parents, and resprouting species where reduced competition favours the resprouting individual and enable clonal spread. There are added benefits of high flammability for species which have heat- or smoke-stimulated seed release or germination, with the greater heat released by more flammable fuels enhancing recruitment from the seedbank (Keeley & Fotheringham, 2000; Moreira et al., 2010; Pausas et al., 2012; Moreira et al., 2014).

How the traits of individual plants collectively influence the flammability of vegetation is an area is a subject that is receiving increasing attention. Evidence so far suggests that the most flammable members of a community may have a disproportionate effect on the flammability of the ecosystem (Blauw et al., 2015; de Magalhaes & Schwilk, 2012; Van Altena et al., 2012; Varner et al., 2017), meaning small changes in plant flammability can have considerable effects on fire regime.

3.0 SELECTION AND PLASTICITY

Plants have adapted to life with fire through changes to their functional traits, and the two mechanisms that allow this adaptation are selection and plasticity. Under selection, the frequency of favourable alleles increases over several generations while that of unfavourable ones tends to decrease. There is growing evidence of selection by fire, particularly in woody species, with different fire regimes creating intraspecific variation in fire-related traits that is genetically determined (Parchman et al., 2012; Budde et al., 2014; Moreira, Castellanos & Pausas, 2014). The role of phenotypic plasticity in adaptation to biotic and abiotic environments is much less known but its importance is becoming increasingly recognised (Bradshaw, 1965; Sultan, 1995). This mechanism of adaptation involves a genotype (individual) being able to express different phenotypes according to its environment (Przybylo, Sheldon, & Merilä, 2000).

As well as responding to current conditions, there is growing evidence that plastic responses of plant phenotypes can be modulated by past environmental conditions (Bruce et al., 2007; Huber et al., 2012; Robertson & Wolf, 2012; Niu et al., 2014; Scholes & Paige, 2015). For example, wheat seedlings that had been submerged showed greater tolerance to waterlogging later on in their life cycle in comparison to those that were not submerged (Li et al., 2011). Primed responses can have profound positive effects on plant tolerance to and performance through environmental perturbations, in addition to reducing costs associated with maintaining a constant high level of phenotypic plasticity (Wang et al., 2017). There is evidence of plants possessing an 'ecological memory' (*sensu* Padisak, 1992) of abiotic stresses such as drought and inundation (e.g. Tahkokorpi, Taulavuori, Laine & Taulavuori, 2007; Onate, Blanc & Munne-Bosch, 2011; Walter et al., 2011; Huber et al., 2012; Wang et al., 2017), but this phenomenon has yet to be explored with regards to fire.

3.1 Exaptation

Traits that are adaptive in fire-prone environments may not be fire adaptations but instead may have originated in response to some other environmental factor but provide fitness benefits in contemporary fire regimes. For example, resprouting is a very widespread trait in the angiosperms so it is unlikely that all resprouting types are a result of adaptation to fire. However, given the long-association between plants and fire, it is likely that post-fire resprouting in some lineages may have originated in response to fire (Keeley et al., 2012). Whether fire-adaptive traits represent adaptations (originating in response to fire) or exaptations (originating in response to other factors) is a key evolutionary question, but one that is not always be possible to answer. However a recent study found that subjection to recurrent fire preceded the evolution of several fire-adapted traits (fire-stimulated flowering, seed-release, and germination) suggesting that these traits represent adaptations to fire, and not exaptations (Lamont & He, 2017). Caution is however required when evaluating the forces that shape trait evolution, as attempting to determine a single evolutionary pressure responsible may be overly simplistic (Keeley et al., 2011).

4.0 FIRE AND GRASS

There is a long and reciprocal relationship between fire and grasses. Fire has played a major role in the historical and contemporary success of the Poaceae family (Bond, Midgley & Woodward, 2005; Keeley & Rundel, 2005; Scheiter et al., 2012; Linder et al., 2017), and grasses have, in turn, fuelled the most frequent fire regimes on Earth (Mouillot & Field, 2005).

Large areas of land in the tropics and subtropics are maintained as grasslands and savannas by fire, despite having the climatic potential to support forest (Bond, Midgley & Woodward, 2005; Beckage, Gross & Platt, 2011). Fire also played a role in the historic creation of these widespread grassy biomes, namely the dramatic late Miocene expansion of C_4-dominated grasslands (Keeley & Rundel, 2005; Scheiter et al., 2012). At this time, increased seasonality saw a surge in fire activity (Keeley & Rundel, 2005) which altered vegetation distributions in large portions of the seasonal tropics and subtropics, by allowing C_4 grasses to dominate in open, high light areas that had been previously forested (Scheiter et al., 2012).

Grasses have profoundly influenced fire regimes by producing high fuel loads quickly. Perennial grasses that dominate tropical grasslands (Allan et al., 2002; Overbeck & Pfadenhauer, 2007) rapidly produce biomass after being burnt through resprouting (Archibald et al., 2013), and can therefore fuel highly frequent fire. The strong influence that grass has on fire regimes can be seen in contemporary landscapes, where invasion by flammable grasses causes dramatic changes in fire characteristics (Rossiter et al., 2003; Grigulis et al., 2005). Such invasions and concomitant changes in fire regime can lead to extensive and long-lasting changes to vegetation composition and structure, such as the elimination of fire-sensitive native trees in the Hawaii dry forest due to increased fire frequency fuelled by invasive grasses (Hughes, Vitousek & Tunison, 1991). Likewise, in the Miocene expansion of the C_4 grasslands, the high fuel loads produced by the elevated productivity of the C_4 grasses further increased fire activity, and thus grassland expansion, in a positive feedback process (Beerling & Osborne, 2006).

With a global average fire return interval of 5.7 years in tropical grasslands, fire represents a highly frequent disturbance in these environments (Mouillot & Field, 2005). In addition, fire is a severe disturbance to grasses, typically removing all above-ground biomass, and is capable of killing mature plants (Zimmerman et al., 2010). The significant selection pressure that fire represents to these species, and the long ~30MY period of shared history between grasses and fire, makes them an ideal study system for fire-plant interactions. Despite these benefits, relatively little attention has been paid to grasses, or herbaceous species in general, with the majority of such studies centred on woody species (e.g. Parchman et al., 2012; Budde et al., 2014; Moreira, Castellanos & Pausas, 2014). The cause of this disparity may be due to a historic assumption that grasses do not vary much in traits that relate to living with recurrent fire (persistence, resistance and flammability) in comparison to woody species, or that fire adaptation has lower fitness benefits in resprouting species, like grasses, in comparison to woody obligate seeder species. This thesis aims to redress this balance and explore the influence of fire on grass functional traits at different biological scales (species, populations, biomes).

Thesis Overview

The thesis constitutes four data chapters, which together broadly aim at exploring the influence that fire has on grass functional traits. The chapters are linked together in ways that are outlined in Figure 1. The narrative of the thesis is provided below, in addition to an overview of each chapter.

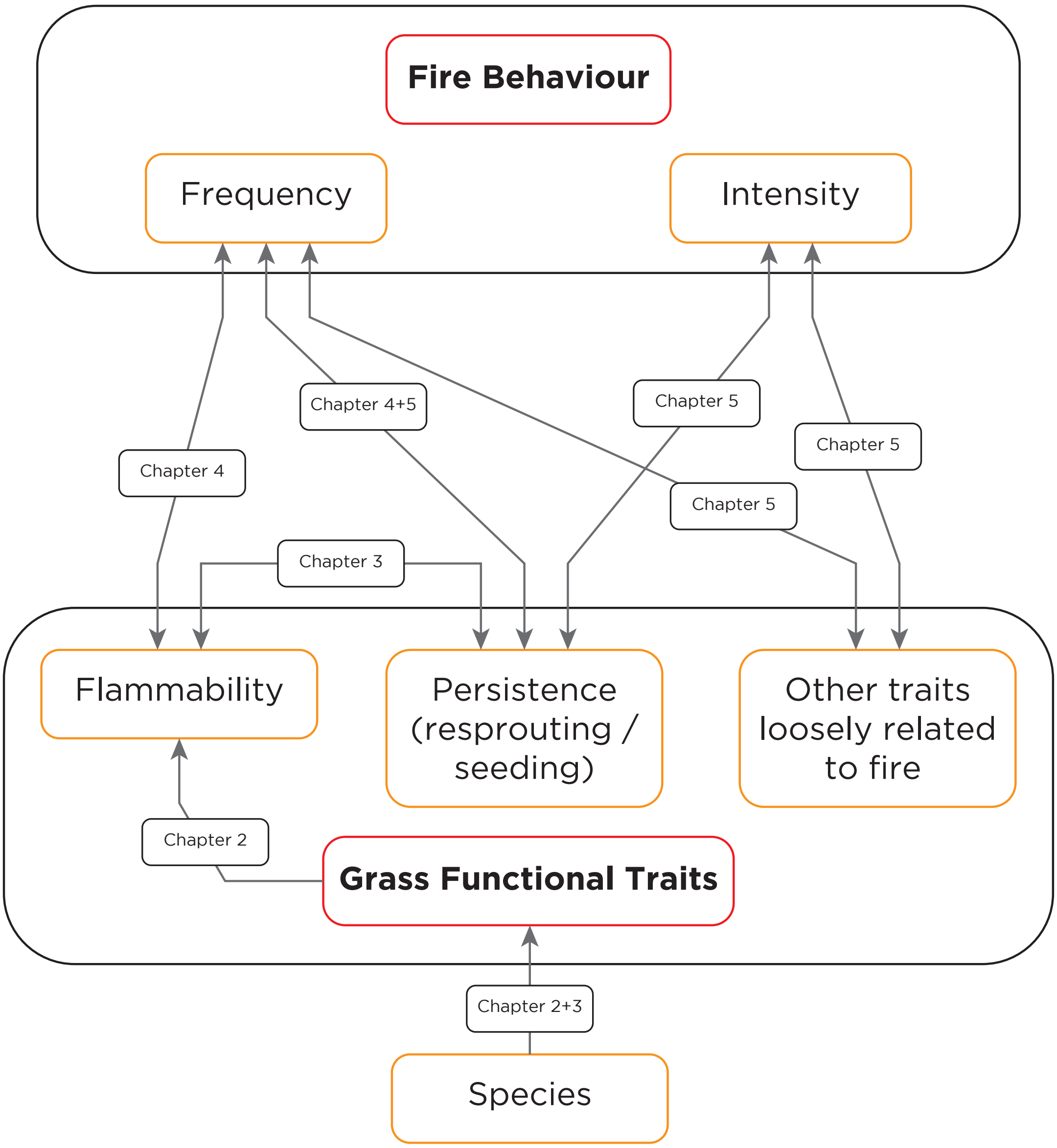

Figure 1. Schematic diagram demonstrating the relationships between fire and grass traits that are investigated in this thesis (including chapter information).

Chapter 2: Determinants of Flammability in Savanna Grass Species

For this thesis, my first focus was on grass flammability, a topic that had previously been little explored, and one in which basic questions remained unanswered. Therefore, in this first data chapter, I try to address some of these questions, such as do grasses differ in their flammability, and what functional traits influence grass flammability?

In fire-prone landscapes, enhanced flammability may be adaptive for grasses via the maintenance of an open canopy and an increase in spatio-temporal opportunities for recruitment and regeneration. In addition, by burning intensely but briefly, high flammability may protect resprouting buds from lethal temperatures. Despite these potential benefits of high flammability to fire-prone grasses, variation in flammability among grass species, and how trait differences underpin this variation, remains unknown. Indeed, there is a historic assumption that grasses are homogenous fuels to fire despite anecdotal evidence to the contrary (e.g. some species stay green into the dry season while others cure rapidly) and findings of significant variation in the flammability of other vegetation types.

By burning leaves and plant parts, I experimentally determined how five plant traits (biomass quantity, biomass density, biomass moisture content, leaf surface-area-to-volume ratio and leaf effective heat of combustion) combined to determine the three components of flammability (ignitability, sustainability and combustibility) at the leaf and plant scales in 25 grass species of fire-prone South African grasslands at a time of peak fire occurrence. I assessed the influence of evolutionary history on flammability using a phylogeny built for the study species.

My results demonstrate that grasses are not homogenous fuels to fire. Rather species differ in functional traits that in turn demonstrably influence all components of flammability. The five measured plant traits predicted components of flammability. Total above-ground biomass was a key driver of combustibility and sustainability with high biomass species burning more intensely and for longer, and producing the highest predicted fire spread rates. Moisture content was the main influence on ignitability, where species with higher moisture contents took longer to ignite and once alight burnt at a slower rate. Grass flammability is influenced by evolutionary history with some components showing phylogenetic signal.

Chapter 3: Flammability and Post-Fire Regrowth are Linked in Savanna Grasses

Following the findings from Chapter 2, I go on to explore how high flammability may be an adaptive trait to fire-prone, resprouting grasses. In this chapter, I address the question of how flammability is linked to post-fire regrowth, with the expectation that they would be positively associated if flammability is a beneficial trait to these plants.

High flammability may increase fitness by creating spatio-temporal opportunities to regenerate or recruit, if it is linked with rapid recovery after fire (via resprouting or establishment from seed). Resprouting grasses of fire-prone savannas and grasslands experience and fuel highly frequent fire regimes, and my work in Chapter 2 showed considerable variation in flammability across coexisting savanna grasses. However, whether this variation is associated with fitness benefits for grasses after fires remains unknown.

By measuring traits influential to flammability, and then burning individual plants *in situ* during the natural fire season and tracking their regrowth, we assessed the relationships between flammability-enhancing traits and regrowth rate after fire in coexisting savanna grass species. Shared evolutionary history was accounted for building a phylogeny of the study species and employing phylogenetic comparative methods.

We found post-fire regrowth was significantly positively related to fuel load, a key predictor of grass flammability. Plants with a higher fuel load regrew more biomass and at a faster rate. Flammability and regrowth traits showed considerable variation across these coexisting grass species.

Chapter 4: Memory of Past Fire Regime Enhances Plant Developmental Response to Fire

If grass traits are shaped by fire, they should show patterns across gradients of fire behaviour. In Chapters 4 and 5, I explore how two key fire characteristics (frequency and intensity) influence grass traits. In Chapter 4, I determine how persistence traits of grass populations are influenced by fire frequency, and whether any trait variation seen is due to selection or plasticity. I also explore whether trait differences endure after the fire regime is lifted, to see if grasses possess a 'memory' of fire.

Fire is a severe disturbance to plants that varies through time. Coping with temporal variation in fire requires plasticity in traits promoting persistence through fire, but how plastic responses to current conditions are affected by prior exposure to fire remains unknown. Here we investigate phenotypic divergence between populations of resprouting savanna grasses exposed to differing experimental fire regimes, and test whether this divergence persists after plants are grown in a common environment.

We took cuttings from four savanna grass species in experimental plots that had been either annually burnt or unburnt for >35 years, and re-established them in a common environment for one year. we measured traits relating to persistence before plants were burnt during the fire season, and their regrowth tracked. To determine if there was genetic differentiation between the populations subjected to differing fire regimes, we compared restriction-site associated genome sequences for a subset of individuals.

The influence of historic fire frequency on traits relating to flowering and below-ground investment could be seen after a year in the common environment, but not regrowth rate after fire. Plants previously subjected to high fire frequency produced more inflorescences and invested a greater proportion of biomass below ground than historically unburnt plants. These results suggest that high fire frequency causes plants to develop a greater capacity for recruitment and resprouting. However, we found no consistent genetic differences between the populations under diverse fire frequencies within each species, suggesting that trait differences arose from developmental plasticity and not natural selection.

Chapter 5: Fire-grass associations at a global scale

In Chapter 5, I explore the influence of fire frequency and intensity on grass functional traits at the biome scale, to determine whether these fire characteristics act as 'filters' for beneficial traits. These traits include both those tightly associated with fire (flammability and persistence traits) and those more loosely associated (e.g. photosynthetic pathway, plant height, specific leaf area).

Fire acts as a community assembling process by selectively excluding species depending on their functional traits. Grassy ecosystems experience the highest fire frequencies on earth, and the distribution and success of many grass species is often linked to fire. Although there is evidence that fire can act as a local filter for grass traits, this mechanism remains untested at the global scale.

Using a dataset of the distribution of 1091 grass species and their functional traits, we examined links with fire regimes. We quantified relationships between fire frequency and intensity related to multiple functional traits: mode of persistence, photosynthetic pathway, culm height, leaf N content, leaf C/N ratio, and SLA.

Fire frequency acts a strong selection pressure for photosynthetic type and mode of post fire persistence, with high fire frequency significantly associated with C_4 resprouter grasses. Fire intensity is also an environmental filter, with high fire intensities associated with a resprouting mode of persistence and shorter statures. Furthermore, leaf traits are structured by fire, with high fire frequency selecting for low leaf nitrogen concentration which is consistent with higher nitrogen use efficiency in frequently burnt environments.

Chapter 6: General Discussion

I summarise and evaluate my findings from the four data chapters within the context of the wider literature. I then consider the limitations and implications of my findings and make recommendations for future research.

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Chapter 2

Chapter 2

Determinants of flammability in savanna grass species

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Table of contents

Figures and tables

Statement of contributions

KJS designed the study with input from GHT, BSR, CMB, CERL and CPO. KJS generated the data with assistance from P-AC (sequence data) and BSR (plant collection). KJS analysed the data with help from P-AC, BSR, GHT and CPO. KJS wrote the chapter, with all authors contributing critically to drafts.

This study has been published in a peer-reviewed journal:

Simpson, K.J., Ripley, B.S., Christin, P.-A., Belcher, C.M., Lehmann, C.E.R., Thomas, G.H. & Osborne, C.P. (2016) Determinants of flammability in savanna grass species. Journal of Ecology, 104, 138-148. doi: 10.1111/1365-2745.12503

A copy of the accepted manuscript has been included as Appendix 1.

Determinants of flammability in savanna grass species

Abstract

1. Tropical grasses fuel the majority of fires on Earth. In fire-prone landscapes, enhanced flammability may be adaptive for grasses via the maintenance of an open canopy and an increase in spatio-temporal opportunities for recruitment and regeneration. In addition, by burning intensely but briefly, high flammability may protect resprouting buds from lethal temperatures. Despite these potential benefits of high flammability to fire-prone grasses, the flammability of such species has been little explored, and the determinants of flammability among grasses remain unknown.

2. By burning leaves and plant-parts, we experimentally determined how five plant traits (biomass quantity, biomass density, biomass moisture content; leaf surface-area-to- volume ratio and leaf effective heat of combustion) combined to determine three components of flammability (ignitability, sustainability and combustibility) at the leaf and plant scales in 25 grass species of fire-prone South African grasslands at a time of peak fire occurrence. The influence of evolutionary history on flammability was assessed based on a phylogeny built here for the study species.

3. Grass species differed significantly in all components of flammability. Accounting for evolutionary history helped to explain patterns in leaf-scale combustibility and sustainability. The five measured plant traits predicted components of flammability, particularly leaf ignitability and plant combustibility in which 70% and 58% of variation respectively could be explained by a combination of the traits. Total aboveground biomass was a key driver of combustibility and sustainability with high- biomass species burning more intensely and for longer, and producing the highest predicted fire spread rates. Moisture content was the main influence on ignitability, where species with higher moisture contents took longer to ignite and once alight burnt at a slower rate. Biomass density, leaf surface-area-to-volume ratio and leaf effective heat of combustion were weaker predictors of flammability components.

4. We demonstrate that grass flammability is predicted from easily measurable plant functional traits, and is influenced by evolutionary history with some components showing phylogenetic signal. Grasses are not homogenous fuels to fire. Rather, species differ in

functional traits that in turn demonstrably influence flammability. This diversity is consistent with the idea that flammability may be an adaptive trait for grasses of fire-prone ecosystems.

Key-words: Biomass moisture content, Biomass quantity, Determinants of plant community diversity and structure, Fire regime, Functional traits, Phylogeny, Poaceae, Resprouting.

Introduction

Fire is a disturbance that has shaped plant traits and floral communities for over 420 million years (Glasspool, Edwards & Axe, 2004; Bond, Woodward & Midgley, 2005), and acts as a powerful selective filter for functional traits related to plant persistence, recovery and recruitment (Emerson & Gillespie, 2008). Fire is also multidimensional and its effects on vegetation depend on the characteristics of the local fire regime (Keeley et al., 2011), which can vary considerably in frequency, intensity, size and season (Archibald et al., 2013). Different fire regimes can lead to the assembly of distinct populations and communities that are functionally clustered for diverse traits (Verdú & Pausas, 2007; Pausas & Bradstock, 2007; Silva & Batalha, 2010; Forrestel et al., 2014). For example, resprouting species are favoured in frequent, low intensity fire regimes, and obligate seeders, that persist via seedling recruitment, are favoured in infrequent, high intensity fire regimes (Pausas & Bradstock, 2007; Pausas & Keeley, 2014).

Plant flammability may both influence and be influenced by fire regime (He et al., 2011; Pausas et al., 2012) but species variation in flammability has received relatively little attention (but see Scarff & Westoby, 2006; Murray, Hardstaff & Phillips, 2013; Grootemaat et al., 2015). Flammability is an emergent property of a plant's chemical and physical traits. However, the identification of these traits in several fire-prone taxa, particularly herbaceous species, has not been achieved. Flammability as a vegetation property consists of several interdependent components (Anderson, 1970) that can each be quantified. Ignitability (the ease of ignition), combustibility (the intensity of combustion) and sustainability (the maintenance of burning over time) are flammability components and can be measured at multiple scales. For example, ignitability is often measured as ignition delay at the leaf or plant scale, whilst the rate of fire spread is a measure of ignitability that operates at the community scale (Gill & Zylstra, 2005).

Plant flammability is a key determinant of fire behaviour (Bond & van Wilgen, 1996; Beckage et al., 2009). In woody plants, flammability varies considerably between and within species (e.g. Fonda, 2001; Saura-Mas et al., 2010; Pausas et al., 2012; Cornwell et al., 2015), and minor changes in vegetation composition have repeatedly demonstrated significant alterations in vegetation flammability and fire regime (Rossiter et al., 2003; Brooks et al., 2004; Belcher et al., 2010). Flammability may act as a means by which plants modify fire regimes to engender favourable conditions (Schwilk, 2003). For example, slow-growing, woody, obligate seeder species, such as *Pinus* species, require infrequent intense fire to complete their life cycle. High temperature crown fires are vital for releasing stored seeds from the retained mature cones of these serotinous species, and to enhance recruitment opportunities of seedlings via mortality of neighbouring trees (Lamont et al., 1991; Keeley et al., 2011). In contrast, resprouting perennial grasses, which dominate grasslands and savannas (Allan & Southgate, 2002; Uys, 2000; Overbeck & Pfadenhauer, 2007), may benefit from very frequent fire (Archibald et al., 2013). These shade-intolerant species require the regular removal of standing dead biomass (Everson et al., 1988) and woody growth (Bond, 2008), which may be aided by high plant flammability. Surface fires in grassy systems are characterized by rapid combustion and spread, low fire residence times and cool burn temperatures (Bradstock & Auld, 1995; Archibald et al., 2013). Such fire characteristics are advantageous to resprouting grass species, protecting basal meristems from excessive heat through biomass that burns rapidly (Gagnon et al., 2010). In addition, high flammability, if linked to efficient post-fire recovery, may provide enhanced regeneration opportunities for these species by killing neighboring plants and reducing post-fire competition (Bond & Midgley, 1995).

Despite these predicted benefits of frequent fire to fire-prone grasses, inter-specific variation in the flammability of such species has been little explored (Ripley et al., 2010), in contrast to knowledge about interspecific variation in post- fire response among grass species (Ripley et al., 2015). A historical belief persists that grasses and other herbaceous plants vary little in their flammability, which has led to the diversity of herbaceous fuels being reduced to one or few fuel classes in fire behaviour modelling (e.g Anderson, 1982). Given the considerable known variation in the flammability of woody species (Schwilk, 2003; Scarff & Westoby, 2006; Pausas et al., 2012; Murray, Hardstaff & Phillips, 2013), such presumptions are unfounded. Substantial changes in grassland community flammability resulting from invasion by non-native grasses provide evidence to suggest considerable inter-specific variation in grass flammability (Hughes, Vitousek & Tunison, 1991; Rossiter et al., 2003). In addition, recent evidence shows that grass traits relating to post-fire recovery are shaped by fire regime (Forrestel et al., 2014; Ripley et al., 2015), suggesting that traits relating to flammability may be responding in similar ways, resulting in intra- and inter-specific variation in flammability.

Physical and chemical traits influencing some or all components of flammability relate to the quantity, quality, moisture content and aeration of biomass (Gill & Moore, 1996; Bond & van Wilgen, 1996). Biomass quantity is critical to combustibility and fire spread rate because it directly influences fire energy output rate (Byram, 1959; Rothermel, 1972). Biomass moisture content determines the extent to which fuels absorb heat energy, with high values associated with delayed ignition and low combustion and fire spread rates (Pyne, 1984, Nelson, 2001). Biomass surface-area-to-volume (SA/V) ratio influences curing and reaction rates within fires (Papio & Trabaud, 1990, Gill & Moore, 1996), with high values linked to rapid ignition, and rapid rates of combustion and fire spread. Increasing biomass density, defined as the mass of biomass per unit volume of fuel bed, raises fuel connectivity, therefore enhancing combustibility and fire spread rate. This relationship applies up to a certain threshold beyond which poor ventilation will limit drying and combustion rates (Rothermel, 1972). Intrinsic properties of plant material, such as heat of combustion, affect combustibility and fire spread rate through the amount of heat energy released during complete combustion. Sustainability is often inversely related to combustibility and ignitability (e.g. de Magalhães & Schwilk, 2012). Therefore plant traits likely to enhance combustion and spread rate may indirectly reduce flaming duration. In contrast, high biomass quantity increases combustion and spread, but is also likely to enhance sustainability, as more fuel takes longer to burn. Plant traits important to flammability have been identified in a number of fire-prone taxa (e.g. Ganteaume et al., 2009; Schwilk & Caprio, 2011). However, the traits that influence grass flammability, and more generally the flammability of herbaceous species, have not been empirically established or explored.

We examined three components of flammability, at multiple scales, for 25 species common in fire-prone South African grasslands. Five structural and chemical plant traits, known to influence vegetation flammability, were measured and correlated with flammability trait values (see Table 1). We hypothesized that 1) there is significant inter-specific variation in flammability among grass species, and that 2) the measured plant traits can explain this variation, with each trait contributing to flammability components in different ways (see Table 1 for specific predictions). We also expected that flammability and plant traits covary due to the interdependent relationships between flammability components and plant traits. The strong phylogenetic patterns in grass distributions across fire frequency gradients (e.g Visser et al., 2012, Forrestel et al., 2014) led us to predict that 3) flammability is influenced by evolutionary history and contains a phylogenetic signal.

Table 1. Matrix summarising the predicted relationships between plant and flammability traits. Flammability traits were determined at different scales (L, leaf; P, plant; C, community) and represent three flammability components. Symbols reflect the direction of the relationship ('+': positive; '-': negative; '0': none; 'N/A': could not be tested). Influence is either direct or indirect (in parentheses)

			Plant trait				
Flammability trait	Flammability component	Scale	Biomass quantity (g)	Biomass density (g cm^{-1})	Biomass moisture content (g g^{-1})	Leaf SA/V ratio	Leaf effective heat of combustion (J g^{-1})
Time to ignition (s)	Ignitability	L	N/A	N/A	-	+	0
Predicted rate of fire spread (m s^{-1})	Ignitability	C	+	+	-	+	+
Flaming time (s)	Sustainability	L, P	+	(-)	(+)	(-)	(-)
Combustion rate (g s^{-1})	Combustibility	L, P	+	+	-	+	+

Materials and methods

PLANT MATERIAL

Plants were collected during the natural fire season in July 2014 in grassland and Nama-Karoo habitats near Grahamstown in the Eastern Cape of South Africa (see Table S1 in Supporting Information for site details). Fire return times over the 2000–2006 period were 2.3 years for vegetation surrounding Grahamstown (Tansey et al., 2007).

Seven individuals of 25 species, representing 5 grass subfamilies, were collected for study (see Table S2). All species were native to the region except *Cenchrus setaceus*, a North African invasive species (Milton 2004). For each species, seven randomly-selected, healthy-looking adult plants were dug up whilst keeping their shoot architecture intact. Plants were stored in sealed plastic bags at room temperature for a maximum of 48 hours to minimise changes in moisture content. A specimen of each species was deposited at the Selmar Schonland Herbarium (Rhodes University).

STRUCTURAL AND CHEMICAL TRAITS

A section of each individual (approximately one third of the entire plant), with its below-ground biomass and soil removed, was used to measure five structural and chemical plant traits. Biomass quantity, density and moisture content were measured at the plant scale, whilst effective heat of combustion (EHoC) and SA/V ratio were measured at the leaf scale.

For measurements of leaf SA/V ratio and EHoC, leaves were removed from a randomly selected tiller of each individual. Total leaf area was measured on digital images using the computer program WinDIAS (Delta-T Devices, Cambridge, U.K.) that determines leaf area by selecting pixels of a pre-defined colour range. Leaf thickness was measured, at the middle of the leaf and excluding the midrib, for three leaves per tiller using digital callipers (accurate to 0.01mm), and an average value was calculated. Leaf SA/V ratio was calculated from the average leaf area and leaf thickness of each species.

The heat of combustion is the energy released as heat when biomass undergoes complete combustion with oxygen, which typically relates to C:N ratio, lignin content and the presence of flammable compounds (Philpot, 1969; Bond & Van Wilgen, 1996). We measured the EHoC, which is the heat of combustion of pyrolysate vapours, and does not assume that all char is consumed. Compared to measurements that involve the full thermal decomposition of biomass (such as in bomb calorimetry), EHoC is a more realistic estimate of the energy released from a wildfire in which combustion is incomplete, and most of the energy is released from burning the pyrolysate vapours. Oven-dried leaf samples of known mass (5.0±0.4mg) were conditioned at room temperature and humidity before being analysed in a microscale combustion calorimeter following the manufacturer's guidelines (FAA Micro Calorimeter, Fire Testing Technology Ltd, East

Grinstead, UK). Each sample was held in nitrogen and heated at a rate of 3°C per second driving off the volatile gases that were ignited and completely oxidised, and heat release was quantified by oxygen depletion calorimetry (Tewerson, 2002). Total heat release was divided by the sample mass to provide the EHoC (kJ g^{-1}). Due to the high repeatability of this trait measurement, material from three randomly-chosen individuals per species was tested in duplicate, to give an average value per individual and per species.

For plant-scale traits, the height (maximum vertical distance from ground level to the tallest point) and width (maximum horizontal spread) of each clump was determined. Biomass density was measured using a novel method, which determined the vertical biomass distribution for each individual. For this, the biomass of each clump was divided at five or more equal intervals along its vertical height, so that intervals were 2.5, 5, 10 or 15cm in length depending on the plant height, and started at ground level. Each clump was cut with scissors at the selected intervals. The fresh and dry biomass of each section were weighed to four decimal places, the latter after oven drying at 70°C to a constant weight. Cumulative dry biomass was calculated at each vertical height interval from ground level. Linear models were fitted to the logged cumulative dry biomass and vertical height for each individual. The slope of this relationship was used as a proxy for biomass density, in g cm^{-1}, with high values indicating densely packed biomass. For each clump, dry biomass values were combined to give the total dry biomass, and moisture content was calculated by dividing the difference between fresh and dry biomass by the dry biomass.

FLAMMABILITY

Flammability was represented by three components: ignitability, combustibility and sustainability (Anderson, 1970). All components were measured for each individual at the leaf scale via epiradiator tests. In addition, combustibility and sustainability were determined at the plant scale by burning partial plant canopies. Plant-scale measurement of ignitability was beyond the scope of this experiment, however a community-level measure was obtained by estimating the rate of fire spread for each individual by parameterising Rothermel's (1972) fire spread model with plant trait data. Leaf- and plant-scale flammability components were measured both on fresh and dry biomass to determine the effect of moisture content. The 'fresh' clump was kept in a sealed plastic bag at room temperature, and the 'dry' clump was first dried at 70°C for a minimum of 48 hours.

Leaf-scale ignitability, sustainability and combustibility were measured as time to ignition, flaming time and mass loss rate respectively using a Quartz infrared 500 W epiradiator (Helios, Italquartz, Milan, Italy) in a fume cupboard with a constant vertical windspeed of 0.1 m s^{-1}. As application of leaf material directly to the epiradiator's silica disk surface always caused instantaneous combustion, 2mm wire mesh was positioned 1cm above the epiradiator's surface. The background temperature at the mesh surface (without fuel), measured by a thermocouple connected to a data-logger, ranged between 370 and 400°C. Samples of 0.2 g (± 0.001 g) leaf material were cut into 2 cm segments to standardise between samples, and applied to the centre of the mesh. The 0.2 g mass was used because preliminary studies found that smaller masses failed to ignite, whilst larger fuel masses increased the risk that other fuel properties, particularly fuel height, influenced flammability values. Smaller samples were used for *Aristida congesta* subsp. *barbicollis* due to the low leaf mass of this species. Each test was filmed at 25 frames s^{-1}, and i) time to ignition (TTI; the time between sample application to the epiradiator and first flaming) and ii) flaming time (FT; the time from ignition to flame extinction) were subsequently determined. As samples were completely combusted by applying them to the epiradiator, an average leaf combustion rate was obtained by dividing the mass of samples by FT. Species average values for TTI and FT were obtained for fresh and dry material. The influence of leaf moisture content on these flammability traits was determined as the difference in values between fresh and dry samples of each individual, and averaged per species.

As canopy architecture influences grass flammability (Martin, 2010), a method that measures plant-scale flammability traits was utilised. Fresh and dry plant material from each individual was clamped on a stand on a four-point balance (Mark 205A; Bel Engineering, Monza, Italy) and burnt in a fume cupboard with a constant 0.1 m s^{-1} vertical wind speed (see Figure S1 for diagram of the set-up used). Samples were ignited by directing a Bunsen burner flame to the side of the base of the clump at a 45° angle and a 5cm distance for a maximum of 3 seconds (less if ignition happened earlier). This resulted in successful ignition in all individuals. Mass loss was logged at 0.2-second intervals and the sigmoidal relationship produced was fitted with a Boltzmann equation. Data were excluded if fitting the relationship was not possible due to noise around the curve (n = 40/350), which occurred if large pieces of plant material fell off the balance during a burn. The width parameter used to fit the Boltzmann curve reflects the time period in which mass was drastically reduced and was used as a plant-scale measurement of sustainability (flaming time). Three seconds of data either side of the inflection point were selected and a linear regression fitted. The slope of this regression represents the maximum combustion rate in g s^{-1}. As preliminary results found this combustibility trait to be strongly driven by the biomass of the sample, interspecific comparisons were standardised for mass. Therefore, maximum combustion rate was plotted against mass change for each species, and linear models were fitted to the fresh, dry and combined datasets. As there was no change in mass common to all 25 species, the y-intercept extracted from the model fitted to the combined dataset was used to characterise the intrinsic combustibility of each species. The combined dataset was used as the slopes of the models fitted to the fresh and dry data did not differ significantly for any species, and model fit was improved by combining the datasets. Any unpaired samples were excluded to ensure a balanced dataset of fresh and dry samples. The y-intercept differed significantly between fresh and dry models for three species (*Panicum* sp., *Hyparrhenia hirta* and *Merxmuellera stricta*) and in these cases, the y-intercept was extracted from linear models fitted to the fresh dataset.

Forward fire spread rate values, the community-scale measure of ignitability, were predicted for each individual using Rothermel's (1972) surface fire spread model as implemented using the "ros" function in the "Rothermel" package (Vacchiano & Ascoli, 2014) in R (R Core Team 2013). Fire behaviour was simulated for each individual by parameterising the model with data for the following traits: leaf SA/V ratio, leaf EHoC, biomass moisture content, plant height, and fuel load (biomass quantity divided by the estimated cover area). See Table S3 for a details of the procedure followed and model assumptions.

PHYLOGENETIC ANALYSIS

We constructed a phylogeny that was initially based on a previously generated dataset for grasses composed of the plastid markers *trnKmatK*, *ndhF*, and *rbcL* (Grass Phylogeny Working Group, II 2012), and augmented here. For ten species not represented in this previous dataset, a fragment of *trnKmatK* was PCR-amplified from genomic DNA, following protocols and primers described previously (Grass Phylogeny Working Group II, 2012). The newly generated sequences have been submitted to NCBI database (Benson et al., 2012) under the accession numbers KP860326 to KP860336. The new markers were manually aligned to the dataset, which consisted of 606 taxa and 5649 aligned bp. This initial dataset was downsized to 70 species, including all the taxa studied here and representatives of all grass lineages. A time-calibrated phylogenetic tree was obtained through Bayesian inference as implemented in BEAST (Bayesian evolutionary analysis by sampling trees; Drummond & Rambaut 2007). A general time-reversible substitution model with a gamma-shape parameter and a proportion of invariants (GTR+G+I) was used. The log-normal relaxed clock was selected. The tree prior was modelled by a Yule process. The monophyly of the BEP-PACMAD clade was enforced, leaving *Puelia olyriformis* as the outgroup. The calibration prior for the age of the BEP-PACMAD crown was set to a normal distribution, with a mean of 51.2 and a standard deviation of 0.001 (mean based on Christin et al., 2014). Two independent runs were conducted for 10,000,000 generations, sampling a tree every 1,000 generations. The convergence of the runs and the appropriateness of the burn-in period, set to 2,000,000 generations, were verified using Tracer (Rambaut & Drummond, 2007; Tracer v1.4, available at http://beast.bio.ed.ac.uk/Tracer). Median ages were mapped on the maximum-credibility tree. The relationships among the species studied here were extracted from this tree, and used for comparative analyses.

DATA ANALYSIS

Statistical analyses were carried out in the R environment (R Core Team, 2013). Data were log-transformed to improve normality and to meet model assumptions where necessary.

Analysis of variance (ANOVA) was used to determine if plant and flammability traits differed significantly between species. The influence of species, and state ('fresh' or 'dry'), on leaf-scale flammability was determined by two-way ANOVA. As biomass quantity values for the plant-scale burns are not representative of the species (i.e for each species, clumps were sub-sampled and a range of masses were burnt), a species effect on the relationship between maximum combustion rate and biomass quantity was tested using the R package "MCMCglmm" (Hadfield, 2010). This approach implements Markov chain Monte Carlo routines for fitting generalized linear mixed models, whilst accounting for non-independence and correlated random effects arising from phylogenetic relationships (Hadfield, 2010). We fitted flammability (maximum combustion rate) and biomass quantity as a bivariate normal response, and species as a random effect. Models were run for 500,000 iterations with a burn-in of 1,000 iterations, a thinning interval of 500 and weakly-informative priors (V=diag(2), nu=0.002). The 95% highest posterior densities (HPD) of within-species and across-species slopes and the difference between slopes were estimated whilst accounting for phylogeny and used to assess whether slopes differed among species.

To test the hypotheses put forward in Table 1 and to establish the strength and direction of plant trait contributions to flammability components, a MCMC multi-response generalized linear mixed model approach was used again. Traits were separated into leaf- and plant-scale to ensure appropriate comparisons, using the same prior and specifications as before. The fit of the models to data was established by fitting linear models between the observed flammability trait values and those predicted by the models. The contribution of plant traits to fire spread rate was tested to determine if strong relationships occurred across species when accounting for phylogeny, whilst acknowledging that some circularity is involved because spread rate was predicted based on the values of these traits.

To explore the pattern of covariance among plant and flammability traits, principal component analyses were performed using the "princomp" function (R Core Team, 2013). Linear regressions were also used to establish the relationships among plant and flammability traits, with the latter being split into leaf-scale and plant-scale traits for analyses to ensure an appropriate comparison. The relationships between flammability traits measured at different scales were also established using linear regressions.

The influence of evolutionary history was established for each plant and flammability trait by testing for the presence of a phylogenetic signal. This was done using the "pgls" function in the "caper" package (Orme et al., 2012) which estimated Pagel's λ.

Results

FLAMMABILITY VARIATION AMONG SPECIES

All flammability components varied considerably across species (Fig. 1; Table S4). At the leaf-scale, significant inter-specific variation was found in ignitability ($F_{24,144}$= 5.02, P<0.001), sustainability ($F_{24,144}$= 3.02, P<0.001) and combustibility ($F_{24,144}$= 2.97, P<0.001). Ignition delays ranged from 1.0 s (*H. hirta*) to 4.0 s (*C. setaceus*) with a mean across species of 1.7 s. The mean flaming duration across species was 6.3 s, and ranged from 4.3 s (*A. congesta* subsp. *barbicollis*) to 7.6 s (*Eragrostis plana*). Connected to flaming duration was average combustion rate, with *E. plana* burning at the slowest rate (27 mg s^{-1}) and *A. congesta* subsp. *barbicollis* at the fastest (49 mg s^{-1}).

At the plant-scale, intrinsic combustibility (for a given biomass) differed by < 2.5-fold across species, ranging from 0.064 g s^{-1} (*Eustachys paspaloides*) to 0.163 g s^{-1} (*Themeda triandra*). When investigating the relationship between combustion rate and biomass, the bivariate mixed effects model revealed that within-species slopes (pooled mean = 0.594, HPD: 0.507 to 0.707) and across-species slopes (mean = 0.797, HPD: 0.067 to 1.385) did not differ significantly (mean slope difference (Δb) = 0.212, HPD: -0.521 to 0.683) when accounting for phylogeny (Fig. 2). This common relationship was extrapolated whilst taking into account intrinsic combustibility differences, allowing combustion values to be predicted for the species mean total biomass. These predicted values of whole-plant combustion rates varied >20-fold among species, ranging from 0.06 g s^{-1} (*A. congesta* subsp. *barbicollis*) to 1.28 g s^{-1} (*M. disticha*; Fig. 2).

Fuel models based on the traits of *C. setaceus* predicted no fire spread, because biomass moisture content values exceeded the moisture of extinction, defined as the fuel moisture content above which a steady rate of fire spread is not possible. Of the remaining species that spread fire, the estimated rate of spread differed substantially (25-fold; Table S4) and varied significantly between species (ANOVA: $F_{24,150}$=42.42, P<0.001).

Substantial inter-specific variation was also found in the five traits measured as explanatory traits for flammability (Fig. 1; see Table S5). Biomass moisture content values of the non-native *C. setaceus* were substantially higher than the other species. However, species still differed significantly for this trait when *C. setaceus* was excluded (ANOVA: $F_{23,144}$=14.39, P<0.001). The measurement of biomass density (i.e. vertical biomass distribution) produced consistent values within species (Fig. S2; species average CV= 28%), but considerable differences among species with slope values ranging from 0.155 (*Eragrostis lehmanniana*) to 0.831 (*M. stricta*).

Collection site did not influence flammability traits. Of the plant traits, vertical biomass distribution (P=0.008) and leaf EHoC (P=0.046) were the only ones affected by collection site (see Table S7).

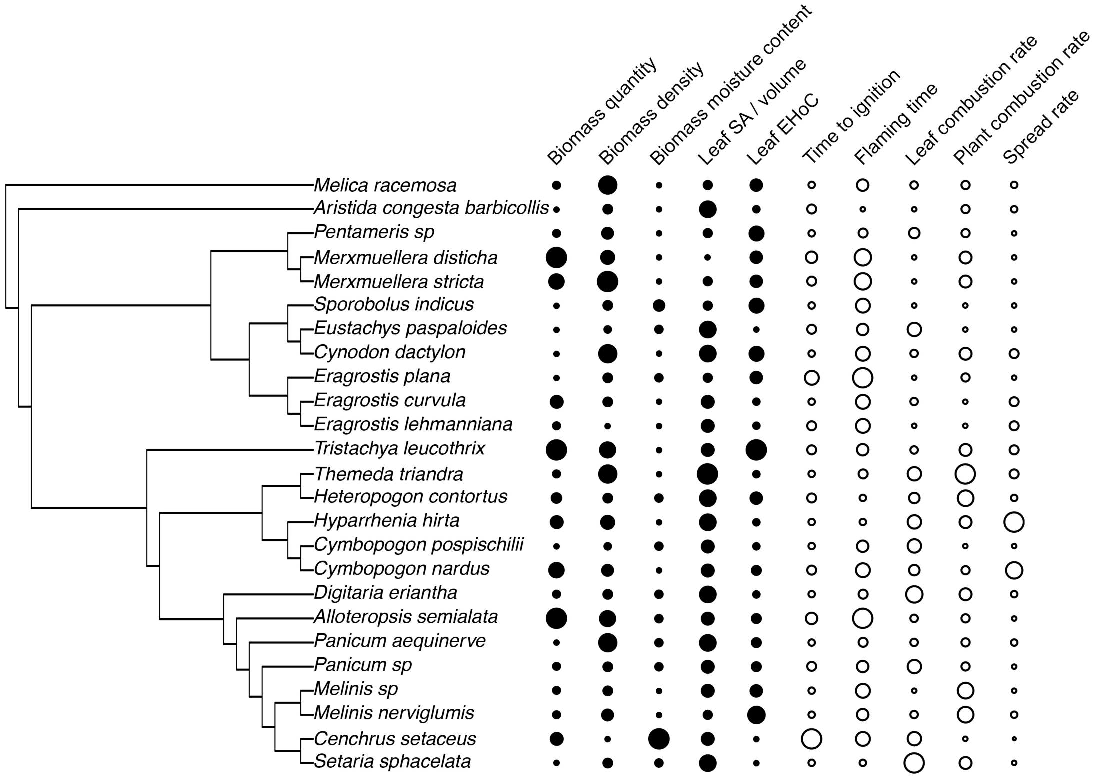


Figure 1. The evolutionary relationships between species and average values of explanatory plant traits (solid circles) and flammability traits (open circles). Trait values are indicated by the size of the circles. A non-zero phylogenetic signal was found for leaf SA/V ratio (Pagel's λ = 1; P=1 for λ=1; P<0.001 for λ=0), leaf flaming time (Pagel's λ = 0.45; P=1.0 for λ=1; P<0.001 for λ=0) and leaf combustion rate (Pagel's λ =0.99; P=0.93 for λ=1; P=0.037 for λ=0).

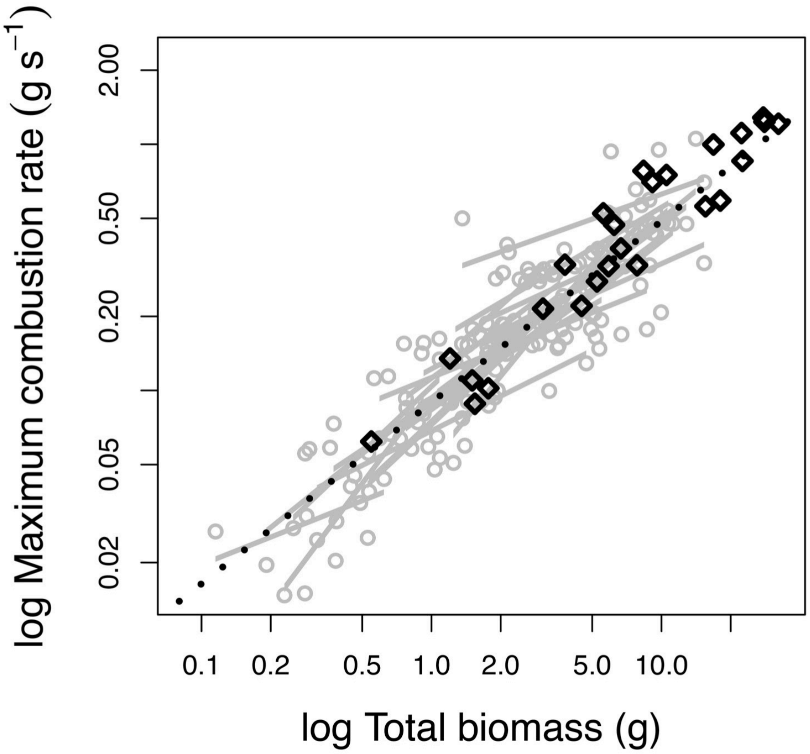


Figure 2. Relationships between biomass quantity and maximum combustion rate across 25 grass species. The mean slopes of within-species relationships (grey lines) and across-species relationships (black dotted line) for maximum combustion rate with biomass burned do not differ significantly when phylogeny is accounted for. Data points are shown as grey circles. Estimates of whole-plant combustion rates (black diamonds) showed substantial variation (>20-fold). These values were calculated by extrapolating the common across-species relationship (black dashed line) to species mean total biomass values whilst taking into account the intrinsic combustibility differences among species.

TRAIT CONTRIBUTIONS TO FLAMMABILITY

Measured plant traits significantly predicted the components of flammability, particularly ignitability and plant-scale combustibility, in which 70% and 58% of variation could be explained by the plant traits respectively (Tables 2 and 3). Variation in sustainability could be explained to a lesser extent by plant traits at the leaf- (47%) and plant-scale (37%), as well as variation in leaf-scale combustibility (39%). The direction of relationships between plant and flammability traits are consistent with those predicted in Table 1, but there are exceptions. Both biomass density and leaf SA/V ratio were expected to correlate positively with predicted spread rate, but instead correlated negatively (Table 3).

Moisture content was key in determining leaf-scale flammability components (Table 2; Table S6). Ignitability was particularly influenced by moisture content, with fresh leaf material taking 42% longer to ignite on average than dry leaf material across species, with a maximum increase of 288% seen for *C. setaceus* (1.0 s dry vs 4.0 s fresh). Once alight, fresh leaf material also burned on average for 7% longer at a 3% lower combustion rate compared to dry leaf material across species. Leaf SA/V ratio significantly influenced sustainability, with high values associated with low flaming duration. The EHoC of leaf material alone contributed little to overall leaf-scale flammability when compared to moisture or SA/V ratio (Table 2).

At the plant-scale, biomass quantity was by far the strongest driver of sustainability and combustibility (Table 3). Plants with greater biomass burnt at a faster rate and for longer. Biomass density and moisture content significantly contributed to plant-scale combustibility, such that plants with high density and low moisture content combusted most rapidly (Table 3). The EHoC of leaf material significantly contributed to sustainability with high values associated with short flaming times (Table 3). Leaf SA/V ratio did not significantly contribute to plant-scale combustibility or sustainability.

Biomass load, moisture content, density and leaf SA/V ratio all contributed highly to predicted fire spread rate when taking phylogeny into account (Table 3). Fuel load contributed directly to reaction intensity, and indirectly to the propagating flux ratio, via bulk density. Biomass moisture content contributed to spread rate by increasing the heat required for ignition and damping the reaction intensity (see Fig. S2). Leaf SA/V ratio influenced reaction intensity and the proportion of this reaching adjacent fuel (propagating flux ratio), as well as the proportion of fuel raised to ignition temperature (effective heating number; Fig. S2). Leaf EHoC contributed to the reaction intensity but played a small part in determining the overall predicted rate of spread (Table 3; Fig. S2).

Table 2. The contribution of plant traits to leaf-scale flammability components as determined by MCMC phylogenetic generalized linear mixed models. Values represent posterior mean estimates of the slopes, the upper and lower 95% confidence intervals and P values (those in bold are significant at P=0.05). In combination, species mean trait values of leaf moisture content, SA/V ratio and effective heat of combustion (EHoC) significantly predicted ignitability ($F_{1,166}$=398.3, P<0.001, R^2=0.70), sustainability ($F_{1,166}$=147.5, P<0.001, R^2=0.47) and combustibility ($F_{1,166}$=105.4 P<0.001, R^2=0.39)

		Leaf moisture content§	Leaf SA/V ratio	log Leaf EHoC
Ignitability (time to ignition)	Estimate	0.691	-0.174e-3	-0.135e-4
	(95% CI)	(0.620 to 0.760)	(-0.420e-3 to 0.872 e-5)	(-0.527e-4 to 0.290e-4)
	P value	**<0.001**	0.17	0.49
Sustainability (flaming time)	Estimate	0.492	-0.876e-3	0.159e-4
	(95% CI)	(0.421 to 0.567)	(-0.142e-2 to -0.359 e-4)	(-0.626e-4 to 0.113e-3)
	P value	**<0.001**	**0.002**	0.741
Combustibility (combustion rate)	Estimate	-0.303e-2	0.522e-5	-0.227e-6
	(95% CI)	(-0.406e-2 to -0.170e-2)	(-0.547e-5 to 0.164e-4)	(-0.254e-5 to 0.193e-5)
	P value	**<0.001**	0.36	0.86

§ Parameter characterized as: the species mean difference in ignition delay (for ignitability) or flaming duration (for sustainability and combustibility) between fresh and dry leaf material for each individual.

Table 3. The contribution of plant traits to plant-scale flammability components as determined by MCMC phylogenetic generalized linear mixed models. Values represent posterior mean estimates of the slopes, the upper and lower 95% confidence intervals and P values (those in bold are significant at P=0.05). Values represent posterior mean estimates of the slopes, the upper and lower 95% confidence intervals and P values (those in bold are significant at P=0.05). In combination, the five plant traits significantly predicted sustainability ($F_{1,151}$=90.07, P<0.001, R^2=0.37), combustibility ($F_{1,151}$=210.8, P<0.001, R^2=0.58) and ignitability ($F_{1,173}$=184.2, P<0.001, R^2=0.51).

		log Biomass quantity	log Biomass density	log Biomass moisture content	Leaf SA/V ratio	log Leaf EHoC §
Sustainability (flaming time)	Estimate (95% CI)	0.434 (0.350 – 0.517)	-0.614 (-2.162 – 0.889)	1.036 (-0.688 – 2.753)	-0.050 (-0.162 – 0.055)	-0.012 (-0.023 – 0.001)
	P value	**<0.001**	0.443	0.252	0.363	0.060
Combustibility (maximum combustion rate)	Estimate (95% CI)	0.035 (0.028 – 0.041)	0.149 (0.021 – 0.277)	-0.108 (-0.250 – 0.027)	0.105e-2 (-0.858e-2) – 0.012)	-0.580e-4 (-0.101e-2 – 0.103e-2)
	P value	**<0.001**	**0.024**	0.116	0.910	0.826
Ignitability (predicted spread rate)	Estimate (95% CI)	2.002 (0.951 – 3.015)	-0.061 (-0.094 – - 0.033)	-0.034 (-0.044 – -0.025)	0.128e-2 (0.789e3 – 0.169e-2)	0.121e-3 (-0.993e-4 – 0.360e-3)
	P value	**<0.001**	**<0.001**	**<0.001**	**<0.001**	0.309

§Species mean values

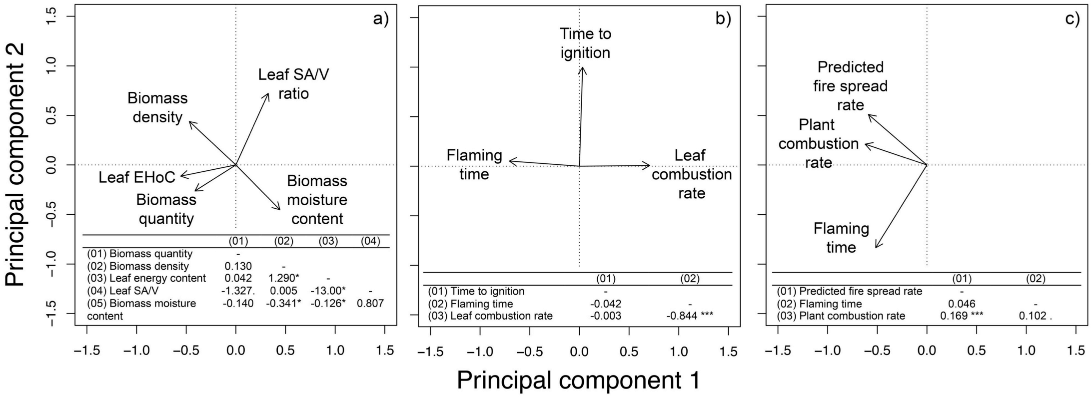
TRAIT COVARIANCE

PCA and linear regressions were used to explore patterns of covariance among the plant and flammability trait variables, with the latter being split into leaf-scale and plant-scale traits (Fig. 3). For the plant traits, the first two principal components accounted for 67.6% of the total variance. The first axis related to the chemical properties of biomass and how it is arranged spatially (leaf EHoC, biomass moisture content and density had the highest axis loadings). Leaf SA/V ratio loaded most heavily on the second axis, followed by biomass moisture content and density. Only biomass quantity did not fall as clearly on the first two principal components, which we believe is due to the high variation within the data (CV = 89.0%). For the leaf-scale flammability traits, the first two principal components accounted for 95.1% of the total variance. Leaf flaming time and combustion rate were negatively correlated ($P<0.001$), and fell in opposing directions on the first PCA axis (Fig. 3), which reflects how combustion rate was derived from flaming time. Time to ignition was unrelated to flaming time and combustion rate, and was orthogonal to both in the PCA (Fig. 3). For plant-scale flammability traits, 71.8% of total variance is accounted for by the first two principal components. Traits did not separate on the first axis, but did on the second axis which related to burning intensity. High rates of plant combustion were associated with rapid predicted fire spread rates ($P<0.001$) and marginally with longer flaming times ($P=0.071$; Fig. 3).

The relationships between flammability traits measured at different scales were variable, with a significantly positive correlation found for ignitability (leaf time to ignition vs predicted rate of spread; $P=0.025$), but no significant correlation for combustibility (leaf-scale combustion rate vs plant-scale combustion rate; $P=0.29$).

INFLUENCE OF EVOLUTIONARY HISTORY ON FLAMMABILITY

Support for a phylogenetic signal was found for leaf-scale combustibility (Pagel's λ =0.99; $P=0.93$ for likelihood ratio test against $\lambda=1$; $P=0.037$ against $\lambda=0$) and sustainability (Pagel's λ =0.45; $P=0.67$ against $\lambda=1$; $P=0.011$ against $\lambda=0$), but not for the other flammability traits. Of the plant traits, there was a strong phylogenetic signal for leaf SA/V ratio (Pagel's λ =1.00; $P=1.00$ against $\lambda=1$; $P<0.001$ against $\lambda=0$), with closely related species tending to have similar values of leaf SA/V ratio. No phylogenetic signal was found in the other plant traits.



	(01)	(02)	(03)	(04)
(01) Biomass quantity	-			
(02) Biomass density	0.130	-		
(03) Leaf energy content	0.042	1.290*	-	
(04) Leaf SA/V	-1.327.	0.005	-13.00*	-
(05) Biomass moisture content	-0.140	-0.341*	-0.126*	0.807

	(01)	(02)
(01) Time to ignition	-	
(02) Flaming time	-0.042	-
(03) Leaf combustion rate	-0.003	-0.844 ***

	(01)	(02)
(01) Predicted fire spread rate	-	
(02) Flaming time	0.046	-
(03) Plant combustion rate	0.169 ***	0.102 .

Figure 3. Principal components analysis biplots of explanatory plant traits (a) and flammability traits at the leaf-scale (b) and plant-scale (c). The tables within each plot indicate the slope and significance of linear regressions between each pair of variables. Data for all traits were log-transformed to improve normality except leaf SA/V ratio. EHoC is the leaf effective heat of combustion. ., $P<0.1$; *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$.

Discussion

This large comparative study of grass flammability provides strong support for the hypothesis that grass species vary significantly in multiple components of flammability. This finding suggests that static classifications of grassy and herbaceous vegetation as homogenous fuels mask considerable inter-specific and community variation in flammability. Consequently, fire behaviour predictions based on such fuel models may lose accuracy when community composition is not accounted for.

A substantial proportion of variation in ignitability and combustibility (70% and 58% respectively) can be explained by a combination of the five plant traits measured here. For sustainability, a smaller proportion of variation was accounted for (37%), perhaps because this component is not only driven by plant traits, but is also directly influenced by combustibility. Additionally, some variation in sustainability could be accounted for by traits relating to leaf chemistry, such as nitrogen, phosphorus and tannin concentrations (Grootemaat et al., 2015), that were not measured in this study. Biomass quantity was the key trait influencing plant-scale flammability components, and also determined the influence of an individual plant on local fire characteristics. The importance of biomass quantity for combustibility, sustainability and fire spread rates in the field is illustrated by the elevated flammability of landscapes caused by the raised fuel load production of non-native grasses (Hughes, Vitousek & Tunison, 1991; D'Antonio & Vitousek, 1992; Rossiter et al*.,* 2003). Whilst making a relatively small contribution to flammability components once alight, biomass moisture content was key to ignitability, with higher moisture contents requiring more energy to dry and heat biomass to the point of ignition (Trollope, 1978; Gill & Moore, 1996; Alessio et al., 2008; Pluckinski & Anderson, 2008). By influencing ignitability, and therefore the likelihood of fire occurring in the first place, moisture content exerts a strong influence on vegetation flammability. Our finding of high inter-specific variation in EHoC (effective heat of combustion) also conflicts with the notion that grass energy content is an almost constant value (Trollope, 1984). However, EHoC contributed little to leaf-scale flammability components, supporting the idea that this intrinsic property is less important in determining flammability than fuel mass, structure and moisture content (Bond & van Wilgen, 1996). Despite this small importance overall, the EHoC marginally contributed to plant-scale flaming time.

The inconsistent relationships between components of flammability, and within flammability components measured at different scales, suggest that descriptions of flammability should incorporate all suitable components and should be taken at an appropriate scale. The mixed covariance between flammability components found here suggests that one cannot always be used as a proxy for the others. Therefore studies that consider one or even two components of flammability may mask the complexity of vegetation flammability (Anderson, 1970). Similar to the findings of Martin (2010), we find support for the importance of incorporating plant architecture into measurements of grass flammability. Inconsistencies between combustibility at the leaf- and plant-scale highlight that other factors (such as biomass quantity and density) are key determinants of combustibility at the plant scale. Bench scale measurements of flammability have been criticised as not being representative of flammability in the field (Fernandes & Cruz, 2012), and our findings emphasize the need for caution when extrapolating flammability traits between different scales. In comparison to leaf-scale studies, the flammability component values obtained here are more representative of flammability in the field because they are measured at the plant scale, and on field-state plants that are at the phenological stage most appropriate to fire occurrence.

The phylogenetic signal found in some flammability components (leaf-scale combustibility and sustainability) suggests that evolutionary history may partially explain patterns of grass flammability and the strong sorting of grass lineages across fire-frequency gradients (Uys, Bond & Everson, 2004; Visser et al., 2012; Forrestel et al., 2014) However, conclusions on phylogenetic signal derived from a small phylogeny (consisting 25 out of ~12,000 recognised grass species) must remain cautious due to low taxonomic coverage and statistical power (Boettiger, Coop & Ralph, 2012).

Through their flammability, plants may modify the fire regime they experience in order to increase their fitness in fire-prone environments (Schwilk, 2003). Resprouting grasses are likely to benefit from frequent fires that remove standing biomass and maintain an open canopy, because they are typically intolerant of shading (Everson et al., 1988; Bond, 2008). The grasses studied here showed high ignitability, combustibility and predicted fire-spread rates, when compared to woody vegetation fuels (e.g Pausas et al., 2012; Ganteaume et al*.,* 2013). Furthermore, grasses are able to regrow quickly after fire. This combination of high flammability and rapid regrowth drives a fire regime characterised by high fire frequency (Grigulis et al., 2005). Plant-scale combustion rate was marginally positively related to flaming time, with high biomass plants burning at a faster rate and for longer. This finding is in contrast with other studies (e.g. de Magalhães & Schwilk, 2012) that found a negative relationship between the two. It also does conflicts with the idea of high flammability providing resprouting plants protection against lethal temperatures (Gagnon et al*.,* 2010), as for grasses that have higher fuel loads, rapid combustion is not associated with lowered burning durations and a subsequent reduction in heat transfer to the soil and below-ground plant parts. The inter-specific variation in flammability components observed across a set of species that commonly coexist in the field further suggests a role for inter-specific competition in promoting flammability as an adaptive trait. Potentially, enhanced plant flammability can increase the mortality of neighbouring, less fire-tolerant individuals, and thereby reduce post-fire competition (Bond & Midgley, 1995). Furthermore, some evidence provides intriguing support for a link between high flammability and ecological success in fire-prone grassland species (Ripley et al., 2015). The influence of flammability at the species level on grassland community-level flammability has not been determined. However findings from other vegetation fuel types show that flammability tends to be driven by the most flammable species of a community, such that fuel loads are non-additive (de Magalhães & Schwilk, 2012; van Altena et al., 2012). The knowledge gained in this study can be used in further work to determine whether high flammability is an adaptation to life in frequently burnt environments for grasses, and has thus been a fundamental trait in grass evolution. In addition, the knowledge of inter-specific variation in grass flammability obtained here can lead to a better understanding of wildfire behaviour, particularly in grassland ecosystems. This could potentially contribute to an improvement of global carbon modelling, and lead to new insights about ecosystem feedback to fire.

Acknowledgements

Research support was provided by a Natural Environment Research Council studentship to KJS, Royal Society University Research Fellowship URF120119 to PAC and URF120016 to GHT, and a European Research Council Starter Grant ERC-2013-StG-335891-ECOFLAM to CMB. We thank Tony Palmer, Claire Adams and Nosipho Plaatjie for their support in the lab and field, Albert Phillimore for assistance with the MCMCglmm analyses, and James Simpson for his help with graphics. We also thank Hans Cornelissen and two anonymous reviewers for their constructive comments on the manuscript.

Data Accessibility

- Trait data: Species average values available in Supporting Information; raw data available in DRYAD entry doi: 10.5061/dryad.2c506
- Sequence data: GenBank accession numbers available as online supporting information
- Phylogeny: Nexus file available in DRYAD entry doi: 10.5061/dryad.2c506
- MCMCglmm R Script: Available in DRYAD entry doi: 10.5061/dryad.2c506

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Supporting Information

Figures and tables

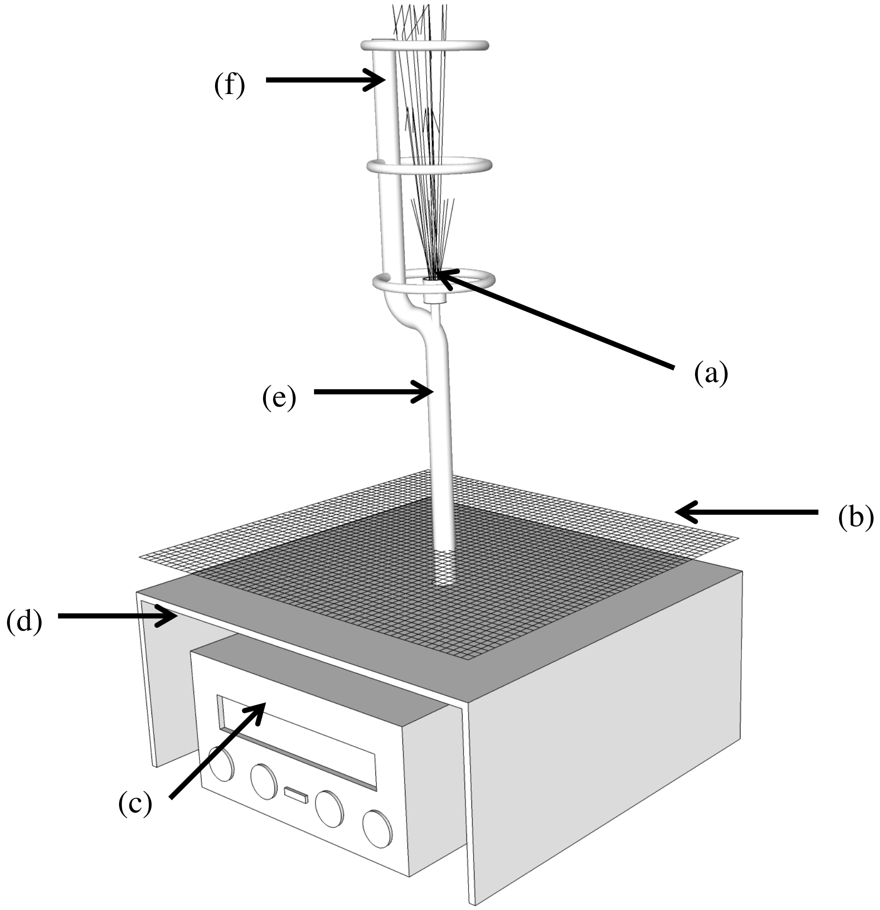

Figure S1. Schematic drawing of the set-up used to measure plant-scale combustibility and sustainability. (a) Clamp for holding grass material; (b) mesh platform for catching falling biomass; (c) four point balance; (d) wooden box (to protect the scales from heat damage); (e) stand; (f) metal wire cage (to prevent biomass from falling off). Samples were ignited by directing a bunsen burner flame to the side of the base of the clump at a 45° angle and a 5cm distance for 3 seconds maximum (less if ignition happened earlier).

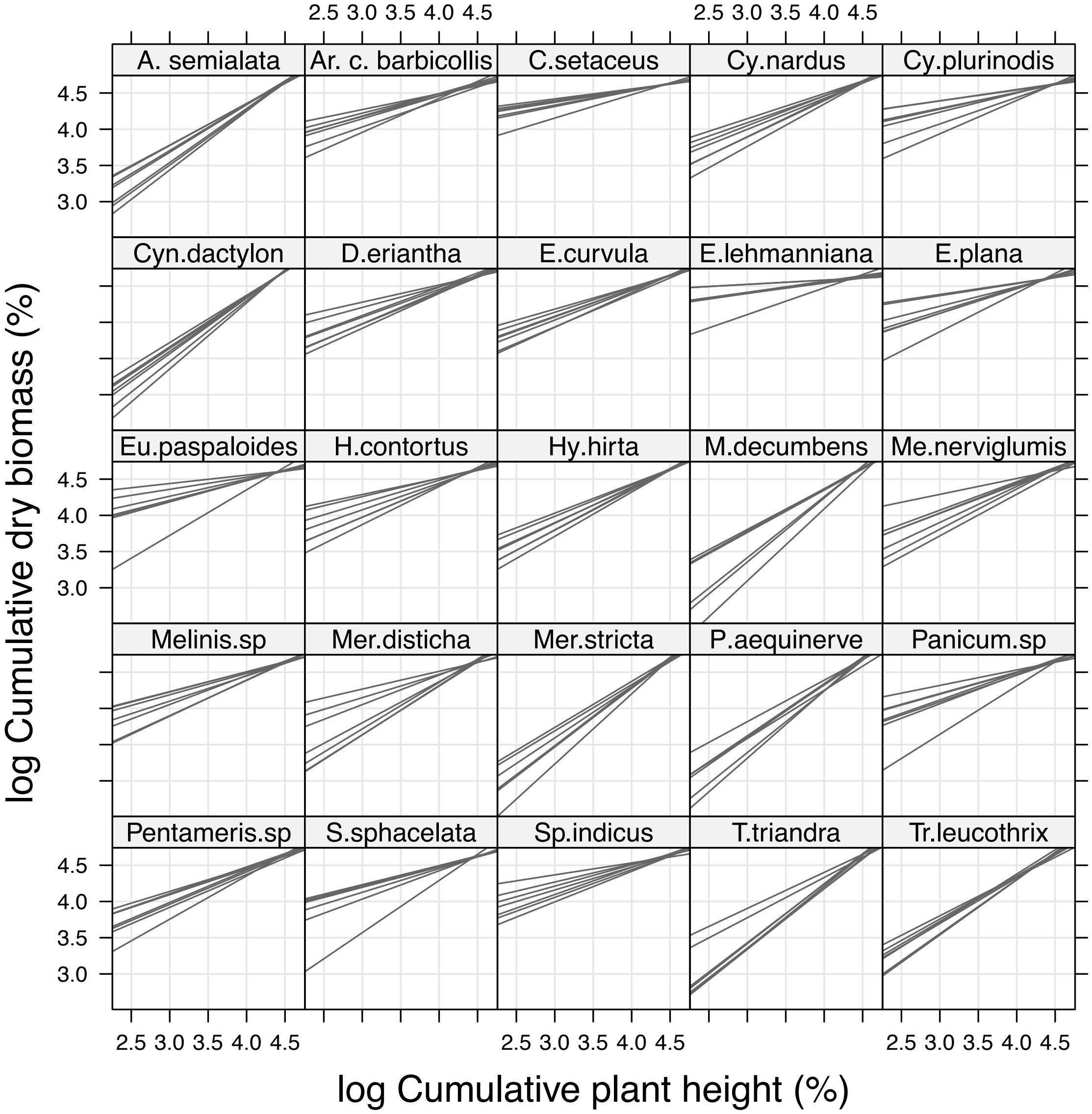

Figure S2. Cumulative dry biomass over vertical plant height for 25 grass species. Each line represents an individual (n=7 per species). Both axes were log-transformed in order to fit a linear model to the data for each individual. Species name abbreviations: A. =*Alloteropsis*; Ar.c.=*Aristida congesta*; C.=*Cenchrus*; Cy.=*Cymbopogon*; Cyn.=*Cynodon*; D.=*Digitaria*; E.=*Eragrostis*; Eu.=*Eustachys*; M.=*Melica*; Me.=*Melinis*; Mer.=*Merxmuellera*; P.=*Panicum*; S.=*Setaria*; Sp.=*Sporobolus*; T.=*Themeda*; Tr.=*Tristachya*.

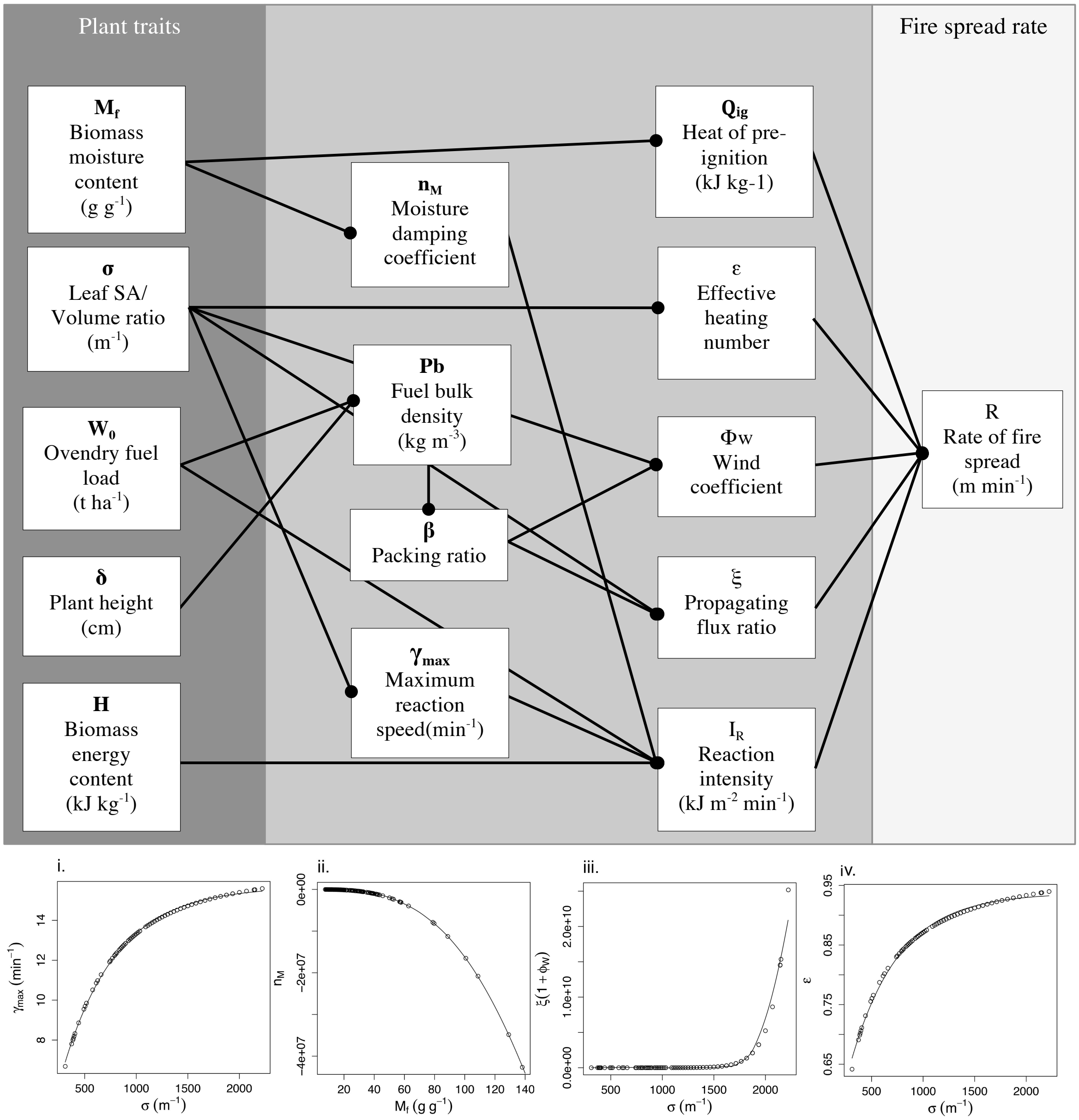

Figure S3. The influence of plant traits on components of Rothermel's (1972) fire spread rate model. The flow chart demonstrates how the input plant traits determine the fire spread components. Graphs *i-iv* show relationships between plant traits and components of the fire spread equation. Points reflect trait values obtained from individual plants, and the lines are the modelled relationships between plant trait and fire spread component.

i. The relationship between plant SA/V ratio (σ) and maximum reaction velocity ($γ_{max}$)

ii. The relationship between biomass moisture content (M_f) and the moisture damping coefficient (n_M)

iii. The relationship between plant SA/V ratio (σ) and the propagating flux ratio (ξ, the proportion of reaction intensity reaching adjacent fuel), taking into account the influence of wind (1 + Φw). Line reflects the relationship between σ and ξ (1 + Φw) when values of δ (fuel height) and W_0 (oven dry fuel load) are held constant.

iv. The relationship between plant SA /V ratio (σ) and the effective heating number (ε; the proportion of fuel that is raised to ignition temperature).

Equations

$$n_M = 1 - 2.59\left(\frac{M_f}{M_x}\right) + \left(\frac{M_f}{M_x}\right)^2 - 3.52\left(\frac{M_f}{M_x}\right)^3$$

Where: M_x = fuel moisture content of extinction (= 0.6)

$$Pb = \frac{1}{\delta}W_0$$

$$\beta = \frac{Pb}{Pp}$$

Where Pp = oven dry particle density (kg m^{-3})

$$\gamma_{max} = \sigma^{1.5}(495 + 0.0594\sigma^{1.5})^{-1}$$

$$Q_{ig} = 250 + 1116M_f$$

$$\varepsilon = \exp\left(\frac{-138}{\sigma}\right)$$

$$\Phi_W = CU^B\left(\frac{\beta}{\beta_{op}}\right)^{-E}$$

Where: $C = 7.47 \exp(-0.133\sigma^{0.55})$; $B = 0.02526\sigma^{0.54}$; $E = 0.715 \exp(-3.59 \times 10^{-4}\sigma)$;

U = midflame wind speed ; $\beta op = 3.348\sigma^{-0.8189}$

$$\xi = (192 + 0.2595\sigma)^{-1}\exp\,[(0.792 + 0.681\sigma^{0.5})(\beta + 0.1)$$

$$I_R = \gamma max W_n H n_M n_s$$

Where: n_s = mineral damping coefficient (= 0.41739)

$$R = (I_R \xi (1 + \Phi_W + \Phi_S))/(P b \mathcal{E} Q_{ig})$$

Where: Φs = slope coefficient (= 0)

Table S1. Climate data from plant collection sites including wind speed, rainfall and temperature. For sites 1 and 2, average wind speed, rainfall and temperature values for July (i.e. the month in which collection occurred) were provided by the South African Weather Service (weather station 0056917 8; www.weathersa.co.za) for years spanning 1993 to 2014. Data for site 3 was provided by the Agricultural Research Council (www.arc.agric.za) and is for years 2012 and 2013

Site name	Coordinates (lat, lon)	Average July daily wind speed (m s^{-1})	Total July daily rain (mm)	Minimum July daily temperature (°C)	Maximum July daily temperature (°C)
Site 1	-33.323, 26.533	3.5	27.8	6.8	18.5
Site 2	-33.278, 26.489	3.5	27.8	6.8	18.6
Site 3	-32.965, 26.082	2.2	0.1	7.6	19.6

Table S2. Grass (Poaceae) species used in this study, collection site and GenBank accession number.

Species	Subfamily	Site	GenBank accession number
Alloteropsis semialata (R.Br.) subsp. *eckloniana*	Panicoideae (Paniceae)	1	(See Grass Phylogeny Working Group II 2012)
Aristida congesta Roemer & Schult subsp. *barbicollis* (Trin & Rupr.) De Winter	Aristidoideae	3	KP860326
Cenchrus setaceus (Forssk.) Morrone	Panicoideae (Paniceae)	2	(See Grass Phylogeny Working Group II 2012)
Cymbopogon plurinodis (K. Schum.) C.E. Hubb.	Panicoideae (Andropogonea)	3	KP860328
Cymbopogon nardus (L.) Rendle.	Panicoideae (Andropogonea)	1	KP860327
Cynodon dactylon (L.) Pers.	Chloridoideae	1	(See Grass Phylogeny Working Group II 2012)
Digitaria eriantha Steud.	Panicoideae (Paniceae)	2	(See Grass Phylogeny Working Group II 2012)
Eragrostis curvula (Schrad.)	Chloridoideae	1	(See Grass Phylogeny Working Group II 2012)
Eragrostis lehmanniana Nees	Chloridoideae	3	KP860329
Eragrostis plana Nees	Chloridoideae	2	KP860330
Eustachys paspaloides (Vahl) Lanza & Mattei	Chloridoideae	3	KP860331
Heteropogon contortus (L.)	Panicoideae (Andropogonea)	1	(See Grass Phylogeny Working Group II 2012)
Hyparrhenia hirta (L.) Stapf.	Panicoideae (Andropogonea)	2	(See Grass Phylogeny Working Group II 2012)

Species	Subfamily	Site	GenBank accession number
Melica racemosa Thunb.	Pooideae	2	KP860332
Melinis sp.	Panicoideae (Paniceae)	1	KP860335
Melinis nerviglumis (Franch.) Zizka	Panicoideae (Paniceae)	2	KP860333
Merxmuellera disticha (Nees) Conert	Danthonioideae	2	(See Grass Phylogeny Working Group II 2012)
Merxmuellera stricta (Schrad.) Conert	Danthonioideae	1	(See Grass Phylogeny Working Group II 2012)
Panicum aequinerve Nees	Panicoideae (Paniceae)	1	(See Grass Phylogeny Working Group II 2012)
Panicum sp.	Panicoideae (Paniceae)	1	KP860334
Pentameris sp.	Danthonioideae	1	KP860336
Setaria sphacelata (Schumach.) Stapf & C.E. Hubb. Ex. M.B.Moss	Panicoideae (Paniceae)	3	(See Grass Phylogeny Working Group II 2012)
Sporobolus indicus (L.) R.Br.	Chloridoideae	2	(See Grass Phylogeny Working Group II 2012)
Sporobolus indicus (L.) R.Br.	Chloridoideae	2	(See Grass Phylogeny Working Group II 2012)
Sporobolus indicus (L.) R.Br.	Chloridoideae	2	(See Grass Phylogeny Working Group II 2012)

Table S3. Plant traits values input to obtain forward rate of fire spread (m min^{-1}). Spread rate values were calculated for each individual by creating surface fire spread models (Rothermel, 1972) based on the plant traits of each individual. The models assumed uniform weather, topography and fuel for the time duration. Biomass density could not be input directly but was calculated as fuel load divided by plant height. All individuals were considered as '1-hour fuels'

Model parameter	Method of calculation
Fuel load (t ha^{-1})	Dry total plant biomass and plant width values were used to calculate a value of biomass per unit area for each individual. Plant width (the maximum spread of photosynthetic material parallel to the soil level) was averaged per species and converted into an area (using area=π(width/2)^2), and the total fresh plant biomass was divided by this. Plant width values were standardised at the species level as small intra-specific variation in this trait had considerable impacts on individual fuel load values.
Fuel SA/ Volume ratio (m^2/m^3)	Leaf SA/V values.
Fuel bed depth (cm)	Plant height values.
Dead fuel moisture of extinction (%)	This parameter is the characteristic moisture content of a dead fuel above which a steady rate of fire spread is not possible. A value of 60% was used for all models as this is the maximum possible for 1-hour fuels.
Fuel heat content (kJ kg^{-1})	Species average effective heat of combustion (EHoC) values were used. As these values fell below the range accepted by the model, all were multiplied by the lowest factor necessary (1.93) to put them into the acceptable range of values. Preliminary tests showed a linear relationship between rate of spread and fuel heat content so multiplying all values by a common factor was deemed acceptable.
Fuel moisture	Biomass moisture content values

content (%)	
Midflame wind speed (km h^{-1})	An average value of July daily wind speed for the three collection sites was used in all models (3 m s^{-1} = 3.6 km h^{-1}).
Site slope (%)	A value of 0 was used for all models.

Table S4. Flammability parameter values (mean ± standard error) for 25 grass species, and the influence of species on these values. The final two rows indicate F values obtained from ANOVA and the degrees of freedom (DF) for all flammability parameters except plant combustion rate. The data for this trait precluded the use of statistical testing because there was only one value per species. ***, $P<0.001$.

Species	Time to ignition (s)	Flaming time (s)	Leaf combustion rate (mg s^{-1})	Plant combustion rate (mg s^{-1})	Fire spread rate (m min^{-1})
Alloteropsis semialata subsp. *eckloniana*	2.19±0.22	7.13±3.19	29.0±2.2	94.0±22.0	1.55± 0.123
Aristida congesta subsp. *barbicollis*	1.75±0.44	4.36±1.65	49.0±5.0	97.0±16.0	2.48± 0.252
Cenchrus setaceus	3.96±1.07	6.02±3.01	39.0±10.1	74.0±9.0	0.00±0.000
Cymbopogon plurinodis	1.93±0.16	6.32±2.39	33.0±2.2	67.0±12.0	0.73±0.173
Cymbopogon nardus	1.23±0.11	5.51±2.08	37.0±2.1	86.0±18.0	5.34±0.310
Cynodon dactylon	1.34±0.18	6.26±2.36	32.0±0.6	121.0±25.0	3.36±0.218
Digitaria eriantha	1.10±0.07	5.31±2.01	41.0±4.4	122.0±28.0	2.25±0.482
Eragrostis curvula	1.40±0.14	6.17±2.33	34.0±2.6	70.0±12.0	2.94±0.406
Eragrostis lehmanniana	1.62±0.15	6.34±2.40	32.0±2.0	71.0±13.0	2.76±0.190
Eragrostis plana	2.53±0.53	7.68±2.90	27.0±1.9	81.0±14.0	0.53±0.100
Eustachys paspaloides	1.56±0.19	5.81±2.20	38.0±5.1	64.0±19.0	0.69±0.158
Heteropogon	1.93±0.20	4.73±1.79	43.0±2.8	132.0±57.0	1.80±0.107

contortus					
Hyparrhenia hirta	1.02±0.08	4.95±1.87	43.0±3.4	124.0±51.0	6.37±0.538
Melica racemosa	1.47±0.14	5.58±2.11	37.0±2.1	82.0±10.0	1.72±0.210
Melinis nerviglumis	1.21±0.13	5.91±2.24	35.0±2.4	137.0±20.0	2.04±0.148
Melinis sp.	1.32±0.12	6.41±2.42	32.0±1.5	147.0±23.0	1.08±0.154
Merxmuellera disticha	2.13±0.23	6.95±2.63	29.0±1.3	111.0±36.0	0.237±0.091
Merxmuellera stricta	1.82±0.26	6.55±2.48	31.0±1.7	113.0±22.0	0.42±0.068
Panicum aequinerve	1.20±0.10	5.23±1.98	38.0±1.1	94.0±25.0	2.03±0.145
Panicum sp.	1.90±0.23	5.62±2.13	37.0±3.5	93.0±22.0	1.32±0.169
Pentameris sp.	1.46±0.17	5.47±2.07	37.0±2.2	86.0±24.0	1.09±0.164
Setaria sphacelata	1.38±0.12	4.79±1.81	44.0±4.3	117.0±15.0	0.69±0.133
Sporobolus indicus	1.07±0.15	6.47±2.64	32.0±2.4	73.0±10.0	0.57±0.058
Themeda triandra	1.13±0.14	5.10±1.93	42.0±4.4	163.0±24.0	2.58 ±0.181
Tristachya leucothrix	1.58±0.06	5.71±2.16	36.0±2.5	106.0±29.0	2.71±0.324
F value	5.02 ***	3.02 ***	2.97 ***	N/A	42.42 ***
DF	24, 144	24, 144	24, 144	N/A	24, 150

Table S5. The influence of species on plant trait values (mean ± standard error) of 25 grass species as indicated by F values obtained from ANOVA. ***, P<0.001.

Species	Biomass quantity (g)	Biomass density (g cm^{-1})	Biomass moisture content (g g^{-1})	Leaf SA/ volume ratio	Leaf EHoC (kJ g^{-1})
Alloteropsis semialata subsp. eckloniana	32.29±5.78	0.690±0.020	0.283±0.030	1402.2±40.2	9.2±0.5
Aristida congesta subsp. barbicollis	0.55±0.04	0.304±0.023	0.121±0.009	1709.2±75.8	8.6±0.1
Cenchrus setaceus	15.55±1.69	0.194±0.015	1.034±0.088	1118.3±58.2	7.3±0.2
Cymbopogon nardus	22.52±1.56	0.452±0.023	0.113±0.007	1489.2±49.6	9.9±0.6
Cymbopogon plurinodis	1.77±0.25	0.267±0.024	0.386±0.047	1378.1±58.0	8.7±0.1
Cynodon dactylon	3.81±0.46	0.762±0.040	0.104±0.005	1729.6±62.7	11.4±0.1
Digitaria eriantha	6.23±0.53	0.386±0.024	0.296±0.052	1869.9±76.6	8.7±0.2
Eragrostis curvula	18.05±2.31	0.391±0.004	0.113±0.018	1135.5±60.6	8.9±0.3
Eragrostis lehmanniana	7.84±0.51	0.170±0.021	0.135±0.013	1292.8±71.7	8.8±0.1
Eragrostis plana	1.51±0.20	0.309±0.028	0.379±0.039	989.6±82.1	10.1±0.2
Eustachys paspaloides	1.55±0.41	0.259±0.032	0.356±0.034	1599.2±60.3	7.7±0.6
Heteropogon contortus	10.52±1.73	0.378±0.027	0.242±0.020	1579.3±93.3	11.0±0.3
Hyparrhenia hirta	16.85±3.82	0.500±0.023	0.158±0.019	1743.9±80.7	8.2±0.4

Melica racemosa	5.25±1.49	0.733±0.046	0.182±0.033	938.7±27.5	10.3±0.5
Melinis nerviglumis	5.59±0.50	0.436±0.028	0.137±0.008	1045.9±52.8	10.1±0.5
Melinis sp.	9.15±1.11	0.362±0.027	0.127±0.011	961.2±43.0	12.4±0.2
Merxmuellera disticha	27.75±6.24	0.479±0.037	0.115±0.010	386.0±14.9	10.9±0.3
Merxmuellera stricta	22.31±2.55	0.792±0.035	0.110±0.012	564.6±22.3	10.7±0.6
Panicum aequinerve	3.06±0.34	0.735±0.034	0.260±0.024	1864.3±82.3	9.6±0.3
Panicum sp	6.67±0.69	0.369±0.031	0.297±0.041	1058.7±99.8	9.1±0.5
Pentameris sp.	5.89±0.68	0.435±0.026	0.158±0.008	871.2±45.7	11.5±0.6
Setaria sphacelata	1.21±0.18	0.362±0.039	0.386±0.034	1597.1±78.7	8.0±0.1
Sporobolus indicus	4.50±0.54	0.306±0.023	0.411±0.030	906.1±70.2	11.2±0.2
Themeda triandra	8.37±1.34	0.753±0.034	0.167±0.010	2078.2±54.7	8.8±0.0
Tristachya leucothrix	28.07±4.88	0.651±0.027	0.165±0.013	1065.0±58.8	13.6±0.4
$F_{24,150}$ *value*	16.25 ***	18.09 ***	39.75 ***	44.38 ***	17.38 ***

Table S6. Results of analysis of variance (two-way ANOVA with interaction) of leaf scale flammability by species and state (fresh or dry). P values in bold are significant at P=0.05. DF= degrees of freedom

Flammability trait	Source	Df	F	p
Time to ignition (s)	State	1	52.42	**<0.001**
	Species	24	5.930	**<0.001**
	State*Species	24	3.650	**<0.001**
Flaming time (s)	State	1	6.147	**0.014**
	Species	24	6.894	**<0.001**
	State*Species	24	0.641	0.904
Combustion rate ($g\ s^{-1}$)	State	1	7.584	**0.006**
	Species	24	7.289	**<0.001**
	State*Species	24	0.648	0.919

Table S7. Mean plant trait values of the three collection sites. Traits that were significantly influenced by site (One way ANOVA; P<0.05) were subjected to a post hoc pairwise test (Tukey HSD). Superscripted letters reflect the significance of pairwise comparisons with different letters signifying significant differences (P<0.05). SD = Standard deviation, n = sample size (number of species)

		Mean site values ± SD				
Site	n	Average leaf EHoC (kJ g^{-1})	Average leaf SA/Volume ratio	Average aboveground dry biomass (g)	Average Biomass water content (g g^{-1})	Average vertical biomass distribution (g cm^{-1})
1	12	10.31 ± 1.38^{a}	13.24±4.43	13.93±10.22	0.179±0.071	0.566 ± 0.183^{a}
2	8	9.87 ± 1.71^{ab}	11.14±4.80	10.85±8.68	0.338±0.303	0.420 ± 0.161^{ab}
3	5	8.34 ± 0.49^{b}	15.15±1.73	2.58±2.97	0.277±0.137	0.277 ± 0.078^{b}

Chapter 3

Chapter 3

Flammability and post-fire regrowth are linked in savanna grasses

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Table of contents

Figures and tables

Statement of contributions

KJS designed the study with input from BSR, GHT and CPO. KJS generated the data with assistance from BSR and JCDT (field measurements) and PAC (phylogeny). KJS analysed the data and wrote the chapter with all authors contributing critically to drafts.

Flammability and post-fire regrowth are linked in savanna grasses

Abstract

1. For grasses of fire-prone grasslands and savannas, which experience and fuel the most frequent fire regimes on Earth, enhanced flammability may provide fitness benefits. Heat-stimulated recruitment, reduced shading from the efficient removal of dead standing biomass, and the protection of basal meristems through short flaming times are all potential benefits of high flammability to these species. In addition, by burning off neighbours, high flammability may increase the space and resources available to resprouting individuals, but only if associated with rapid regrowth after fire. However, links between flammability and post-fire regrowth in recurrently-burnt grasses have not been tested.

2. Here we investigate the relationship between flammability and regrowth traits in 19 species of fire-prone South African grasslands. Structural traits known to influence grass flammability were measured on plants in the field before they were burnt *in situ* using small-scale fires during the natural fire season. Regrowth was then tracked for several weeks after the fire. The relationships between flammability and regrowth were established whilst taking into account evolutionary history by building a phylogeny of the study species.

3. Post-fire regrowth showed a significant positive relationship with fuel load, a key predictor of grass combustion rate and flaming duration, but was unrelated to fuel moisture content, a trait important to ignitability. After accounting for shared evolutionary history, plants with a higher fuel load regrew more biomass ($P<0.001$) and at a faster rate ($P<0.001$). Considerable variation across these coexisting grass species was seen in flammability (35-fold variation) and regrowth traits (5-fold variation).

4. Our finding of a positive link between flammability and post-fire success in fire-prone grasses adds to the growing body of evidence that enhanced flammability may be beneficial in fire-prone environments. Grass species may be altering local fire conditions in order to optimise their fitness, through increasing their resprouting success. However, the fitness benefits of high flammability cannot be determined here as the traits studied here are putatively adaptive. The significant interspecific variation in flammability and regrowth suggests that changes in savanna or grassland community composition could alter fire behaviour through fire frequency and intensity.

Key-words: Biomass quantity, Determinants of plant community diversity and structure, Fire regime, Fuel load, Functional traits, Phylogeny, Poaceae, Resprouting.

Introduction

Flammability, or the propensity to burn, is a complex plant trait that may be adaptive in recurrently burnt environments (He et al., 2012; Pausas et al., 2012; Moreira et al., 2014). By influencing local fire conditions through their flammability, plants can maintain favourable conditions and create spatio-temporal opportunities to regenerate or recruit after fire. For grasses of fire-prone savannas and grasslands that experience and fuel the most frequent fires on Earth (Mouillot & Field, 2005), being highly flammable is likely beneficial. However empirical evidence of the fitness benefits of flammability to these species is currently lacking.

Plant flammability is highly variable, with considerable differences seen across and within species in all flammability components (ignitability, combustibility and sustainability; Anderson 1970). The physical properties of plants influence all components of flammability, and largely relate to the amount, density, surface area and moisture content of fuels (Scarff & Westoby, 2006; Murray, Hardstaff & Phillips, 2013; Grootemaat et al., 2015; Simpson et al., 2016). In addition, chemical traits, such as the energy content or the presence of high levels of flammable compounds, may be important to the flammability of particular vegetation types (Ormeño et al., 2009; Pausas et al., 2016). Differences in these traits generates variation in flammability, which in turn drives variation in fire behaviour. Vegetation flammability is a key determinant of local fire regime, and changes in flammability (via changes in community composition) have repeatedly caused temporal (e.g. Belcher et al., 2010) and spatial (e.g. Rossiter et al., 2003; Brooks et al., 2004) alterations to the fire regime.

There is growing evidence that plants may engender favourable conditions under recurrent fire through their flammability (Schwilk & Ackerly, 2001; He et al., 2012; Pausas & Moreira, 2012), leading to the prediction that plants in fire-prone environments may follow a number of overarching 'flammability strategies' (Pausas, Keeley & Schwilk, 2017). Some plants, such as woody trees in flammable grasslands, possess traits that result in low flammability (e.g thick corky branches and large dense leaves that do not ignite; Dantas, Batalha & Pausas, 2013) and are thus little affected by the frequent fire in their habitat (the "non-flammable" strategy *sensu* Pausas, Keeley & Schwilk, 2017). Plants that do burn can increase survival or reproduction by following one of two strategies. Some plants fuel very hot fires, with long flaming durations, via traits that elevate combustibility and lower the fire spread rate (e.g. large, dense fuel loads and highly-branched architecture; the "hot-flammable" strategy *sensu* Pausas, Keeley & Schwilk, 2017). These plants are killed by the fire, but their offspring benefit from the increased availability of space and the resource-rich post-fire conditions. A possible mechanism underlying the evolution of the hot-flammable strategy is provided by the 'kill-thy-neighbour' hypothesis (Bond

& Midgley, 1995). This hypothesis states that traits driving hot-flammability could be favoured in individuals if they increase recruitment opportunities through the elevated mortality of nearby competitors. The hot flammable strategy must be linked to robust post-fire recruitment (Bond & Midgley, 1995; Kerr et al., 1999; Pausas & Moreira, 2012), and is therefore particularly relevant in species that possess fire-enhanced recruitment such as serotiny. Evidence for the fitness benefits of the hot-flammability strategy has been provided by a number of studies that show the correlated evolution of flammability enhancing traits and fire-stimulated recruitment (Keeley & Zedler, 1998; Schwilk & Ackerly, 2001; He et al., 2012), or selection for flammability-enhancing traits under frequent fire (Pausas & Moreira, 2012; Pausas et al., 2012; Moreira, Castellanos & Pausas, 2014). In the other flammable strategy (the "fast-flammable" strategy; Pausas, Keeley & Schwilk, 2017), plants burn in recurrent fire but survive and regenerate from protected meristems. They possess traits that make them ignite easily and burn intensely for a short time (e.g. large amounts of fine fuels with high surface area/volume ratio), generating fast fire spread and low burn temperatures. Such a strategy may increase fitness if the traits that drive fast-flammability reduce fire duration and the heat shock to meristems (Gagnon et al., 2010). An alternative mechanism for the evolution of flammability in fast-flammable, resprouting plants is provided by the 'kill-thy-neighbour' hypothesis (Bond & Midgley, 1995). Flammability can evolve in these species by increasing neighbour mortality, but is more likely to do this if flammability-enhancing traits have additional benefits, or are linked to traits that increase fitness after fire, such as rapid resprouting. However, evidence of the fitness benefits of fast-flammability remains limited.

Grasses of fire-prone grasslands and savannas fuel frequent, cool, fast-moving surface fires, and are expected to follow the 'fast-flammability' strategy. These grasses typically resprout from basal meristems that are protected from the heat of the fire (Allan et al., 2002; Overbeck & Pfadenhauer, 2007). Their traits that promote rapid combustion and spread, and low fire residence times, may therefore reduce heat-induced mortality (Gagnon et al., 2010). Fires can still cause high mortality in savanna grasses (Zimmerman et al., 2010), and open up opportunities for recruitment via seedlings (which may be heat-stimulated; Sarmiento, 1992; Baxter et al., 1994; Verboom, Stock & Linder, 2002; Ghebrehiwot et al., 2012) or clonal spread that are otherwise highly restricted in these habitats. Individuals that survive the fire benefit from the resource-rich conditions afterwards, however the quick recovery of grasses after fire means the reduction in competition is short-lived. Therefore, high flammability may benefit fire-prone grasses if it increases neighbour mortality, but this is more likely if linked to rapid post-fire regrowth. The recent identification of significant variation in all flammability components between coexisting grass species and the traits underlying that variation (Simpson et al., 2016) makes fire-prone grasses an ideal study system for uncovering the fitness benefits of being fast-flammable. Indeed, tantalising evidence of a link between flammability and post-fire success in savanna grasses comes from a recent study by Ripley and colleagues (2015), in which they found some evidence of a correlation between fuel load and rapid regrowth in a sample of savanna grass species.

However, more research is needed to uncover the fitness benefits of flammability in savanna grasses.

Here we investigate the relationship between flammability-enhancing traits and regrowth after fire in 19 species common in fire-prone South African grasslands. Structural traits known to influence grass flammability (aboveground biomass quantity and moisture content) were measured on plants in the field before they were burnt *in situ* in small-scale fires. Their regrowth was then tracked for several weeks after the fire. The relationships between flammability and regrowth were established whilst taking evolutionary history into account. We predicted that flammability would vary between these grass species (as found previously; Ripley et al., 2015; Simpson et al., 2016) and would be positively associated with post-fire regrowth. We predict that fast-flammability is a beneficial strategy for these species, but we cannot test this here as the plant traits investigated are only putatively adaptive to fire regimes.

Materials and Methods

PLANT SELECTION

Seven healthy, undamaged individuals from 19 Poaceae species, representing four grass subfamilies, were selected from two sites (one grassland and one grassy Karoo shrubland) in the Eastern Cape of South Africa (see Table S1 in Supporting Information for site details). At both sites the vegetation burns periodically but at different frequencies: the average fire return interval for the grassland site (Site 1) is 2.3 years (Tansey et al., 2007), whereas the drier Karoo site (Site 2) burns every 10-50 years (Stuart-Hill & Mantis, 1982). Two species (*Eragrostis curvula* and *Themeda triandra*) occurred and were sampled at both sites (n=7 for each site). A specimen of each species was identified and deposited at the Selmar Schonland Herbarium (Rhodes University).

FLAMMABILITY TRAIT MEASUREMENT

Two traits (fuel load and biomass moisture content) known to influence grass flammability were measured on each individual plant (Simpson et al., 2016). Approximately one quarter of the aboveground biomass of each individual was removed in order to measure these traits. A piece of wire bent at a 90° angle was used to separate a quarter of the aboveground biomass, which was then removed by clipping 1cm above the ground level. Clipped material was stored in sealed plastic bags and transported to the lab, where total fresh weight was measured using a four-point balance. After drying the biomass at 70ºC for a minimum of 48 hours, the fuel load was measured as total dry biomass (Simpson et al., 2016). Plant moisture content was calculated by expressing the difference between fresh and dry biomass relative to the dry biomass (Simpson et al., 2016).

CONTROLLED BURNS

On the same day that plant material was removed for measuring pre-fire traits, plants were burnt *in situ.* The experimental burns happened over a period of 6 days in 2015 for Site 1 (14.07.15 – 20.07.15) and on one day for Site 2 (15.07.16). This period coincides with the natural end of winter fire season and thus plants were in a phenological stage most relevant to burning. Burns were carried out on days that had a low wind speed and were preceded by at least two rain-free days.

The burns were carried out using the set-up shown in Figure S1. A steel barrel was first placed over the plant, such that the plant was central in the barrel. The barrel itself is a modified 45-gallon oil drum (85 cm tall and 61cm diameter) with the top and base removed. A spark arrestor was fitted into the top of the barrel, and wet hessian sacking was placed around the base to minimize risk of the fire spreading. The plant was ignited by inserting a lit blowtorch through a hole for ~3 seconds and then removing it. The fire was left until it went out naturally. After this point, the equipment was removed and the ground around the plant doused with water to ensure the fire was completely out. Any remaining plant material was removed to 1 cm above the ground level, both to ensure equivalence in the severity of damage across individuals, and to avoid confusion when measuring regrown plant material. In the vast majority of cases, very little material was cut off. Small cloches were put over each burned individual to protect regrowing plants from herbivory and late-season frosts, and to simulate springtime air temperatures. The cloches consisted of two ~70cm pieces of 5mm steel wire manipulated to form a four-legged metal frame. The legs were pushed into the ground around the burned plant and a transparent plastic bag was fitted over the frame.

The temperature within the cloches was recorded using duplicate or triplicate thermochron data loggers (i-buttons; model DS1922L) placed under multiple, randomly chosen plants at each of the sites. The thermocouples recorded air temperature at hourly intervals.

REGROWTH AFTER FIRE

After the controlled burns, plants were monitored regularly for signs of resprouting. Regrowth measurements commenced when resprouting biomass was visible for the majority of individuals (after 10 days for Site 1; after 12 days for Site 2).

Several regrowing leaves were randomly selected for each plant, and marked with a small ring of coloured wire around the leaf base. The number of leaves measured per plant ranged from four to seven. The length of the resprouted leaf was measured using digital callipers (accurate to 0.01 mm) 10-12 days after the burn and on a further three occasions, each being at least two days apart, and the final measurement were taken 18-27 days after the burn.

Regrowth rate depends upon air temperature, with high temperature values stimulating plant growth and low values reducing or even stopping plant growth. Therefore air temperature during the regrowth period was incorporated into the measurement of post-fire regrowth using the single triangulation method (Wilson & Barnett, 1983; McMaster & Wilhelm, 1997; Snyder et al., 1999). Daily minimum and maximum temperature values were extracted from the i-buttons and averaged for each site. The number of growing degree-days (GDD, °C-day) accumulated for each regrowth period was calculated using the following equations:

$$GDD = \left[\frac{T_{MAX}+T_{MIN}}{2}\right] - T_{BASE} \qquad (1)$$

$$GDD = \left[\frac{(T_{MAX} - T_{Base})^2}{2(T_{MAX} - T_{MIN})}\right] \qquad (2)$$

Where T_{MAX} is the daily maximum air temperature and T_{MIN} is the daily minimum air temperature. T_{BASE} is the base temperature for growth (i.e below which growth does not occur) and is related to temperature adaptation of species or cultivars. A value of 10°C was selected for T_{BASE} which represents an intermediate value for published temperate and tropical grass T_{BASE} values (Cooper & Tainton, 1968; McWilliams, 1978; Osborne et al., 2008; Steduto et al., 2009; Bartholomew, 2014; Moreno et al., 2014). Equation (2) was used to correct for when the average temperature (i.e. $[(T_{MAX} + T_{MIN})/2]$) was less than base temperature. This method is the most commonly used method for calculating GDD (McMaster & Wilhelm, 1997). A rate of leaf length regrowth was calculated for each individual by fitting linear models to the cumulative leaf length and GDD data.

In addition to the final growth measurement, all aboveground regrown biomass was clipped and weighed. To convert the rate of leaf length regrowth to a rate of leaf biomass regrowth, the lengths and dry masses (after 48 hours at 70ºC) of 3-6 leaves of each individual were measured. The relationship between leaf length and mass was determined for each species by fitting linear models to the data, with all models showing a good fit (mean R^2 value: 0.92; range: 0.76-0.98; Table S3 and Figure S4). The slope of these relationships allowed the species average rate of leaf length regrowth to be converted into a rate of leaf biomass regrowth. Regrowth rate was also calculated using a second method that takes account of plant size. Leaf relative growth rate (RGR) was determined by dividing the logged change in average leaf mass (calculated from the length) between the first and last regrowth measurements by cumulative growing degree days.

PHYLOGENETIC ANALYSIS

We constructed a phylogeny that was based on previously generated, published sequences of the plastid markers *trnKmatK*, *ndhF*, and *rbcL* (Grass Phylogeny Working Group, II 2012; see Table

S2 for GenBank accession number details). A time-calibrated tree was inferred with BEAST (Bayesian evolutionary analysis by sampling trees; Drummond & Rambaut, 2007), using the concatenated alignment. Two independent analyses were run for 10,000,000 generations each, using a GTR+G+I substitution model, a log-normal relaxed molecular clock, and a Yule process speciation prior. The monophyly of the PACMAD clade was enforced by adding *Briza maxima* and *Oryza nirvana* (both BEP clade) as the outgroup. The calibration prior for the age of the BEP-PACMAD crown was set to a normal distribution, with a mean of 51.2 and a standard deviation of 0.001 (mean based on Christin et al., 2014). Run convergence was verified using Tracer (Rambaut & Drummond, 2007; Tracer v1.6, available at http://beast.bio.ed.ac.uk/Tracer), and the burn-in period was set to 2,000,000. The trees sampled after burn-in from the two analyses were combined, and the median ages of nodes were mapped on the maximum credibility tree. Two species (*Poaceae* sp1 and *Poaceae* sp2) were excluded from the phylogenetic analysis because they could not be sequenced due to contamination of leaf samples. The relationships among the other species were extracted from the tree and used for comparative analyses.

STATISTICAL ANALYSIS

Statistical analyses were carried out in the R environment (R Core Team, 2017). Data were log-transformed if necessary to improve normality and meet model assumptions. Analysis of variance (ANOVA) was used to establish if traits differed significantly between species and sites.

The relationships between pre-fire flammability traits (fuel load, biomass moisture content) and regrowth traits (leaf regrowth rate, leaf RGR, total resprouted dry biomass) were determined whilst accounting for phylogeny using a MCMC generalized linear mixed model approach from the MCMCglmm R package (Hadfield, 2010). We fitted each flammability and regrowth trait combination as a bivariate normal response, and species as a random effect. Models were run for 500,000 iterations with a burn-in of 1,000 iterations, with a thinning interval of 500 and weakly-informative priors (V=diag(2), nu=0.002). The relationship between leaf length expansion and cumulative GDD across species was also established using the same methods. The fit of the models to data was established by fitting linear models between the observed flammability trait values and those predicted by the models.

Results

PRE-FIRE TRAITS

Species varied considerably in the measured pre-fire traits, with species having a significant influence on every trait (Table 1, Figure 1). Fuel load, the key determinant of grass combustibility and sustainability, differed by 35-fold among species (1.3g for *Poaceae* Sp1 vs. 47.9g for

Alloteropsis semialata subsp. *ecklonianа*). Biomass moisture content, an important factor in ignitability, was low for all species but still varied considerably (0.01 – 1.07 g g^{-1}). As these traits are known to influence flammability (Simpson et al., 2016), these results suggest that the coexisting species sampled here vary considerably in their flammability. Plants at Site 1 had significantly higher values for all of the pre-fire traits measured (ANOVA: $P<0.05$) than the drier Site 2 (e.g. average fuel load: 12.65g (Site 1) vs. 7.37g (Site 2); average moisture content: 0.44 g g^{-1} (Site 1) vs. 0.13 g g^{-1} (Site 2)).

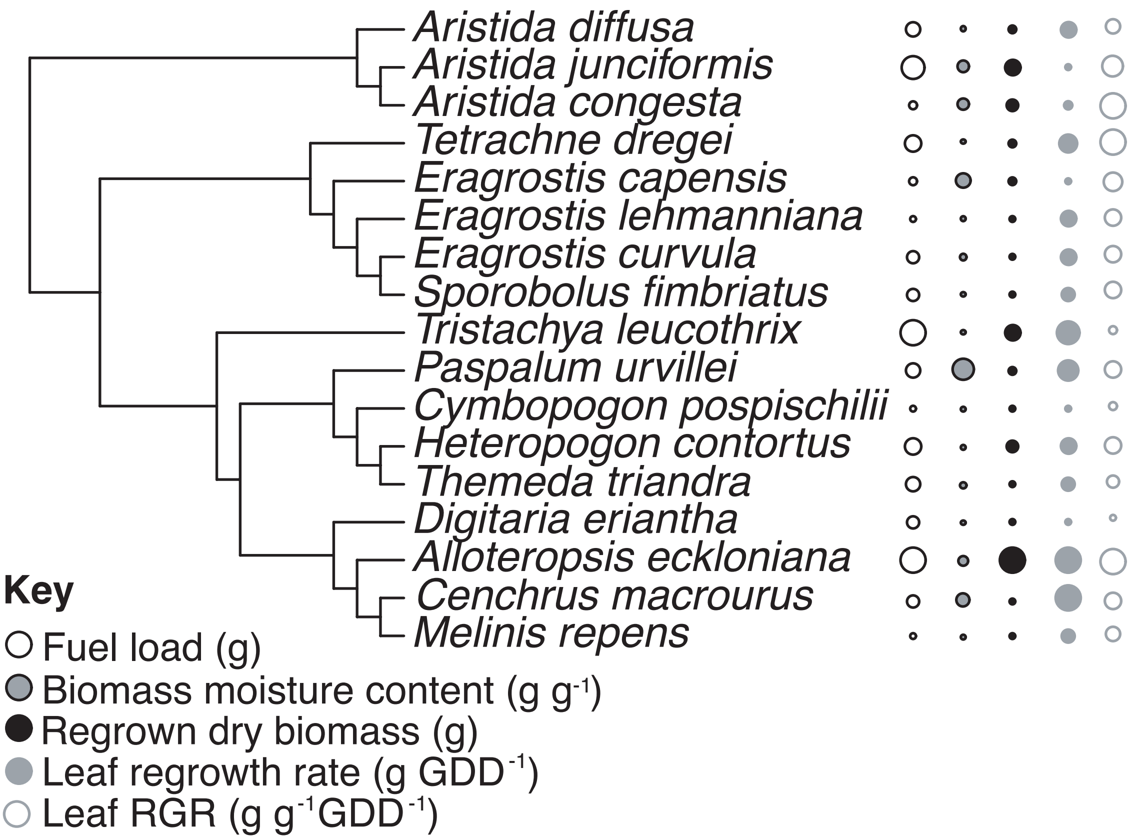

Figure 1. The evolutionary relationships between the study species and average values of flammability traits (fuel load, biomass moisture content) and regrowth traits (regrown dry biomass, leaf regrowth rate and leaf relative growth rate). Trait values are indicated by the size of the circles.

Table 1. Flammability and regrowth trait data for 19 Poaceae species. The final row indicates the influence of species on trait values (F values obtained from ANOVA). ***, P<0.001

		Flammability traits			**Regrowth traits**		
	Site	Total fresh plant mass (g)	Fuel load (g)	Biomass moisture content ($g\ g^{-1}$)	Regrown dry mass (g)	Regrowth rate ($mg\ GDD^{-1}$)	Leaf RGR ($mg\ mg^{-1}\ GDD^{-1}$)
Alloteropsis semialata	1	60.08	47.89	0.34	1.87	0.299	0.869
Aristida congesta	1	6.86	4.62	0.48	0.41	0.051	0.839
Aristida diffusa	2	9.34	8.67	0.12	0.35	0.138	0.345
Arisida junciformis	1	29.58	20.72	0.44	0.82	0.047	0.636
Cenchrus macrourus	1	6.84	4.73	0.50	0.09	0.276	0.414
Cymbopogon pospischilii	2	7.55	4.89	0.09	0.09	0.038	0.140
Digitaria eriantha	2	6.30	5.58	0.10	0.15	0.035	0.078
Eragrostis capensis	1	5.37	3.12	0.61	0.31	0.043	0.540
Eragrostis curvula	1	11.15	9.33	0.21	0.19	0.129	0.714
	2	5.91	4.76	0.24	0.19	0.138	0.240
Eragrostis lehmanniana	2	2.42	2.67	0.11	0.08	0.137	0.478
Heteropogon contortus	2	12.96	11.78	0.09	0.55	0.134	0.434
Melinis repens	1	2.18	1.87	0.16	0.01	0.088	0.319
Paspalum urvillei	1	17.08	8.34	1.06	0.29	0.197	0.460
Poaceae sp1	1	2.01	1.34	0.52	0.06	0.039	0.487
Poaceae sp2	1	14.95	10.21	0.45	0.40	0.133	0.776
Sporobolus fimbriatus	2	6.47	5.75	0.10	0.17	0.077	0.494
Tetrachne dregei	2	12.99	11.01	0.18	0.24	0.164	0.807
Themeda triandra	1	5.90	4.12	0.44	0.03	0.036	0.162
	2	12.51	11.21	0.12	0.26	0.159	0.417
Tristachya leucothrix	1	47.30	43.35	0.09	0.98	0.215	0.176
F value		12.63 ***	13.98 ***	6.91 ***	12.28 ***	12.85 ***	11.99 ***

POST-FIRE REGROWTH

Regrowth temperatures varied considerably within and across days, and between the two sites (Figure S2). Mean daily minimum and maximum temperatures for Site 1 were 5.5ºC (range: 1.0 to 9.2ºC) and 20.7ºC (9.7 to 28.8ºC) respectively; and -0.6ºC (-7.5 to 6.5ºC) and 23.8ºC (8.8 to 37.0ºC) for Site 2. Comparisons of i-button data with weather data (South African Weather Service 2016) showed that the cloches caused a small increase in temperature of approximately 2-4.5ºC. Temperature had a significant impact on the rate of leaf regrowth, with a significant positive relationship seen between leaf length increase and GDD when phylogeny is accounted for (Figure 3; slope= 0.437 (95% CI= 0.3923, 0.4747), $P<0.001$).

The majority of plants of all species had initiated resprouting 10-14 days after burning, although nine plants of six species failed to resprout at all within the 20 days following the burn (4/84 for Site 1; 6/63 for Site 2). Species showed considerable differences in the rate of leaf regrowth rate, with 5-fold variation seen in species average values, ranging from 0.035 mg GDD^{-1} (*Digitaria eriantha*) to 0.299 mg GDD^{-1} (*A. semialata* subsp. *eckloniana*; Figure 1 and Table 1). The other measure of regrowth rate, Leaf RGR, was significantly positively correlated to leaf regrowth rate (linear regression: $F_{1,136}=8.457$, $P=0.004$). Species average values of leaf RGR ranged from 0.078 mg GDD^{-1} (*D. eriantha*) to 0.869 mg GDD^{-1} (*A. semialata* subsp. *eckloniana*; Figure 1 and Table 1). In addition, the total amount of regrown biomass differed considerably between individuals, with species mean values ranging between 0.01g (*Melinis decumbens*) and 1.87g (*A. semialata* subsp. *eckloniana*). Both regrowth traits were significantly related to pre-fire fuel load across species, such that plants with a higher fuel load regrew more biomass ($P<0.001$) and at a faster rate ($P<0.001$; Table 2). However, neither regrowth trait was significantly associated with biomass moisture content (both $P>0.05$; Table 2).

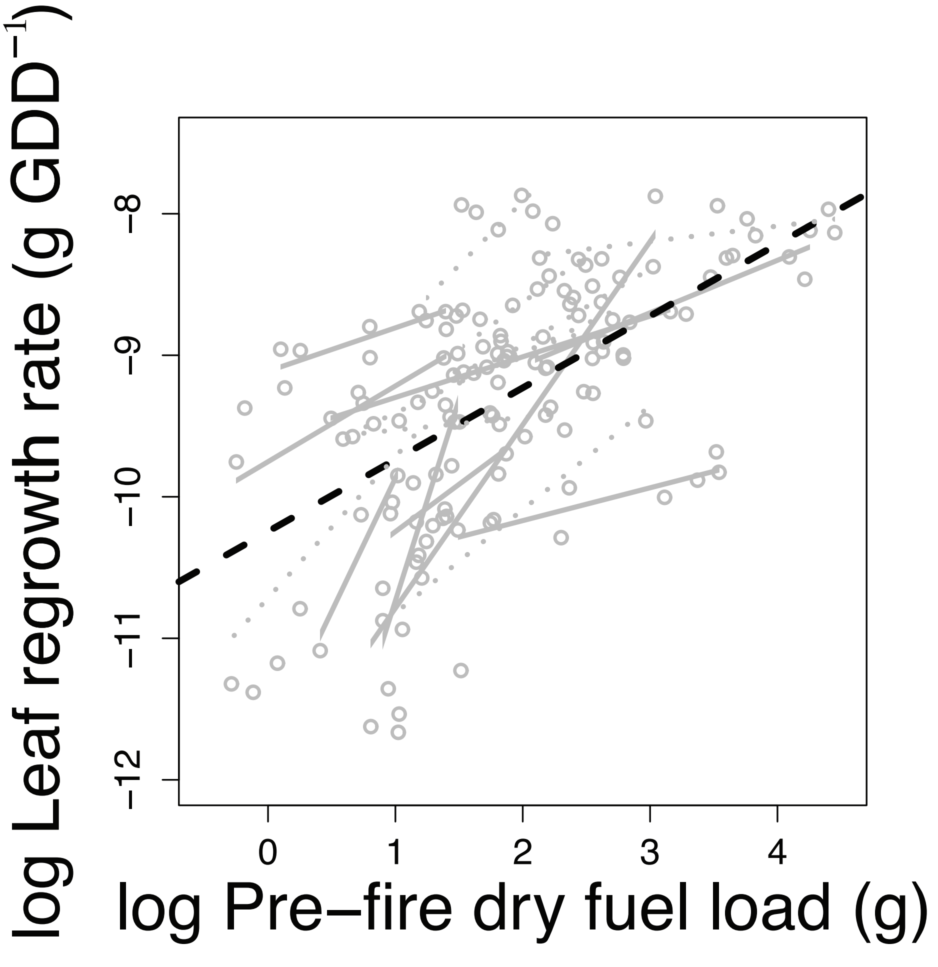

Figure 2. Relationships between fuel load and leaf regrowth rate across 19 grass species. The mean slopes of within-species relationships were significant for 11 out of the 19 species (grey lines; significant - solid line; non significant – dotted line). The across-species relationship (black dashed line) is highly significant ($P<0.001$) when phylogenetic associations are taken into account. Data points are shown as grey circles.

Table 2. The relationship between pre-fire flammability and post-fire regrowth traits as determined by MCMC phylogenetic generalized linear mixed models. Values represent posterior mean estimates of the slopes, the upper and lower 95% confidence intervals and P values (those in bold are significant at P=0.05). Fuel load, but not biomass moisture content, significantly predicted leaf regrowth rate ($F_{1,123}$=44.2, P<0.001, R^2=0.26), leaf relative growth rate ($F_{1,123}$=35.9, P<0.001, R^2=0.23) and regrown biomass ($F_{1,123}$=143.0, P<0.001, R^2=0.54).

			Pre-fire flammability	
			Biomass moisture content (g g^{-1})	Fuel load (g)
Post-fire regrowth	Leaf regrowth rate (g GDD^{-1})	Estimate (95% CI)	0.0003 (-0.0095 to 0.0090)	0.0025 (0.0015 to 0.0035)
		P value	0.956	**<0.001**
	Leaf relative growth rate (g g^{-1} GDD^{-1})	Estimate (95% CI)	-0.0966 (-0.8015 to 0.6007)	0.3023 (0.1608 to 0.4541)
		P value	0.774	**<0.001**
	Total dry regrown biomass (g)	Estimate (95% CI)	-0.0019 (-0.2436 to 0.2558)	0.0224 (0.0157 to 0.0283)
		P value	0.968	**<0.001**

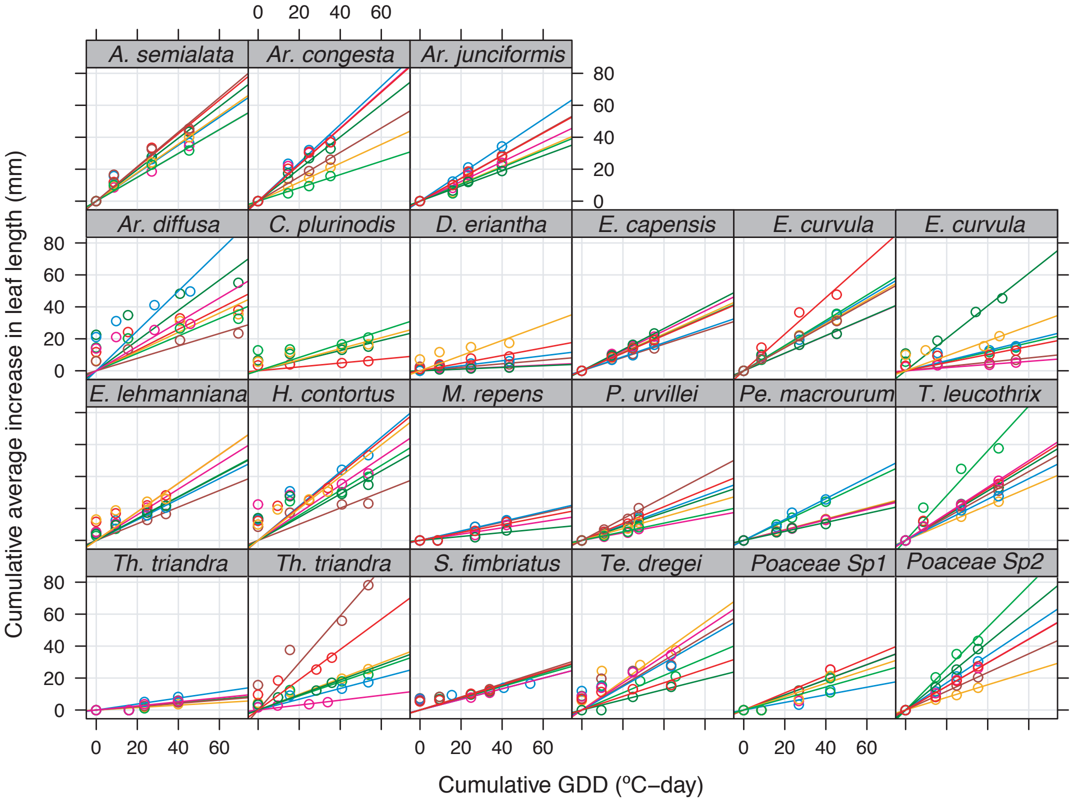

Figure 3. The rate of leaf regrowth after fire by growing degree day (GDD) for 19 Poaceae species. Each colour (line and points) represents one individual. *A.*, *Alloteropsis*; *Ar.*, *Aristida*; *C.*, *Cenchrus*; *Cy.*, *Cymbopogon*; *D.*, *Digitaria*; *E.*, *Eragrostis*; *H.*, *Heteropogon*; *M.*, *Melinis*; *P.*, *Paspalum*; *S.*, *Sporobolus*; *T.*, *Tetrachne*; *Th.*, *Themeda*; *Tr.*, *Tristachya*.

Discussion

Using a novel, ecologically-relevant method of burning individual plants *in situ* during the natural fire season and then tracking their regrowth in the field, we carried out a large experimental study of grass flammability and regrowth after fire in a phylogenetic comparative context. We found support for the hypotheses that considerable variation exists in both of these traits for co-occurring grass species, and that high fuel loads were associated with rapid post-fire regrowth. This link between flammability and post-fire success builds upon the work done by Ripley and colleagues (2015). In their study, a non-significant, positive relationship between fuel load and regrowth rate was found, although this relationship was lost when phylogenetic structure was accounted for. Here, by increasing statistical power with a larger sample of species, we found a highly significant positive association between fuel load and regrowth.

Fuel load is the key driver of flammability in grasses (Simpson et al., 2016), with high values associated with rapid combustion, long flaming times and fast fire spread rates. The considerable variation in fuel load found here between species therefore suggests a considerable disparity in flammability too, supporting previous work (Ripley et al., 2010; Ripley et al., 2015; Simpson et al., 2016). Fuel load is not only a product of plant growth rate and time since the last fire, but also the decomposition rate of aboveground biomass. Traits such as C:N ratio and the presence of tannin-like substances influence decomposition, with low C:N values and high concentrations of these substances both aiding the accumulation of fuel load by slowing decomposition, as well as discouraging herbivory (Ellis, 1990; Aerts, 1997). The influence of decomposition rate on the variation in fuel load across the study species is of interest but was not possible within the scope of this study. Whilst fuel load is a product of functional traits that may be shaped by evolution, there are problems with this being considered adaptive. Fuel load is closely related to plant size, but size can be controlled by factors (e.g water, light and nutrient availability) which are independent of fire regime. Therefore this is unlikely to be a specific adaptation to fire. The finding here that large plants are more flammable and resprout faster may just be consequence of their size and not the fire regime experienced. In order to explore the evolution of flammability in grasses, evolutionary-relevant, flammability-enhancing traits which may be responding specifically to fire need to determined and examined in relation to fire regimes.

Biomass moisture content, another trait that influences grass flammability, differed considerably between species although all values were low (<1.06 g g^{-1}) likely due to sampling during the winter dry season. This trait largely determines whether ignition is successful or not, with high moisture contents requiring large amounts of heat energy to be absorbed before ignition can be achieved. However, the relatively low moisture content values obtained here suggests that the all individuals would be highly ignitable. This trait, similar to fuel load, is likely driven by factors other than fire, and so applying evolutionary hypotheses about the evolution of flammability to biomass moisture content is inappropriate.

For fire-prone grasses, there are considerable fitness benefits to rapidly resprouting or establishing from seed after a fire. Surface fires in grasslands and savannas typically remove the majority of aboveground biomass. Therefore, the immediate post-fire environment is characterised by high light availability, reduced competition, and the deposition of ash, which results in a flush of available nutrients (Grove, O'Connell & Dimmock, 1986; Knapp & Seastedt, 1986). The considerable variation in resprouting rate found here for these coexisting species suggests those that resprout quickly after fire will benefit the most from the resources available. The traits underlying the disparity in resprouting were not investigated here but are likely to be associated with photosynthetic capacity (stomatal density and size), the cost of producing leaf biomass (specific leaf area) and the possession of below-ground carbohydrate reserves (Reich et al., 1999; Verdaguer & Ojeda, 2002; Paula & Pausas, 2011).

Whilst the majority of grasses survived the experimental burns carried out here and resprouted afterwards, several failed to do so within the timeframe of the study (10/147 plants) and were assumed to have died. Indeed, fire can be a significant mortality factor for savanna grasses if excessive heat reaches the vulnerable basal meristem (Zimmerman et al., 2010). Therefore, if grasses can alter local fire behaviour through their elevated flammability and increase neighbour mortality but survive themselves, they stand to benefit from increased availability of resources. This is particularly true for free physical space in which to resprout, recruit or for clonal spread, which can be a rarity in grasslands (Defossé, Robberecht & Bertiller, 1997; Milton & Dean, 2000). Whilst the 'kill thy neighbour' hypothesis (Bond & Midgley, 1995) is largely associated with the "hot-flammable" strategy of post-fire seeders, it may also be applicable to the "fast-flammable" strategy of resprouting, fire-prone grasses (Pausas, Keeley & Schwilk, 2017). Our understanding of how the flammability of a single species influences community-level flammability in fire-prone grasslands needs greater development. If flammability in these ecosystems is driven by the most flammable species of a community, as has been found for other vegetation types (de Magalhães & Schwilk, 2012; van Altena et al*.,* 2012), then a link between flammability and post-fire success suggests that high flammability may be a 'niche construction' trait (Keeley et al., 2011). However, the application of these evolutionary hypotheses to the findings here are limited due to the putatively adaptive traits used to underpin flammability.

For fire-prone grasses there are several potential benefits to being highly flammable. Firstly, many of these species are shade-intolerant and their survival is dependent upon the maintenance of an open canopy (Sage et al., 1999). They are not only tolerant of fire, but also dependent upon it to remove standing dead and woody biomass, as demonstrated by their elimination from experimental plots where fire is supressed (Everson et al*.,* 1988; Uys et al*.,* 2004; Fynn et al., 2005; Fynn et al., 2011). A high flammability can aid the removal of dead biomass, thus stimulating new growth and reducing mortality from self-shading (Zimmerman et al., 2010). Secondly, although not extensively studied, there is growing evidence of heat-stimulated recruitment (flowering and germination; Sarmiento, 1992; Baxter et al., 1994; Verboom, Stock & Linder, 2002; Ghebrehiwot et al., 2012) in savanna grasses, which may be enhanced by high flammability. Finally, high biomass flammability may protect basal meristems from excessive heat by burning rapidly but briefly, which results in reduced heating of the soil (Gagnon et al., 2010). However, empirical testing of this 'pyrogenicity as protection' hypothesis is lacking in the context of resprouting grasses and requires further research in order to establish whether it is a potential fitness benefit.

The considerable differences in grass flammability and regrowth rate found here suggests that grassland and savanna communities of varying composition may fuel very different fire behaviour. Plant traits influential to flammability impact upon the likelihood of fire, and the intensity and spread of that fire. Regrowth rates also affect future fire behaviour by influencing future fuel loads, which in turn determines the frequency of fire that can be sustained in a landscape. Therefore, changes in community composition through time may drastically alter fire behaviour, as evidenced by the introduction of invasive, flammable grasses into a number of ecosystems (D'Antonio & Vitousek, 1992; Grigulis et al., 2005).

Acknowledgements

Research support was provided by a Natural Environment Research Council studentship to KJS, Royal Society University Research Fellowship URF120119 to PAC and URF120016 to GHT. We thank Sean Gallagher, Sam Mzangwa, Barry Hartley and the Makana Municipality fire department for their assistance in the lab and field.

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Supporting Information

Figures and tables

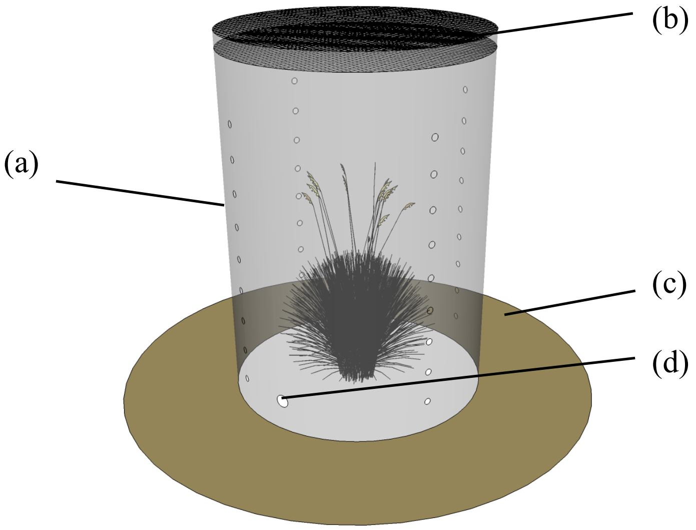

Figure S1. Schematic drawing of the set-up used to burn plants *in situ*. (a) Modified, cleaned 45-gallon oil drum (85 cm tall and 61cm diameter) with the top and base removed, and a series of holes drilled in four vertical lines down its side for ventilation; (b) double layer of fine mesh fitted into the top of the drum to act as a spark arrestor; (c) wet hessian sacking placed around the base of the barrel to minimize risk of the fire spreading; (d) the ignition hole through which a lit blowtorch was applied

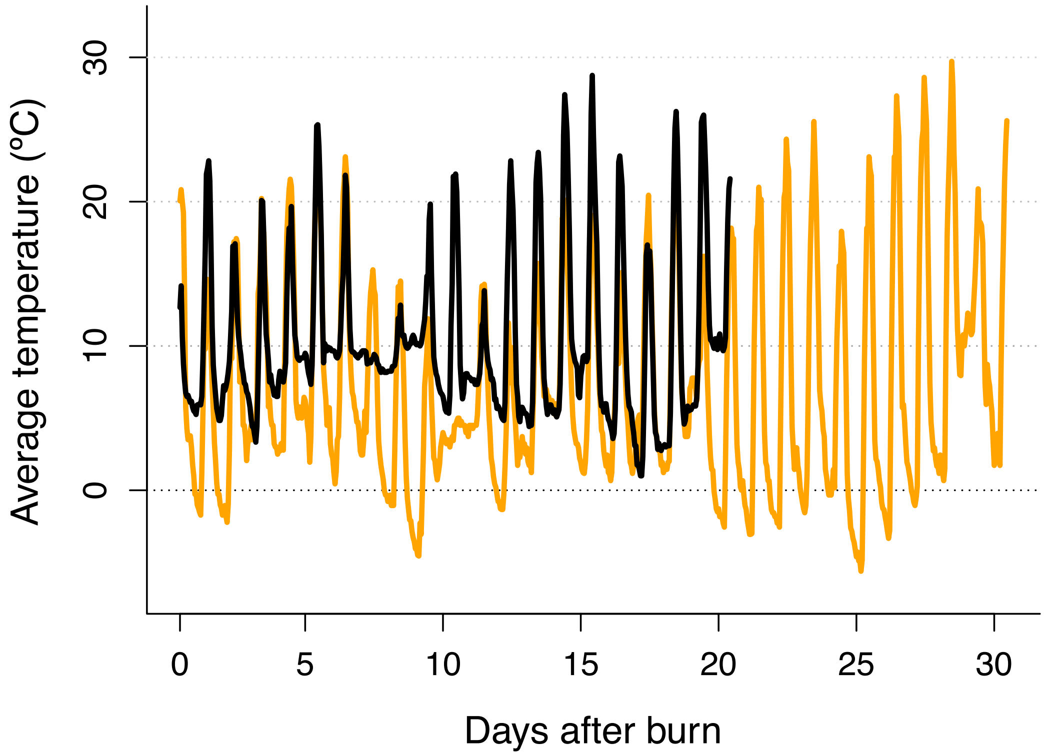

Figure S2. Temperatures of the regrowth period for Site 1 (black line) and Site 2 (orange line).

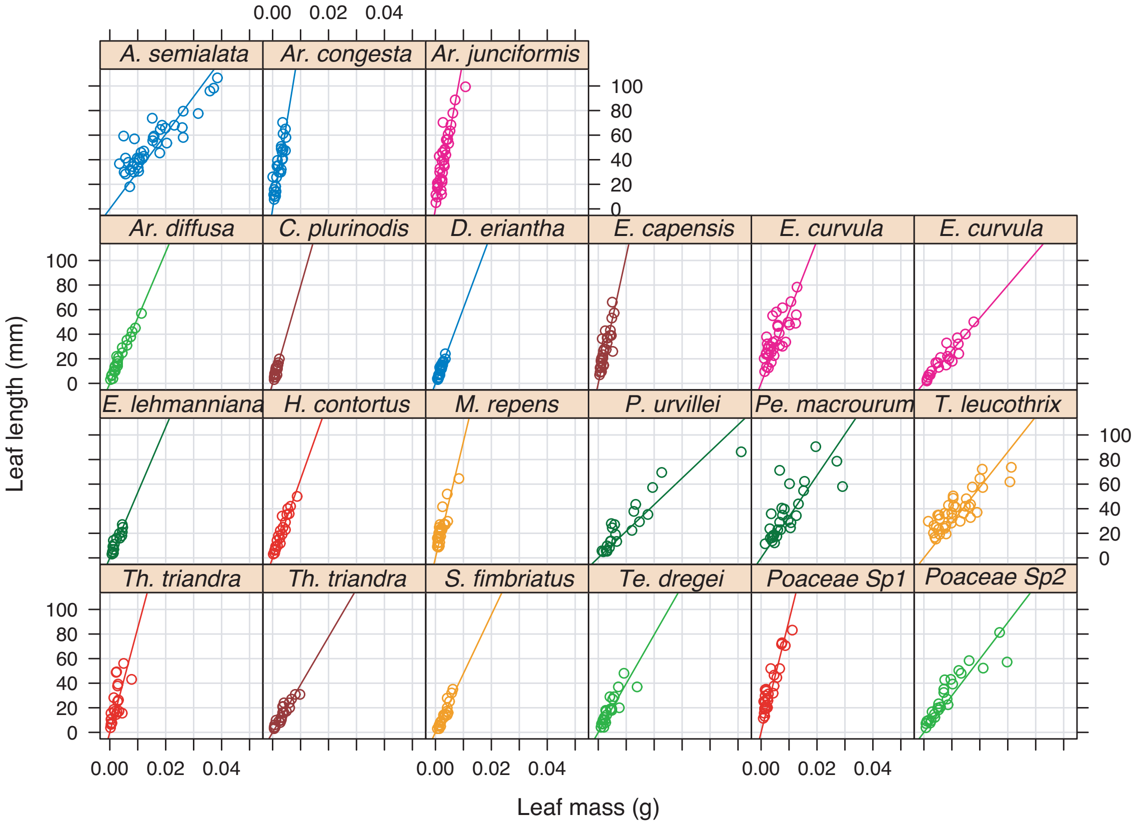

Figure S3. The relationship between leaf length and leaf mass for 19 Poaceae species. Regression coefficients are provided in Table S3. Each colour (line and points) represents one individual. *A.*, *Alloteropsis*; *Ar.*, *Aristida*; *C.*, *Cenchrus*; *Cy.*, *Cymbopogon*; *D.*, *Digitaria*; *E.*, *Eragrostis*; *H.*, *Heteropogon*; *M.*, *Melinis*; *P.*, *Paspalum*; *S.*, *Sporobolus*; *T.*, *Tetrachne*; *Th.*, *Themeda*; *Tr.*, *Tristachya*.

Table S1. Climate and location data from the sites where plants were burnt in situ. Site 1 is a grassland site located about 5 km outside of Grahamstown in the Eastern Cape of South Africa. Site 2 is a typical Karoo grassy shrubland in a section of the Grootfontein Agricultural Development Institute research farm, situated in the north of South Africa's Eastern Cape. For both sites, average rainfall and minimum and maximum temperature values for July and August (i.e. the months over which this experiment occurred) were provided by the South African Weather Service (weather station 0056917 8 for Site 1 and 0144899 5 for Site 2 (rainfall only; www.weathersa.co.za) for years spanning 1993 to 2016. Temperature data for Site 2 was provided by www.meteoblue.com (site = Middelberg, Eastern Cape).

Site name	Coordinates (latitude, longitude)	Average daily rainfall (mm)	Average minimum daily temperature (°C)	Average maximum daily temperature (°C)
Site 1	-33.323, 26.533	1.2	7.3	19.1
Site 2	-31.529, 24.908	0.2	2.5	17.0

Table S2. Grass (Poaceae) species used in this study and GenBank accession numbers used for phylogeny construction

Species	**Subfamily**	**GenBank accession numbers**
Alloteropsis semialata (R.Br.) subsp. *eckloniana*	Panicoideae (Paniceae)	KT281150
Aristida congesta Roemer & Schult	Aristidoideae	FR821317, GQ924369, FR821351
Aristida diffusa Trin.	Aristidoideae	GQ924376
Aristida junciformis Trin. & Rupr.	Aristidoideae	HE586078, GQ924398, HE575768
Cenchrus macrourus Trin. Morrone	Panicoideae (Paniceae)	AY116266, GU561525
Cymbopogon pospischilii (K. Schum.) C.E. Hubb.	Panicoideae (Andropogonea)	KP860328, AF117406
Digitaria eriantha Steud.	Panicoideae (Paniceae)	HE574068, KP057660, HE573497
Eragrostis capensis (Thunb.) Trin.	Chloridoideae	HE573970, DQ655881, AM849209
Eragrostis curvula (Schrad.)	Chloridoideae	HE586097, DQ655876, U21989
Eragrostis lehmanniana Nees	Chloridoideae	KP860329, GU990398, HM346984
Heteropogon contortus (L.)	Panicoideae (Andropogonea)	KY596145, KY596145, KY596145
Melinis repens (Willd.) Zizka	Panicoideae (Paniceae)	HE574080, GU594561, FJ486532
Paspalum urvillei Steud.	Panicoideae (Paniceae)	HF558483
Sporobolus fimbriatus (Trin.) Nees	Chloridoideae	DQ655901
Tetrachne dregei Nees	Chloridoideae	AF312363, GU990366, JN681761
Themeda triandra Forssk.	Panicoideae (Andropogonea)	KY707771
Tristachya leucothrix Trin ex Nees.	Panicoideae (Paniceae)	KY432801

Table S3. Regression coefficients for relationships between leaf length and leaf mass for 19 Poaceae species. Values are species averages (± standard error) that were determined by fitting linear models to leaf length and leaf mass data. P and R^2 values and the sample size (n) for these models are given. Site information is also provided. For the two species that occurred in both sites (*Eragrostis curvula* and *Themeda triandra*), there were significant inter-site differences in the relationship between leaf length and mass (ANOVA: *E. curvula*, F_2=56.6, P<0.001; *T. triandra*, F_2=20.8, P<0.001).

Species	Site	Leaf mass per unit length (mg mm^{-1})	n	P value	R^2 value
Alloteropsis semialata	1	3.04 (±0.14)	39	<0.001	0.930
Aristida congesta	1	13.875 (±0.68)	28	<0.001	0.939
Arisida junciformis	1	12.22 (±0.57)	35	<0.001	0.931
Aristida diffusa	2	5.34 (± 0.15)	21	<0.001	0.982
Cenchrus macrourus	1	3.35 (±0.27)	28	<0.001	0.854
Cymbopogon pospischilii	2	7.89 (±0.43)	14	<0.001	0.963
Digitaria eriantha	2	6.11 (± 0.20)	18	<0.001	0.982
Eragrostis capensis	1	10.37 (±0.60)	27	<0.001	0.920
Eragrostis curvula	1	5.81 (±0.38)	33	<0.001	0.882
	2	2.66 (±0.12)	21	<0.001	0.960
Eragrostis lehmanniana	2	5.32 (±0.28)	15	<0.001	0.962
Heteropogon contortus	2	6.39 (±0.24)	21	<0.001	0.973
Melinis repens	1	9.45 (±0.62)	24	<0.001	0.911
Paspalum urvillei	1	2.16 (±0.16)	21	<0.001	0.905
Poaceae Sp1	1	9.12 (±0.49)	28	<0.001	0.926
Poaceae Sp2	1	2.98 (±0.18)	26	<0.001	0.915
Sporobolus fimbriatus	2	4.78 (±0.24)	18	<0.001	0.959
Tetrachne dregei	2	3.97 (±0.30)	21	<0.001	0.900
Themeda triandra	1	8.49 (±1.04)	22	<0.001	0.761
	2	3.88 (± 0.18)	21	<0.001	0.958
Tristachya leucothrix	1	2.87 (±0.15)	38	<0.001	0.905

Chapter 4

Chapter 4

Memory of past fire regime enhances plant developmental response to fire

Kimberley J. Simpson, Jill K. Olofsson, Brad S. Ripley & Colin P. Osborne

Table of contents

Figures and tables

Statement of contributions

KJS designed the study with input from JKO, BSR and CPO. KJS generated the data with assistance from BSR (plant collection) and JKO (RAD sequence data). KJS analysed the data with help from JKO (population genetics) and CPO (general data analysis). KJS wrote the chapter with all authors contributing critically to drafts.

Memory of past fire regime enhances plant developmental response to fire

Abstract

1. Fire is a severe disturbance to plants that varies through time. Coping with temporal variation in fire requires plasticity in traits promoting persistence through fire, but how plastic responses to current conditions are affected by prior exposure to fire remains unknown. Here we investigate phenotypic divergence between populations of resprouting savanna grasses exposed to differing experimental fire regimes, and test whether this divergence persists after plants are grown in a common environment.

2. Cuttings were taken from four savanna grass species in experimental plots that had been either annually burnt or unburnt for >35 years, and re-established in a common environment for one year. Traits relating to persistence through fire were then measured before plants were burnt during the fire season, and their regrowth tracked. To determine if there was genetic differentiation between the populations subjected to differing fire regimes, restriction-site associated genome sequences were compared for a subset of individuals.

3. After a year in the common environment, the historic fire frequency influenced traits relating to flowering and below-ground investment, but not regrowth rate after fire. In comparison with historically unburnt plants, those previously subjected to high fire frequency produced more inflorescences and invested a greater proportion of biomass below ground. These results suggest that high fire frequency causes plants to develop a greater capacity for recruitment and resprouting. However, we found no consistent genetic differences between the populations under diverse fire frequencies within each species, suggesting that trait differences arose from developmental plasticity and not natural selection.

4. The development of four perennial grass species is influenced by prior exposure to fire, independent of the current environmental conditions, suggesting a ‘memory’ for fire. This developmental priming may improve the fitness of individual plants when exposed to fires in future.

Key-words:

Flammability, Flowering, Functional traits, Phenotypic plasticity, Poaceae, Resprouting, Stress memory

Introduction

The role of phenotypic plasticity in adaptation to biotic and abiotic environments is becoming increasingly recognised, as is the influence of past environmental conditions in modulating future plastic changes (Huber et al., 2012; Niu et al., 2014). Plants may possess an ‘ecological memory’ (*sensu* Padisak, 1992), responding to extreme events differently depending upon their previous experiences (Bruce et al., 2007; Robertson & Wolf, 2012; Walter et al., 2013; Scholes & Paige, 2015; Wang et al., 2017). This primed response can have profound positive effects on plant tolerance to and performance through environmental perturbations, in addition to reducing costs associated with maintaining a constant high level of phenotypic plasticity (Wang et al., 2017).

Fire is a major and ancient environmental perturbation that has significantly influenced plant populations and communities for 420 million years (Glasspool, Edwards & Axe, 2004; Bond, Woodward & Midgley, 2005; Keeley et al., 2011). Plants have adapted to life with fire through changes to their functional traits, with growing evidence of this phenomenon from woody species, where different fire regimes create intraspecific variation in fire-related traits that is genetically determined and thus responding to natural selection (Parchman et al., 2012; Budde et al., 2014; Moreira, Castellanos & Pausas, 2014). However, the priming effect of exposure to fire on plastic responses remains unexplored. Fire-prone tropical grasses are an ideal study system for exploring the role of fire on priming, as these perennial species persist through the most frequent fire regimes on Earth (Mouillot & Field, 2005).

In recurrently burnt environments, plasticity in the traits relating to how a plant population can persist through fire are important. The two main mechanisms for post-fire persistence are resprouting from protected organs or recruiting from a fire-resistant seed bank (i.e. resprouter verses seeder strategies). For resprouting species, traits that increase the protection of vulnerable meristems from lethal temperatures, such as basal budbanks and insulating bark (He et al., 2012; Pausas, 2015; Midgley et al., 2015), and that allow an individual to resprout quickly after fire (Cavender-Bares & Reich, 2012; Forrestel, Donoghue & Smith*,* 2014), are likely to be favoured. The immediate post-fire environment is rich in sunlight and nutrients (Knapp & Seastedt, 1986; Grove, O’Connell & Dimmock*,* 1986), making plant competition intense. Individuals that can resprout quickly after fire will benefit the most from the resources available. The possession of traits such as high specific leaf area (SLA) and stomatal density, and large stomatal size will aid in rapid post-fire regeneration (Reich et al., 1999). Having large below-ground carbohydrate reserves also facilitates robust resprouting after fire (Verdaguer & Ojeda, 2002; Paula & Pausas, 2011). For seeder species, rapid recruitment is important in gaining access to the high post-fire resources, and is enhanced by fire-stimulated flowering, seed release or germination (Keeley & Fotheringham, 2000).

Grasses are taxa whose historical and contemporary success has largely been tightly linked to fire (Bond et al., 2005; Keeley & Rundel, 2005; Scheiter et al., 2012; Linder et al., 2017). With a global average fire return interval of 5.7 years in tropical grasslands, fire represents a frequent disturbance in these environments (Mouillot & Field, 2005). In addition, fire is a severe disturbance to grasses, typically removing all above-ground biomass, and is capable of killing mature plants (Zimmerman et al., 2010). The majority of grasses that inhabit fire-prone grasslands and savannas are perennial, resprouting species (Allan et al., 2002; Overbeck & Pfadenhauer, 2007). Successful recruitment in many perennial grasslands is infrequent, with seedlings competing with established plants for below-ground resources (Defossé, Robberecht & Bertiller, 1997; Milton & Dean, 2000). However, fire has a positive effect on several stages of the recruitment process in perennial grasses (Zimmerman et al., 2008), such as through the stimulation of flowering and germination (Sarmiento, 1992; Baxter et al., 1994; Verboom, Stock & Linder, 2002; Ghebrehiwot et al., 2012; but see Overbeck et al., 2006) and by changing the competitive environment seedlings are exposed to (Bennett, Judd & Adams, 2002; Snyman, 2004). Therefore, in frequently burnt environments, traits that enhance post-fire recruitment and/or regrowth are likely to be adaptive, leading to the expectation of phenotypic divergences between populations experiencing different fire frequencies.

We tested the expectation that fire causes divergences in persistence (recruitment and regeneration) traits using individuals of four perennial savanna grass species sampled from experimental plots that had been either unburned or annually burned for >35 years. To test whether any trait differences between populations persisted independently of the current environment, cuttings were taken from plants in the fire plots and grown in a common environment for one year, after which traits were measured again. In comparison to unburnt plants, we predicted that annually burnt plants would have traits advantageous under recurrent fires, including rapid post-fire resprouting (high investment in below-ground biomass, high regrowth rate, high SLA) and enhanced recruitment (fire-stimulated flowering). To evaluate whether any phenotypic differences had a genetic component or were due to plasticity, we used restriction-site associated genome sequencing to test for allelic divergence among populations.

Materials and Methods

PLANT COLLECTION

This study utilized plants collected from experimental burn plots located at the University of Fort Hare Research Farm, in the Eastern Cape of South Africa (32° 47′S, 26° 52’ E). These plots, set up in 1980, comprise six treatments (no burn, annual, biennial, triennial, quadrennial and sexennial burns) replicated twice in a completely randomized design on 100 m x 50 m plots, each with a 5m mowed boundary (as described in Trollope, 2004). The area in which the plots are situated is semi-arid savanna (“False Thornveld”; Acocks, 1975) with a mean annual rainfall of 440mm, the majority of which falls in the Austral summer (78% rain falling between October – April; Table S1). The temperature regime is moderate, with hot summers and cold winters (Table S1). The soil at the site is a shallow silty loam of the Glenrosa series (Soil Classification Working Group, 1991).

Plants of four Poaceae species (*Cymbopogon pospischilii, Digitaria eriantha, Melica racemosa* and *Themeda triandra*) were collected on 07/07/15, shortly before the winter controlled burns, from plots experiencing two contrasting fire frequency treatments: annual burn and no burn, which had not been burnt in the 35 years since the plots were created (Trollope, 1983). Both of these treatments represent a departure from the natural fire return interval of the site (approximately 15-20 years; W.S.W. Trollope, 2017, pers. comm). These species occurred abundantly in both treatment plots, and are perennial, resprouting species from three grass subfamilies (Table S2). In the 35 years of treatment, it is predicted that the populations will have undergone several rounds of reproduction and recruitment based on the reported longevity of savanna grasses (McCarthy, Tolhurst & Chatto, 1999). Thirty-five mature individuals of each species were dug up from open areas of grassland, minimizing root damage, from across the two replicate plots (n=17 or 18 from each plot; plot number was noted for each individual). Within 48 hours of collection, a clump of 5 tillers was carefully removed from each individual. The roots were washed carefully to remove soil, in order to limit the effects of any soil nutrient differences on plant growth. The clumps were subsequently planted into 10L pots containing locally sourced natural topsoil. A voucher specimen of each species was deposited at the Selmar Schonland Herbarium (Rhodes University; see Table S2 for specimen numbers).

To determine whether there were differences in plant traits between annual-burn and no-burn populations at the time of sampling from the experimental burn plots, traits were measured on 14 plants per treatment per species (n=7 from each plot). For this, the remainder of biomass (with the five tiller clump having been removed) from each plant was used to measure plant height. The above-ground biomass was subsequently separated into culm, live leaf and dead leaf, and the fresh and dry (after 48 hours at 70°C) mass of each fraction was measured using a four-point balance. Total biomass moisture content values (in g g^{-1}) were calculated by dividing the difference between fresh and dry biomass by the dry biomass. The dry mass ratio of living vs dead leaves in the canopy was also calculated.

GROWTH CONDITIONS

The plants were grown for 12 months (July 2015 – July 2016) in a common environment (a naturally lit polytunnel at Rhodes University, Eastern Cape, South Africa) in a fully randomized block design, and were weeded and watered regularly. In the polytunnel, average monthly temperatures ranged from 13.9°C (July) to 26.4°C (January) and average relative humidity was 68% (as recorded by thermochron data loggers: i-buttons, model DS1923, Maxim Integrated Products, California, USA). A 12-month growth period was chosen so that plants could become well established in the pots and to minimise any environmental effects arising from the different treatment plots. Several plants died during this period (n=37/280) but mortality was not associated with a particular species, treatment or plot (ANOVA: P>0.05). Watering was reduced in frequency and eventually stopped two weeks prior to burning to imitate the winter dry season and to force the plants into a phenological stage most relevant to burning.

PRE-FIRE TRAITS

On the day prior to the experimental burns (03/07/16), the number of flowering tillers was recorded and a sample of above-ground biomass (~1/4 of the total biomass) was removed for the remainder of the plants. The harvested biomass was divided into live leaves, dead leaves and culm, and the fresh and dry (after 48 hours at 70°C) mass for each fraction was measured using a four-point balance. For each species, eight annual-burn and no-burn plants were also randomly chosen, destructively harvested and used to measure the allocation of biomass to above and below the ground. The above-ground biomass was separated from the roots, oven dried and weighed . Roots were carefully washed over a fine sieve and then dried at 70°C for 7 days. Root dry mass was measured and expressed as a proportion of the total dry plant biomass.

EXPERIMENTAL BURN

All remaining plants were burnt on a warm day with little wind (04/07/16) in an order determined using a random number generator. An area of land was cleared of vegetation and series of holes were dug. Each plant was carefully removed from its pot to minimize root disturbance, and lowered into a hole. The depth of the hole was adjusted to ensure that the soil surface for all the plants was at the same height and burning was even. Each plant was burned in turn in a controlled way (see Fig. S1 for diagram of the set-up). After burning, plants were returned to their pots and any ash on the soil surface was removed to standardize any fertilizing effect this would have across individuals. The plants were then put back into the polytunnel in a randomized block design and watered.

POST-FIRE REGROWTH

The plants were visually checked for signs of regrowth in the days following the burns, and the majority had initiated regrowth six days after fire. Five regrowing leaves were randomly selected for each plant, and tagged with a small ring of coloured wire around the leaf base. The length of the resprouted leaf was measured using digital callipers six days after the burn and on a further four occasions (each being 5-7 days apart), with the final measurement being taken 30 days after the burn. For the duration of regrowth, plants were watered regularly and air temperature in the polytunnel was recorded hourly using thermochron data loggers as before. Daily average temperatures were slightly higher (2.7°C on average) in the polytunnel than outside (see Fig. S3), thus the plants experienced conditions similar to early spring without the complication of late season frosts. Some of the plants belonging to two species failed to resprout within 30 days (*M. decumbens*= 10/47; *T. triandra*= 6/44), but this fire-induced mortality was not associated with the burn treatment that plants were sampled from (ANOVA: P>0.05).

After the last measurement was taken, all regrown biomass was removed from each plant and stored in sealed plastic bags containing moist tissue paper. Total regrown leaf area was then measured within 72 hours using digital images and the program WinDIAS (Delta-T Devices, Cambridge, U.K.) that determines leaf area by selecting pixels of a pre-defined colour range. The regrown material was subsequently dried at 70°C for 48 hours and the dry mass was determined using a 4-point balance. Specific leaf area (SLA) was calculated by dividing the regrown leaf area by the regrown dry mass.

A regrowth rate was calculated using the leaf length and air temperature data. Daily minimum and maximum temperature values were used to calculate growing degree-days (GDD, °C-day) for each time period between measurements using the equation:

$$GDD = \left[\frac{T_{MAX}+T_{MIN}}{2}\right] - T_{BASE}$$

Where T_{MAX} is the daily maximum air temperature and T_{MIN} is the daily minimum air temperature. A value of 10°C was selected for T_{BASE} (the base temperature for growth), which represents an intermediate value for published temperate and tropical grass T_{BASE} values (Cooper & Tainton, 1968; McWilliams, 1978; Osborne et al., 2008; Steduto et al., 2009; Bartholomew, 2014; Moreno et al., 2014). An average rate of leaf length regrowth was calculated for each individual by fitting linear models to the cumulative leaf length and GDD data.

To convert the rate of leaf length regrowth to a rate of leaf biomass regrowth, the fresh length and dry mass of three leaves of each individual were measured. The relationship between leaf length and dry mass was determined for each species by fitting linear models to the log-transformed data. The fit of the models to the data was good (R^2 values >0.87 for all species; Fig. S2), and the slopes of these relationships were used to convert leaf length regrowth rate into leaf biomass regrowth (in mg GDD^{-1}).

STATISTICAL ANALYSIS

All analyses were performed using R (version 3.4.1; R Core Team, 2017). The effect of fire frequency on plant traits (plant height, number flowering tillers, above-ground biomass, total biomass, root proportion of total biomass, regrown leaf biomass, regrown leaf area, regrowth rate and regrown leaf SLA) was determined by fitting a linear mixed-effects model to the data ("lme4" package; Bates et al., 2015). The fixed effects were "treatment" (annual burn vs no burn) and "species", and an interaction term between these effects was added if it improved the quality of the model (as indicated by the Akaike information criterion value). "Plot" (i.e the replicate plot the plant was taken from) was added as random effect. Above-ground biomass was also added as a fixed effect for models in which the trait is likely influenced by the size of the plant before fire (number of flowering tillers, height and some regrowth traits after fire). To determine whether fire frequency was significantly influencing plant traits, this model was then compared to a grand mean model using a parametric bootstrapping method ("pbkrtest" package, Halekoh & Højsgaard, 2015) with 10,000 simulated generations.

To establish the relationships between pre-fire aboveground biomass and post-fire regrowth, mixed models were fitted using the methods above. The regrowth trait (either regrown dry biomass, regrown leaf area, or regrowth rate) was set as the dependent variable. Fixed effects were "pre-fire aboveground biomass" and "species", and "plot" was added as random effect. Model comparison was carried out using the same methods as above.

DNA EXTRACTION AND SEQUENCING ANALYSES

For each species, silica-dried leaf material from a subset of individuals (n=3-5) per treatment was selected and total genomic DNA extracted using the DNeasy Plant Mini Kit (Qiagen) following the manufacturer's protocol with the exception that DNA was eluded in 50 µl AE buffer.

Double digested restriction associated DNA (ddRAD) libraries were built using the protocol of Nosil et al. (2012) with small modifications following Peterson et al. (2012) to allow for paired-end sequencing. In short, 7 µl DNA extract (150-350 ng DNA) was double-digested using *Eco*RI and *Mse*I after which barcoded (8-10 bp) adaptors were ligated to the *Eco*RI side and a common adaptor to the *Mse*I side. The common adaptor of Peterson et al. (2012) was used to allow for paired-end sequencing. The 34 libraries were pooled with 62 other libraries of different projects and the library pool was gel size selected (300-600 bp) and purified using the QIAquick Gel Extration kit. The size-selected library pool was paired-end sequenced (125 bp) on one Illumina HiSeq2500 lane following standard protocols.

The raw sequencing data was cleaned using the trimmomatic tool kit (Bolger, Lohse & Usadel, 2014). Adaptor and primer sequences were removed using the ILLUMINACLIP option in palindrome mode, supplying the program with the expected sequences and allowing for a maximum of two mismatches to the supplied adaptor and primer sequences. The cleaned reads were further trimmed removing low quality (<3) bases from both the 3' and 5' end of the reads as well as all bases with a minimum quality of 15 in a four-base sliding window. Finally, all reads shorter than 36 bases after trimming were removed. The library pool was de-multiplexed and the barcodes were removed using the processRADtag.pl script supplied with the program Stacks (Catchen et al., 2011). After de-multiplexing, each species was treated separately in all following analyses.

Reads mapping to the chloroplast were separated from the rest of the reads to avoid combining chloroplast and nuclear data into one assembly. Representative whole chloroplast genomes were downloaded from Genbank (Table S3) and used as references. Cleaned reads were mapped to the respective reference genome using Bowtie2 v.2.2.3 (Langmead & Salzberg, 2012) with default settings for paired end reads. All reads not mapping to the chloroplast were then retrieved using SAMtools v.1.2 (Li et al., 2009) and BEDtools v.2.19.1 (Quinlan & Hall, 2010) and used to *de novo* assemble nuclear RAD loci in ipyrad v.0.7.2 (Eaton & Overcast, 2016). A cluster threshold (sequence similarity for homology) of 0.85 was used and only loci with a cluster depth below 100 and less than 50% missing data were output. All other parameters were kept at the default settings.

One random single nucleotide polymorphism (SNP) with a minor allele count of three was extracted in VCFtools from each assembled RAD loci and used for all downsteam analyses. This ensures that only unlinked SNPs with the minor allele observed in at least two individuals were retained. The retained SNPs were used in a principal component analysis (PCA) in the R package "adegenet" (Jombart, 2008; Jombart & Ahmed, 2011) to test whether the two treatments were genetically distinct from each other. An analysis of similarity was used to evaluate the significance of clustering of the samples (R package "vegan"; Oksanen et al., 2017). Signatures for genetic differences between the two treatments, possibly indicating differences in selective pressures, were further evaluated by calculating the genetic distances between the two treatments for each species. Pairwise F_{ST} for each SNP were calculated in VCFtools v.0.1.15 (Danecek et al., 2011). The average F_{ST} across all SNPs was then estimated after all negative values of were converted to zero. Jackknifing was used to evaluate the significance of the average F_{ST} value. For each species, individuals were randomly sampled with replacement into 1,000 two-population comparisons and the average F_{ST} between the two populations were calculated as outlined above for each comparison. Significance was evaluated as the percentage of the jacknifed F_{ST} values that were greater than or equal to the observed F_{ST}. As individuals within a species were assumed to be closely related, and thus average F_{ST} might not be sensitive enough to detect small significant differences between the treatments, the number of SNPs showing extreme F_{ST} values (>0.8) was also assessed.

Finally, the SNPs were concatenated to an alignment and used to estimate a maximum likelihood phylogenetic tree for each species using RAxML (Stamatakis, 2006) under a GTR+G substitution model. Node support was evaluated with 100 bootstrap replicates.

Results

INITIAL PLANT TRAITS

Plants in the annual-burn and no-burn populations differed significantly in their initial (field-state) traits. In comparison to annual-burn plants, no-burn plants were taller (+29.6%; likelihood ratio test (LRT)=35.1, df=1, P<0.001) and had a larger above-ground biomass (+33%; LRT=62.5, df=1, P<0.001; all model coefficients given in Table S4).

PRE-FIRE PLANT TRAITS

Significant trait differences between no-burn and annual-burn populations persisted after plants had been reduced to a small, uniform number of tillers and regrown in a common environment for 12 months. After this growth period, all plants were well established and had greatly increased in size (~500-700% increase from the initial number of tillers, data not shown). Past fire frequency had a significant effect on the number of flowering tillers, with annual-burn plants having 49.6% more flowering tillers on average than no-burn plants (LRT = 11.11, df=1, P<0.001; Fig. 1). Treatment had no effect on the total (above- and below-ground) dry biomass (LRT=0.62, P=0.43), but the way this was allocated differed significantly - annual-burn plants invested significantly more of their total biomass below-ground (+23.0% on average; LRT=19.98, df=1, P<0.001; Fig. 1) than no-burn plants. Plant height was unassociated with treatment (LRT=0.09; df=1, P=0.77; model coefficients in Table S5).

POST-FIRE REGROWTH

All regrowth traits differed significantly between species (ANOVA: P<0.05), but none were affected by fire frequency. Treatment had no significant effect on regrowth rate (LRT=0.69, df=1, P=0.41), regrown leaf area (LRT=0.11; df=1, P=0.73) or regrown leaf SLA (LRT=1.22, df=1, P=0.27; all model coefficients in Table S6). Overall, there was no treatment effect on regrown dry biomass across species (LRT=0.46; df=1, P=0.49) but significant intra-specific differences existed within *C. pospischilii* and *T.triandra* populations where annual-burnt plants regrew a larger aboveground biomass after fire in comparison to no-burn plants.

Regrowth traits were largely driven by the above-ground biomass of the plant before it was burnt. Pre-fire live leaf dry biomass was the key predictor of post-fire regrown mass (LRT=98.26; P<0.001), regrown leaf area (LRT=103.4; P<0.001), and regrowth rate (LRT=41.28; P<0.001) for individual plants. Total above-ground dry biomass was also a significant predictor of these three regrowth traits (regrown mass: LRT=30.03, P<0.001; regrown area: LRT=30.28, P<0.001; regrowth rate: LRT=15.25, P<0.001; model coefficients in Table S7). Across all individuals (i.e. plants from both treatments), there was a significant positive relationship between pre-fire fuel load and post-fire regrowth rate for all four species (P<0.001; Fig. 2). This relationship was significant within the annually burnt *C. pospischilii* and

D. eriantha popultions and the unburnt *M. decumbens* populations but not in the other treatment populations (Fig. 2).

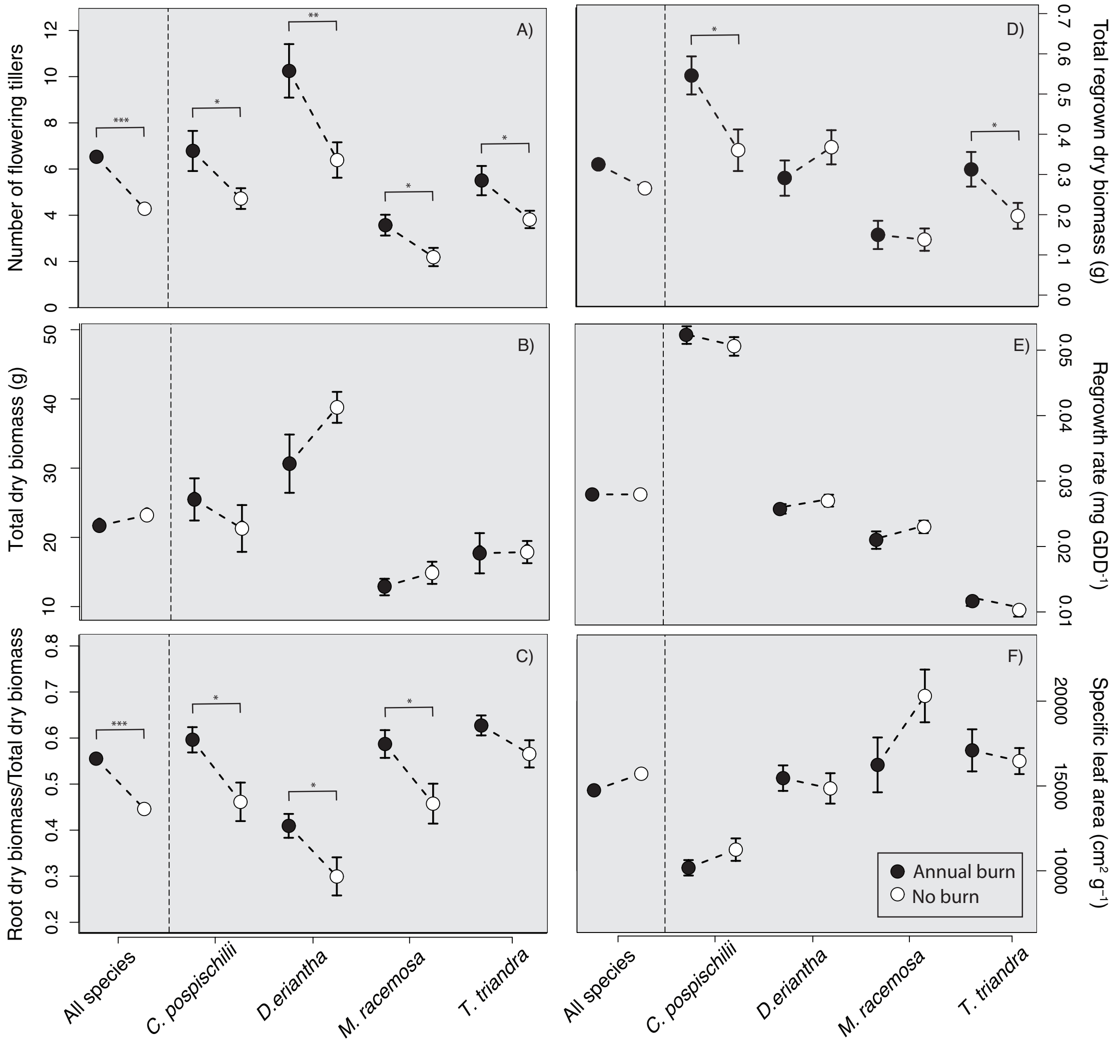

Figure 1. Grass traits under contrasting fire frequencies. Annual-burn plants had significantly more flowering tillers than no-burn plants (P<0.001; panel A). Treatment had no effect on total (above- and below-ground) dry plant biomass (panel B) but did on the allocation of biomass with annual-burn plants investing a higher proportion of their biomass below ground (P<0.001; panel C) in comparison to no-burn plants. After burning all individuals, there was no effect of treatment on total regrown biomass (panel D), regrowth rate (panel E) or the specific leaf area of regrown leaves (panel F) *C.*=Cymbopogon; *D.*=Digitaria; *M.* =Melica; *T.*=Themeda. *, P < 0.05; **, P<0.01; ***, P < 0.001.

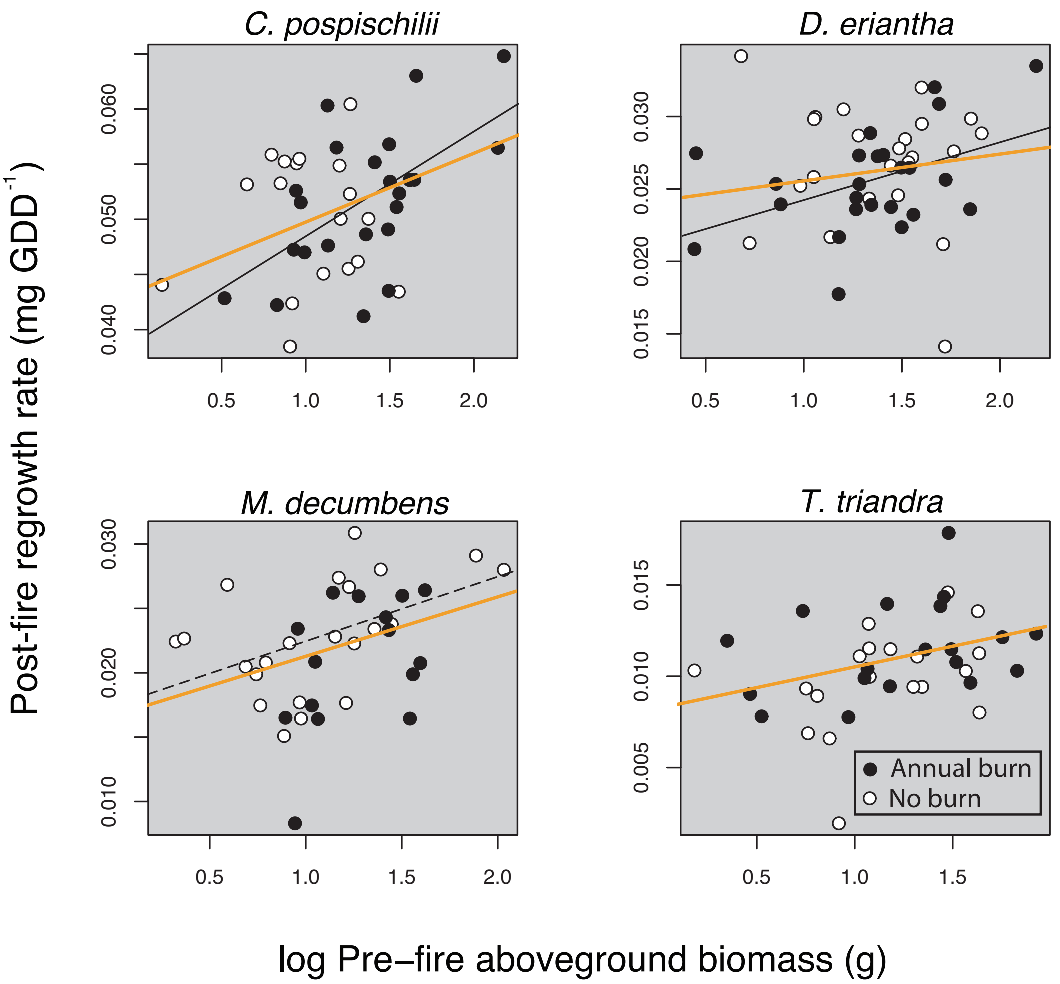

Figure 2. Relationships between pre-fire aboveground biomass and post-fire regrowth rate in savanna grass species. Pre-fire aboveground biomass was a significant predictor of post-fire regrowth rate across all individuals for all species (orange lines; P<0.001). Significant relationships within annually burnt plants (black solid line) and unburnt plants (black dashed line) are shown. *C.*=Cymbopogon; *D.*=Digitaria; *M.*=Melica; *T.*=Themeda.

POPULATION GENETIC ANALYSES

The species *M. decumbens* was excluded from the genetic analyses, because failure of some samples resulted in a low sample size. The number of assembled RAD loci for the three other species varied between 40,031 (*T. triandra*) and 11,716 (*D. eriantha*), with the number of retained SNPs varying between 9,977 (*T. triandra*) and 4,611 (*D. eriantha*; Table 1). The number of assembled loci and SNPs is a function of the sequencing quality and depth, and the divergence between the individuals within each species, and therefore variations in the assembly statistics are expected between the three species. We found no significant clustering of the individuals within each species based on treatment (Fig. 3, analysis of similarity: *C. pospischilii,* P=0.22; *D. eriantha,* P=0.417; *T. triandra* P=1), and none of the species showed a significant genetic difference (as estimated by average F_{ST} and number of F_{ST} outliers) between the two treatments (Table 1). Furthermore, with a few exceptions, the bootstrap support in the maximum likelihood trees were generally low (<95) indicating that there is no significant phylogenetic clustering in any of the investigated species.

Table 1. Assembly statistics for the analysis of genetic differences between populations of three savanna grass species under contrasting fire regimes [annual burn (AB) and no burn (NB)]. RAD; restriction-site associated DNA sequencing, SNPs; single nucleotide polymorphisms. *Melica decumbens* was removed from this analysis because failure of some samples resulted in a low sample size.

Species	Number individuals (NB:AB)	Number assembled nuclear RAD loci	Number SNPs	Average F_{ST} (p-value)	Nr. Outlier F_{ST} (p-value)[a]
Cymbpogon pospischilii	10 (5:5)	21,649	5,528	0.083 (0.074)	33 (0.136)
Digitaria eriantha	9 (5:4)	11,716	4,611	0.095 (0.107)	22 (0.361)
Themeda triandra	8 (5:3)	40,031	9,977	0.107 (0.076)	104 (0.122)

[a] $F_{ST} > 0.80$

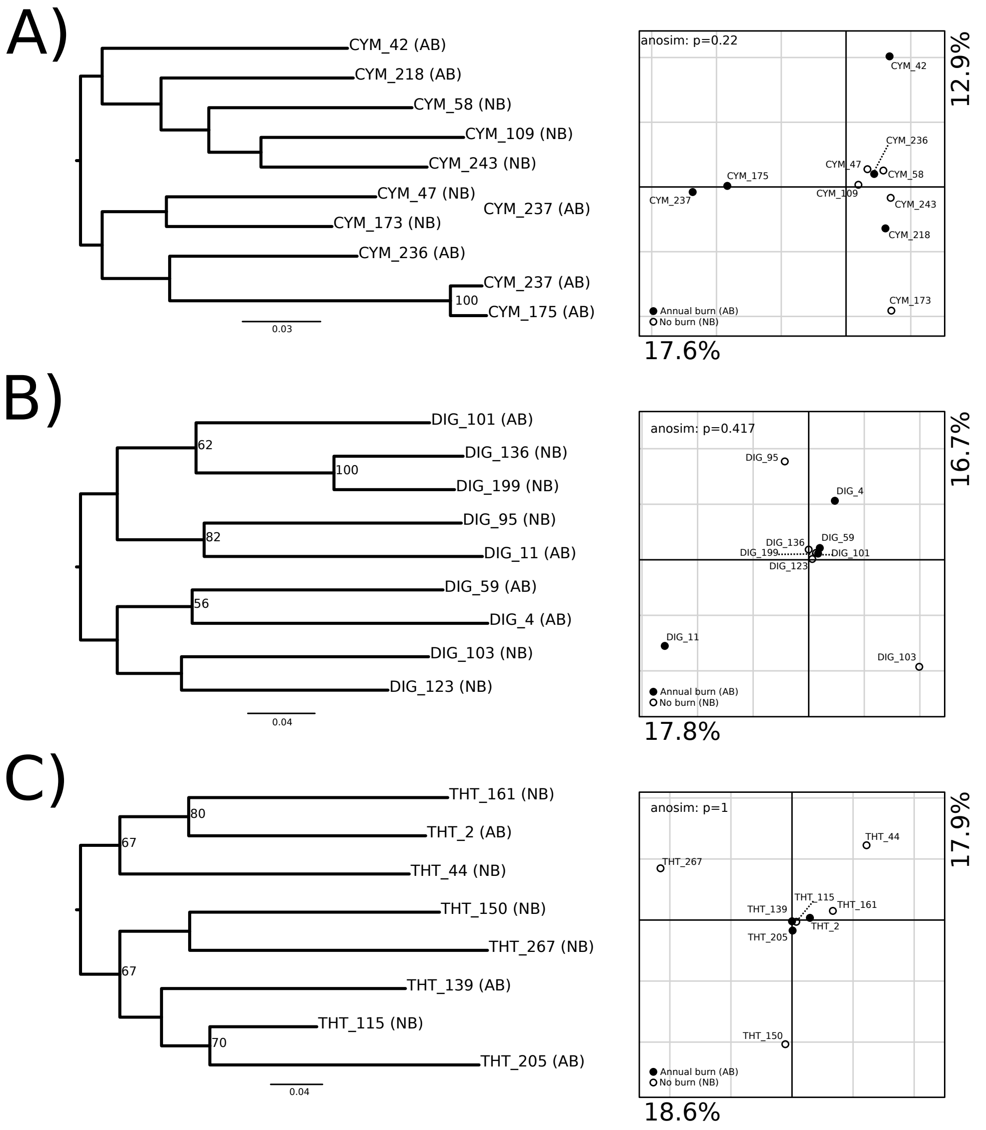

Figure 3. No genetic differentiation between grass populations under contrasting fire frequencies. Phylogenies and PCA plots reveal no clustering based on treatment for individuals of *Cymbopogon pospischilii* (A), *Digitaria eriantha* (B), and *Themeda triandra* (C). Analysis of similarity (anosim) results are indicated in the top left of the PCA plots. Values on nodes represent support evaluated with 100 bootstrap replicates. PCAs are based on all single nucleotide polymorphisms.

Discussion

This study of grass functional traits under differing fire frequencies supports the hypothesis that fire has strong direct effects upon plant structure and function (Keeley et al., 2011). Previous studies have found evidence of a genetic basis of fire-related traits such as serotiny in pines (Parchman et al., 2012) and flammability in a Mediterranean shrub (Moreira et al., 2014). However, we found no evidence of significant genetic groupings based on the two fire regimes indicating that any potential selective pressure imposed by fire has not been strong enough to create significant population structures between the two treatments. This implies that the trait differences observed here arose from plasticity in development rather than divergent natural selection. Previous evidence of selection for traits relating to flammability and recruitment after fire has been obtained from obligate seeder species (Moreira et al., 2014). Such species are expected to experience stronger selection pressures for fire adaptations than resprouting species, such as those studied here, due to their short and non-overlapping generations, and the higher cost of being burnt. The lack of genetic differentiations between the annual-burn and no-burn grasses may therefore be a result of their resprouting mode of persistence through fire.

The trait differences observed between the diverse fire treatments could potentially be explained by environmental effects carried over from the long-term treatments into the common environment, rather than by differential developmental responses to the treatments. However, this is unlikely for three reasons. Firstly, we washed the roots of soil before potting the plants. Fire causes a release of nutrients into the soil, and may result in increased soil fertility and faster plant growth in burnt areas (Maclean et al., 1983; Coutinho, 1990; Úbeda et al., 2009). Indeed, a study on the experimental burn plots used here has found higher available phosphorus and exchangeable cations in the soil of annual burnt plots when compared to the unburnt plots (Oluwole, Sambo & Sikhalazo, 2008). However, any possible carry-over fertilisation effects were limited by soil removal from the roots. Secondly, we found no significant difference in the total (above- and below-ground) biomass between the treatments after one year in a common environment, implying that any carryover of internal resource stores from annually burnt plots did not enable plants to grow larger. Finally, the long period of growth in a common environment resulted in the initial transplanted biomass (five tillers) constituting only a small fraction of the final plant biomass (30-40 tillers).

This study constitutes the first documentation, as far as we are aware, of plants having an 'ecological memory' for fire, as found for some other abiotic stresses such as drought and inundation (e.g. Tahkokorpi et al., 2007; Onate, Blanc & Munne-Bosch, 2011; Walter et al., 2011; Huber et al., 2012; Wang et al., 2017). Traits relating to flowering and growth allocation showed a priming response to fire history. These primed physiological and morphological changes may improve tolerance and/or responses to future fires. Fire is a major disturbance to plants but, by priming these traits, current performance can be maximised whilst avoiding the potential costs of maintaining a life-long high-fire-suited phenotype. The mechanism of this 'memory' is not addressed here, but such responses to previous conditions could involve epigenetic, metabolic, physiological or morphological changes (Bruce et al*.,* 2007; Robertson & Wolf, 2012; Walter et al., 2013; Scholes & Paige, 2015).

Similar to findings on the effect of crown fires on woody species (He et al., 2012; Crisp et al., 2011; Pausas et al., 2012), this study shows that surface fires cause trait divergence in the above- and below-ground allocation strategies of herbaceous plants. Annually burned plants invested more of their biomass below ground compared to no-burn plants, which likely equates to them having greater stored energy reserves to initiate and support early resprouting. The greater frequency of disturbance experienced by the annual-burn plants means they are regularly subjected to the near complete removal of above-ground biomass and frequently encounter the competitive, post-fire environment. Thus, greater investment below ground results in a smaller proportion of total plant biomass being consumed by fire, and provides plants with a greater competitive ability for conditions after fire. However, a higher proportional investment in root biomass in annual-burn plants did not cause a faster initial resprouting rate compared to no-burn plants, as expected. Similarly, specific leaf area, a trait indicative of resource acquisition (Reich et al., 1999), did not differ between annual-burn and no-burn plants. However, recurrent fire could instead select for more robust resprouting (i.e. lower post-resprouting mortality) rather than a faster rate of resprouting (i.e. resprouting vigour *sensu* Moreira, Tormo & Pausas, 2012), although this was not measured here. Drivers of the initial ability to resprout are not necessarily related to those driving longer-term survival and success after resprouting (Lloret & López-Soria, 1993; Moreira et al., 2012). Whilst we saw no effect of the diverse fire treatments on the ability to initiate resprouting and the rate of regrowth after one burn, the influence of recurrent burns may have shown differences, with a potential decline in the fitness of plants from the no-burn treatment.

Grasses showed plasticity in reproduction that is likely to be adaptive in fire-prone environments, and this plasticity depended on previous experiences of fire. A history of high fire frequency favours grass traits relating to vigorous post-fire recruitment, with the heightened flowering in annual-burn plants suggesting that flowering and seed production is stimulated by fire in these plants. Fire-stimulated flowering has been demonstrated in other savanna grass species (Howe, 1994; Ellsworth & Kauffmann, 2010), but this study represents the first documentation of fire having a priming effect on flowering in a grass. In many perennial grasslands, successful recruitment is a rare event (Defossé et al., 1997; Milton & Dean, 2000). However, fire can play an important role in population turnover (Zimmerman et al., 2008) by enhancing seedling emergence and growth through reduced below-ground competition (Bennett et al., 2002; Snyman, 2004).

Fire-prone ecosystems, including grasslands and savannas, are vulnerable to global change drivers (Dubinin, Luschekina & Radeloff, 2011; Pausas & Fernández-Muñoz, 2012), with fire regimes changing in terms of fire frequency and intensity (Andela et al., 2017). As fire behaviour influences plant traits, a consequence of such changes may be transformed functional and phylogenetic diversity of fire-prone communities, such as tropical savannas and grasslands. However, the finding here that grasses may have a 'memory' of fire, resulting in trait changes that are adaptive to frequently-burnt environments, may result in a community composition that is more resistant to future fire regime changes (Walter et al., 2013).

Acknowledgements

Research support was provided by a Natural Environment Research Council studentship (1371737) to K.J.S. and a European Research Council grant to J.K.O. (ERC-2014-STG-638333). We thank the Winston Trollope and the staff at the Department of Livestock & Pasture Science, University of Fort Hare, for allowing us to use their experimental burn plots and providing information about the plots. We also thank William Tleki for his support in the lab, and Pascal-Antoine Christin and Gavin Thomas for their insightful comments.

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Supporting Information

Figures and tables

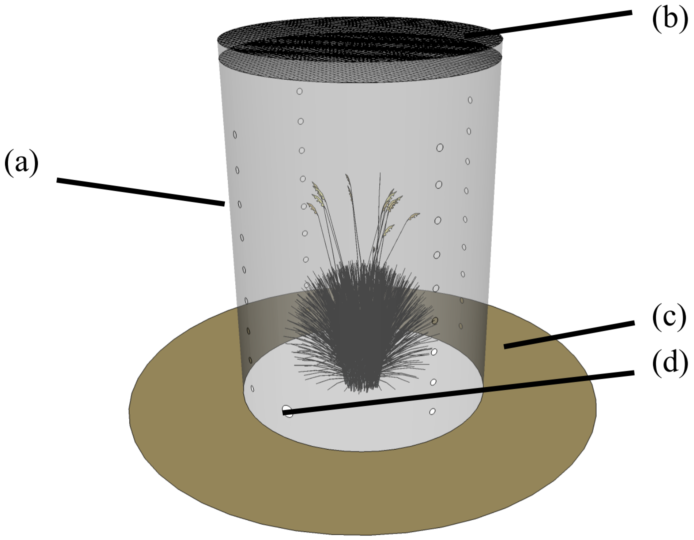

Figure S1. Schematic drawing of the set-up used to burn plants. (a) Modified, cleaned 45-gallon oil drum (85 cm tall and 61cm diameter) with the top and base removed, and a series of holes drilled in four vertical lines down its side for ventilation; (b) double layer of fine mesh fitted into the top of the drum to act as a spark arrestor; (c) wet hessian sacking placed around the base of the barrel to minimize risk of the fire spreading; (d) the ignition hole through which a lit blowtorch was applied. Each plant was carefully removed from it's pot to minimize root disturbance, and lowered into a hole (not shown). The depth of the hole was adjusted to ensure that the soil surface of all the plants was at the same height and thus burned evenly.

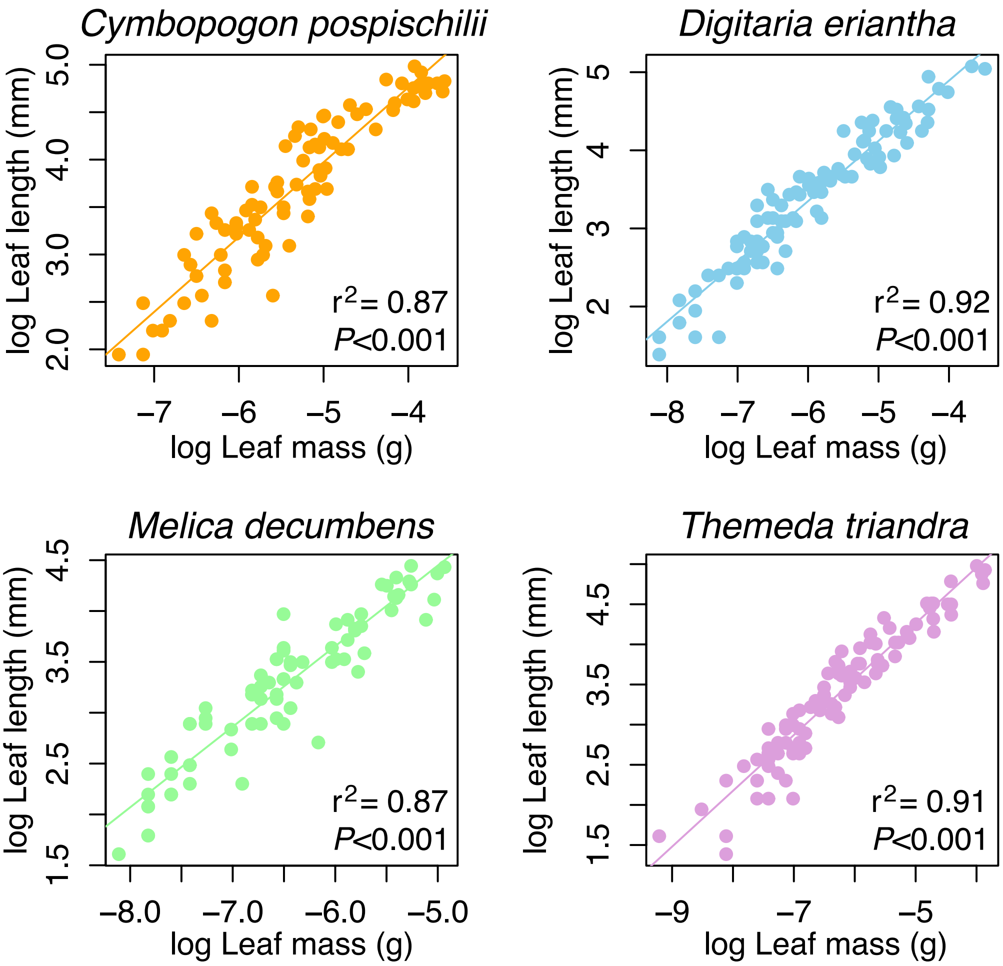

Figure S2. The relationship between leaf length and dry mass for the four studied grass species, as determined by fitting linear models to the log-transformed values of both variables. The fit of the models to the data was good (R^2 values <0.87 for all species) and the slope of these relationships was used to convert leaf length regrowth rate into leaf biomass regrowth (in mg GDD^{-1}).

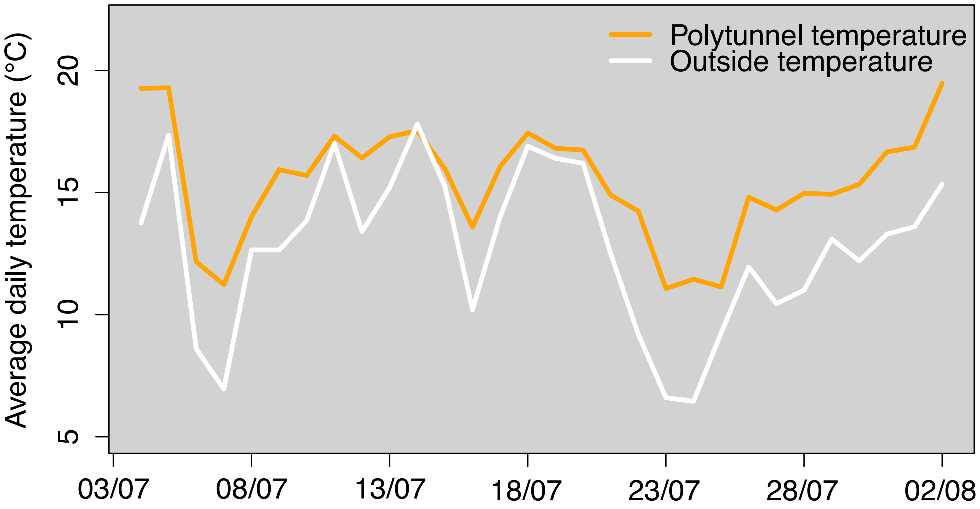

Figure S3. A comparison of temperatures inside and outside the polytunnel during the period of plant regrowth following the experimental burn. Daily average temperatures were slightly higher (2.7°C on average) in the polytunnel than outside, thus the plants experienced conditions similar to early spring without the complication of late season frosts. Minimum and maximum daily temperatures provided by the South African Weather Service (weather station 0056917 8; www.weathersa.co.za) were averaged to give the outside temperature. Temperatures inside the polytunnel were measured hourly and averaged to give daily values.

Table S1. Climate data for the Fort Hare experimental burn plots. Values represent monthly average daily minimum temperature, daily maximum temperature and rainfall for years spanning 1997 to 2015. Data was provided by the South African Weather Service (weather station 0078227A3; www.weathersa.co.za).

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Monthly rain (mm)	56.1	50.1	56.7	43.8	16.3	17.4	17.8	21.3	23.0	37.1	43.4	54.4
Daily Max. Temperature (°C)	30.1	30.0	28.6	25.6	23.7	21.4	21.3	22.8	24.4	25.7	27.0	28.5
Daily Minimum Temperature (°C)	16.8	17.2	15.4	12.3	9.3	6.7	6.1	7.4	9.0	11.6	13.4	15.5

Table S2. Details of the species used in this study and herbarium numbers of specimens of each species deposited at the Selmar Schonland Herbarium, Rhodes University.

Species	**Subfamily**	**Herbarium number**
Cymbopogon pospischilii (K. Schum.) C.E. Hubb.	Panicoideae (Andropogoneae)	GRA:Simpson s.n. 29
Digitaria eriantha Steud.	Panicoideae (Paniceae)	GRA:Simpson s.n. 27
Melica racemosa Thunb.	Pooideae	GRA:Simpson s.n. 28
Themeda triandra Forssk.	Panicoideae (Andropogoneae)	GRA:Simpson s.n. 26

Table S3. Details of reference chloroplast genomes downloaded from GenBank.

Study species	**Reference plastid species**	**GenBank reference**
Cymbopogon pospischilii	*Cymbopogon flexuosus*	NC_035040.1
Digitaria eriantha	*Digitaria exilis*	NC_024176.1
	Digitaria exilis	KJ513091.1
Themeda triandra	*Themeda* sp	KU291484.1

Table S4. Mixed model coefficients for the two initial (field-state) plant traits (plant height and total dry biomass). The intercept represents the species *Cymbopogon pospischilii* and the annual burn treatment. Dig = *Digitaria eriantha*; Mel = *Melica decumbens*; NB = no burn; SE = standard error; The = *Themeda triandra*.

	Plant height		**Total biomass**	
	Estimate ± SE	t value	Estimate ± SE	t value
(Intercept)	28. 197 ± 2.770	10.18	2.564 ± 0.122	20.99
NB treatment	8.359 ± 1.302	6.42	0.853 ± 0.093	9.17
Dig	-5.776 ± 1.677	-3.44	-0.932 ± 0.132	-7.09
Mel	5.221 ± 2.180	2.39	-1.688 ± 0.131	-12.83
The	-4.889 ± 1.498	-3.26	-0.535 ± 0.131	-4.07
log (Total dry mass)	2.311 ± 0.990	2.33		

Table S5. Mixed model coefficients for pre-fire plant traits (number of flowering tillers, proportion of total biomass in roots, total biomass (above and below ground) and plant height). The intercept represents the species *Cymbopogon pospischilii* and the annual burn treatment. Dig = *Digitaria eriantha*; Mel = *Melica decumbens*; NB = no burn; SE = standard error; The = *Themeda triandra*.

	Flowering tillers		Proportion root biomass		Total biomass		Plant height	
	Estimate ± SE	t value	Estimate ± SE	t value	Estimate ± SE	t value	Estimate ± SE	t value
(Intercept)	1.169 ± 0.165	7.05	0.583 ± 0.025	23.19	3.044 ± 0.097	31.28	3.531 ± 0.112	31.59
NB treatment	0.066 ± 0.158	-0.42	-0.109 ± 0.022	-4.84	0.068 ± 0.087	0.79	0.008 ± 0.038	0.22
Dig	0.326 ± 0.144	2.27	-0.174 ± 0.032	-5.48	0.429 ± 0.123	3.48	-0.372 ± 0.062	-6.01
Med	0.480 ± 0.149	-3.22	-0.007 ± 0.032	-0.21	-0.460 ± 0.123	-3.73	0.017 ± 0.054	0.31
The	0.096 ± 0.148	0.15	0.068 ± 0.032	2.13	-0.250 ± 0.123	-2.03	-0.288 ± 0.053	-5.37
NB Treatment ·Dig	-0.362 ± 0.213	-1.69						
NB Treatment · Med	-0.243 ± 0.212	-1.15						
NB Treatment ·The	-0.118 ± 0.215	-0.55						
log(Total dry mass)	0.571 ± 0.097	5.91					0.053 ± 0.048	1.10

Table S6. Mixed model coefficients for post-fire regrowth traits (regrown dry biomass, regrown leaf area, regrown leaf SLA and regrowth rate). The intercept represents the species *Cymbopogon pospischilii* and the annual burn treatment. Dig = *Digitaria eriantha*; Mel = *Melica decumbens*; NB = no burn; SE = standard error; The = *Themeda triandra*.

	Regrown SLA		Regrown area		Regrown mass		Regrowth rate	
	Estimate ± SE	t value	Estimate ± SE	t value	Estimate ± SE	t value	Estimate ± SE	t value
(Intercept)	10305.8 ± 796.0	12.95	6.932 ± 0.289	23.96	0.473 ± 0.047	9.96	0.046 ± 0.001	34.17
NB treatment	793.3 ± 717.9	1.11	0.049 ± 0.148	0.34	-0.016 ± 0.024	-0.68	0.0007 ± 0.001	0.83
Dig	4462.6 ± 968.0	4.61	-0.222 ± 0.199	-1.12	-0.129 ± 0.033	-3.95	-0.025 ± 0.001	-27/36
Mel	7910.3 ± 1047.8	7.55	-1.179 ± 0.213	-5.32	-0.299 ± 0.035	-8.56	-0.029 ± 0.001	-28.99
The	6080.7 ± 1020.0	5.96	-0.335 ± 0.208	-1.61	-0.168 ± 0.034	-4.97	-0.040 ± 0.001	-41.50
log(Total pre-fire dry mass)			1.098 ± 0.191	5.75	0.189 ± 0.031	6.05	0.003 ± 0.001	4.21

Table S7. Mixed model coefficients for post-fire regrowth traits (regrown dry biomass, regrown leaf area and regrowth rate) when pre-fire traits (live leaf dry biomass, total dry biomass) are the model's fixed effect. The intercept represents the species *Cymbopogon pospischilii*. Dig = *Digitaria eriantha*; Mel = *Melica decumbens*; NB = no burn; SE = standard error; The = *Themeda triandra*.

		Regrown mass		Regrown leaf area		Regrowth rate	
		Estimate ± SE	t value	Estimate ± SE	t value	Estimate ± SE	t value
Fixed effect = Live leaf dry biomass	(Intercept)	-0.384±0.106	-3.63	8.927 ± 0.116	77.05	-2.037 ± 0.220	76.66
	log(live leaf dry mass)	0.792±0.067	11.71	0.881 ± 0.072	12.15	0.890 ± 0.155	6.88
	Dig	-0.032±0.132	-0.24	0.355 ± 0.142	2.50	-0.536 ± 0.161	-27.03
	Mel	-0.578±0.164	-3.53	0.066 ± 0.176	0.37	-1.380 ± 0.183	-23.19
	The	-0.616±0.135	-4.56	-0.191 ± 0.146	-1.32	-0.782 ± 0.168	-44.59
Fixed effect = Total dry biomass	(Intercept)	-2.037±0.220	-9.25	7.097±0.242	29.34	0.047 ± 0.001	36.83
	log(total dry biomass)	0.890±0.155	5.76	0.982±0.170	5.78	0.004 ± 0.001	4.00
	Dig	-0.536±0.161	-3.33	-0.202±0.177	-1.14	-0.025 ± 0.001	-27.45
	Mel	-1.380±0.183	-7.55	-0.826±0.201	-4.12	-0.027 ± 0.001	-26.62
	The	-0.782±0.168	-4.66	-0.334±0.184	-1.81	-0.040 ± 0.001	-41.67

Chapter 5

Chapter 5

Fire-grass trait associations at a global scale

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Table of contents

Figures and tables

Statement of contributions

KJS designed the study with help from GHT, CERL and CPO. KJS sourced and generated the data, with assistance from ECJ (who provided the leaf-scale trait data) and SA (who extracted fire frequency data). KJS analyzed and interpreted the data with assistance from GHT and CPO. KJS wrote the chapter with all authors contributing critically to drafts.

Fire-grass trait associations at a global scale

Abstract

1. Fire acts as a community assembling process by selectively allowing species into frequently-burnt environments depending on their functional traits. Grassy ecosystems experience the highest fire frequencies on earth, and the distribution and success of many grass species is often linked to fire. Although there is evidence that fire can act as a local filter for grass traits, this mechanism remains untested at the global scale.

2. We used data on species occurrence, evolutionary history and fire-related functional traits in combination with the frequency and intensity of fire to investigate the relationships between fire and grass functional traits.

3. Fire frequency acts a strong selection pressure for photosynthetic type and mode of post fire persistence, with high fire frequency significantly associated with C_4 resprouter grasses. Fire intensity also influences grass traits, with high fire intensities associated with a resprouting mode of persistence and shorter statures. Furthermore, leaf traits are structured by fire, with high fire frequency selecting for low leaf nitrogen concentration which is consistent with higher nitrogen use efficiency in frequently burnt environments. We show that fire characteristics sort grasses at a global scale for specific functional traits. Understanding how fire influences the structure of grassy ecosystems is vital for understanding the impacts of anthropogenic changes in the fire regime.

Key words: Environmental filter, Drought, Fire frequency, Fire intensity, Functional traits, Phylogeny, Poaceae, Resprouting, Seeder.

Introduction

Fire regimes can shape the functional diversity of plant communities (Woodward & Diament, 1991; Keddy, 1992; Keeley & Zedler, 1998; Webb et al., 2002; Lebrija-Trejos et al., 2010), just as fire regimes are strongly influenced by the quantity and characteristics of the vegetation that fuel them (Bond & van Wilgen, 1996; Bond & Keeley, 2005; Beckage, Platt & Gross, 2009), in tandem with climate and weather (Keeley & Syphard, 2016). This disturbance can assemble communities based on functional traits that relate to persistence, recovery and flammability. In woody plant communities, there is abundant evidence of fire regimes creating functionally-clustered communities (Cavender-Bares et al., 2004; Verdú & Pausas, 2007; Ojeda, Pausas & Verdú, 2010; Bathalha et al., 2011; Myers & Harms, 2011; Cianciaruso et al., 2012; Hollingsworth et al., 2013; Shryock, DeFalco & Esque, 2014). However, in grass-dominated communities, that are responsible for the majority of fires on Earth today (Mouillot & Field, 2005), the global relationship between fire regimes, functional traits and phylogeny has yet to be explored and understood.

Fire is a multidimensional disturbance, with fire regimes varying spatially and temporally in terms of frequency, seasonality and intensity (Gill, 1975; Belcher et al., 2010; Archibald et al., 2013; Keeley & Syphard, 2016). Therefore, fire can assemble plant communities based on traits that are adaptive to a particular fire regime (Verdú & Pausas, 2007; Pausas & Verdú, 2008). Frequency and intensity are two characteristics of a fire regime relevant for plant growth and performance. Fire frequency limits periods of plant growth, whilst intensity relates to the temperature experienced by the plant, and thus both likely impact upon plant mortality and reproductive success (Moreno & Oechel, 1991; Wade, 1993; McCaw, Smith & Neal, 1997). Fire intensity and frequency are not independent, but intrinsically linked, as long fire return periods enable the development of significant fuel loads that can result in high intensity fires (Bond & van Wilgen, 1996; Archibald et al., 2013). Heightened frequency and intensity can strengthen the influence fire has on assembling communities (Cianciaruso et al., 2012; Hollingsworth et al., 2013; Forrestel, Donoghue & Smith, 2014), and spatio-temporal variability in fire regime characteristics can increase the number of species that persist in an environment (pyrodiversity begets biodiversity – Martin and Sapsis, 1992). However, whilst fire frequency and intensity can influence the functional traits of plants, the flammability of plants that burn drive the fire's characteristics. Therefore disentangling the cause and effect in plant-fire relationships is challenging.

Fire limits plant growth through the removal of biomass and heat-induced damage to plant hydraulic systems, frequently with fatal consequences (Midgley, Kruger & Skelton, 2011). Plant species persist in fire-prone environments by resprouting from surviving tissue protected from the fire, or recruiting from seedbanks (Bond & Midgley, 2001; Pausas et al., 2004). The relative benefit of these two modes of persistence (resprouter versus seeder strategies) probably depends on fire frequency and intensity (Keeley, 1986; Bellingham & Sparrow, 2000; Knox & Morrison, 2005; Pausas & Keeley, 2014), and evidence exists for fire filtering these strategies in woody plants (Cavender-Beres et al., 2004; Verdú & Pausas, 2007; Hollingsworth et al., 2013; but see Pekin et al., 2011). In places that burn frequently, seeders are susceptible to 'immaturity risk' (Zedler, 1995) due to the time it takes for an individual to reach maturity versus the likelihood of fire, and thus the seeder strategy becomes more viable as fire frequency decreases. In contrast, resprouters are predicted to dominate in productive, frequently burnt environments. Here, stored resources that support a bud bank enables rapid post-fire regrowth. Seeders are predicted to do better than resprouters in places that experience more intense fires. This is because the seeds of many fire-prone seeder species are protected from the heat of the fire (Keeley & Fotheringham, 2000), for example through a hard seed coat or serotinous cone, and are thus much less vulnerable to heat-related damage than adult resprouting plants (Pausas & Keeley, 2014). In addition, the likelihood of mortality for mature plants increases with fire intensity, meaning resprouting is less viable.

Fire alters the environment and local availability of resources such that the conditions before fire are very different to those afterwards. Therefore fire can indirectly filter for traits related to surviving in and exploiting post-fire environments (Cavender-Bares & Reich, 2012; Forrestel, Donoghue & Smith, 2014), which are characterised by high light (Knapp & Seastedt, 1986), elevated surface temperatures (Sharrow & Wright, 1977; Raison et al., 1986; Santana et al., 2010) and a flush of nutrients (Viro, 1974; Vitousek & Howarth, 1991). Fire frequency dictates the number of times that resprouting individuals experience these post-fire environments during their lifetimes, and thus the fitness benefits of traits related to rapid post-fire recovery.

Grass species dominating tropical grasslands and savannas experience the highest fire frequencies of any biome (Mouillot & Field, 2005), and indeed these biomes are largely maintained by fire (Bond, Midgley & Woodward, 2005; Beckage, Gross & Platt, 2011). However, in comparison to woody fire-prone species, the role of fire in influencing functional traits in grasses has received less attention. The grass functional traits that are most likely to be associated with frequent fire enable rapid post-fire recovery (e.g. high specific leaf area (SLA), low leaf nitrogen (N), high leaf C/N ratio; Forrestel, Donoghue & Smith, 2014). Other traits that may be influenced by fire are photosynthetic pathway, plant height and the mode of post-fire persistence (i.e. seeder vs resprouter strategies). The C_4 photosynthetic pathway may be advantageous in fire-prone grasslands (Tix & Charvat, 2005; Ratnam et al., 2011), in which higher productivity allows plants to regrow rapidly after fire, and higher water- and N-use efficiencies may be beneficial in the warm, dry, low-N, post-fire environment (Knapp & Medina, 1999; Long, 1999; Sage, 2004; Keeley & Rundel, 2005). Plant height in grasses has been recently linked to fire frequency, with taller grasses associated with places that burn more frequently (Lehmann et al., in prep), suggesting that this may be another plant trait influenced by fire.

Here we use a dataset of the functional traits and geographical distribution of 1091 grass species to examine links with fire regimes. We focus on the influence of fire on grass traits here but acknowledge that the dual direction of fire-plant interactions and the difficulty in determining causation. We quantified the associations between fire frequency, fire intensity and multiple functional traits (mode of persistence, photosynthetic pathway, culm height, leaf N content, leaf C/N ratio, and SLA), hypothesizing a number of specific relationships (Table 1).

Table 1. Predicted relationships between the functional traits of grasses and fire characteristics. '+' indicates a positive relationship and '-' a negative relationship. Indirect relationships are in parentheses.

Trait	Unit	Relationship with fire frequency	Relationship with fire intensity	Functional significance for fire tolerance	Functional significance for promoting fire
Maximum culm height	m	+	(-)	Positively related to competitive vigour and disturbance tolerance	Promotes an aerated fuel bed and increases fire spread probability
Mode of post-fire persistence	*Categorical*:			Strategy for persisting in a fire-prone environment	
	Resprouter	+	-		
	Seeder	-	+		
Photosynthetic pathway	*Categorical*:			Productivity, water- and nitrogen-use efficiency. Shade intolerant C_4 grasses require frequent defoliation	Rapid regrowth can increase fuel accumulation and fire frequency
	C_3	-	(+)		
	C_4	+	(-)		
Specific leaf area	mm^2 mg^{-1}	+	(-)	Related to resource uptake	Rapid regrowth can increase fire frequency
Leaf N concentration	mg g^{-1}	-	(+)	Nitrogen-use efficiency	
Leaf C/N ratio		+	(-)		Related to decomposition rate and the accumulation of a flammable fuel load

Materials and methods

GRASS SPECIES OCCURRENCES

We extracted all georeferenced occurrence records (~10.2 M records) for Poaceae taxa from the Global Biodiversity Information Facility (GBIF) web portal (http://www.gbif.org/) via the R statistical computing package "rgbif" (Chamberlain et al*.,* 2014; data accessed 18th January 2014). This and all statistical analyses were carried out in the R environment (R Core Team, 2013). The extracted data consisted of four fields: species name, longitude, latitude and year.

Steps were taken to control the quality and suitability of the occurrence records. First, species names were standardized against the Kew grass synonymy database (Clayton et al*.,* 2006) using the software package Taxonome (Kluyver & Osborne, 2013), and records were discarded if they could not be given an accepted species name. Second, longitude and latitude data were checked to ensure values were sensible (e.g. removal of records with: reversed longitude/latitude values, longitude/latitude values of 0, and those within 20km of the GBIF headquarters) and were recorded with at least three decimal places, to represent the small scale over which fire regimes change. Finally, to ensure that records represent individuals grown in a natural environment, and are therefore subject to an evolutionarily relevant fire regime, domesticated grass species were excluded (Meyer, DuVal & Jensen, 2012; Kluvyer, 2013) and all other species records were filtered to only include those falling within Kew GrassBase distributions (Clayton et al*.,* 2006), mapped to the TDWG level 3 regions (Brummitt et al*.,* 2001). Records from highly transformed landscapes, where humans have greatly altered fire regimes through changes in ignition frequency and fuel properties, were also removed from the dataset. To do this, records from protected areas were identified (using the World Protected Areas map; http://www.wdpa.org/) and kept. For the remaining records, a human influence index (HII) value was obtained (Sanderson et al., 2002). This indicates how impacted the landscape is by human activities by incorporating information on population density, human access, land-use and infrastructure. Records with a HII value above 30 are from highly transformed landscapes in terms of fire characteristics (Archibald et al., 2013) and were excluded. Records made before 1980 were removed. This cut off represents a compromise between removing records taken before satellite-derived fire characteristic datasets began and not removing a large proportion of the total records. After all of these cleaning steps, species were excluded if they were sampled by a low (<50) number of unique occurrence points. This stringent data cleaning process resulted in a dataset containing 1091 species, whose distribution covers the majority of tropical and subtropical grassland landscapes (see figure S1 for the distribution of occurrence data).

FIRE CHARACTERISTICS

Fire frequency

The MODIS global monthly burnt area (MCD45A1) satellite data product provides fire data for the Earth's surface at a 500-metre resolution. By utilising the characteristic changes in surface reflectance following a fire (deposits of charcoal and ash, removal of vegetation), the MODIS algorithm uses changes in daily surface reflection inputs to detect the approximate date of burning (accurate to within 8 days; Royer et al., 2005).

Following the methods of Archibald et al. (2010), fire date information was extracted for each GBIF location for the 14-year dataset (April 2000 to March 2014) and used to calculate inter-fire intervals. A Weibull distribution was fitted to the interval data for each species using the "survival" package in R (Therneau, 2015). The first and last fires of the MODIS dataset were open-tailed (i.e. unconstrained interval) and the survival analysis allows these open-tailed intervals to be included, maximising the information provided. Estimations of the Weibull shape and scale parameters and their confidence intervals were determined for each species using data from pixels that burnt at least once during the 14-year period. The shape and scale parameters were used to determine the median fire interval (MEI) using the equation from Moritz et al. (2009):

$$\mathrm{MEI} = \mathrm{b}(\ln 2)^{\frac{1}{\mathrm{c}}}$$

where b is the scale parameter and c is the shape parameter. For species in infrequently burnt places (i.e. where the fire return period is much greater than the 14 years of the MODIS dataset), the algorithm would converge either on an unrealistically large MEI or not converge at all. We focused on species where fire is a major disturbance and frequent enough to act as a selective pressure over their range, and hence we defined whether species were 'fire-exposed' or not. To do this, the proportion of records that fell within pixels that burnt, relative to the total number of records for each species, was used. This proportion was plotted against the number of species and a break-point regression fitted to the curve (Fig. S2) using the "segmented" library (Muggeo, 2003). An estimated break-point value of 0.16 was calculated and species which had <16% of their occurrence records in burnt locations were therefore excluded from fire frequency analyses. The impact of any spatial bias in the records on the proportion burnt was tested by subsetting the records such that there was a maximum of 3 records per species in each 10km^2 grid cell. This had a negligible effect on proportion values, with little difference seen between the original (mean=0.282; median=0.139) and subsetted datasets (mean=0.282; median=0.142), suggesting there is no pervasive spatial bias in the original dataset. Finally, any species with fire-return intervals that were not expected for grass-dominated ecosystems (FRI values over 100 years or less than one year) were removed.

Fire intensity

Values of fire radiative power (FRP), or the rate of radiant energy released, can be obtained from satellite measurements of middle infrared emission over actively burning areas. FRP is frequently used as a proxy for fire intensity (Dwyer et al., 2000; Archibald et al., 2013), and is available globally from the MODIS global monthly fire location product (MCD14ML). This product maps active fires at 1 km resolution and calculates the rate of radiative energy emitted per pixel.

FRP values (in megawatts per 1 km pixel) were extracted for all active fire points over the duration of the dataset (2002-2015) for each record. Any FRP values with a detection confidence <50% were discarded. These data were then grouped by species, and the 95th quantile extracted. There is typically a bias towards low FRP values, due to the high variation in this measurement over the duration of a fire (Dwyer et al., 2000) and low values during the night. The 95th quantile was therefore used, as done elsewhere (Archibald et al., 2013), to indicate the maximum value that a headfire could attain in a particular environment and to avoid errors caused by outliers. The 95th quantile also corresponded strongly with median values and the range of values across all species.

PRECIPITATION AND SEASONAL DROUGHT

One difficulty in determining the role of fire as a community assembling process is that fire characteristics are often correlated with, and covary with, climate. To address this issue, we also controlled in the analysis for climatic factors that are commonly associated with fire regime.

High fire frequencies typically occur in areas with high rainfall, where greater and more spatially connected fuel loads are produced (Pausas & Bradstock, 2007; Pekin et al., 2011). The WorldClim dataset (www.worldclim.org; accessed February 2016) was used to obtain values for mean annual precipitation values for each record and this was averaged for each species.

Fire is common in seasonally dry environments and is thus linked to drought characteristics, such as length and intensity. High intensity droughts create dry fuels, which will ignite readily and burn more intensely, but drought length will also negatively influence the fuel load, so the relationship is not straightforward. Therefore a proxy for seasonal drought intensity (precipitation of the driest quarter; BIO17) was obtained from the WorldClim dataset for each record, and the 5th quantile for each species was extracted. Seasonal drought length was calculated using the WorldClim mean annual precipitation dataset as the number of successive months where mean precipitation was below 30mm for each record. The 95th quantile was extracted for each species.

PLANT TRAITS

Species-level plant traits (maximum culm height, photosynthetic type, mode of post-fire persistence, SLA, foliar N content and foliar C/N ratio) were collated from a number of sources.

Data on maximum culm height was acquired from the Kew Grassbase dataset (Clayton et al., 2006); and photosynthetic type from Osborne et al. (2014). Mode of post-fire persistence was collected from a literature review and available databases (e.g. FEIS-USDA Forest Service; Crowley, Gardner & Marrinan, 2007; Paula et al., 2009). We classed species as either 'seeders' or 'resprouters' based on the response to fire, as opposed to clipping or other disturbances. Resprouters were defined in a number of ways depending upon the type of data available in the literature. For categorical data, resprouting species were defined as those where resprouting is the main post-fire persistence strategy. For quantitative data, species that experienced <30% mortality when subjected to 100% leaf scorch were classed as resprouters (*sensu* Crowley, Gardener & Marrinan, 2007). Conversely, a species was classified as a seeder if the main post-fire persistence strategy was from seeds, or if plants of this species experienced >70% mortality when subjected to 100% leaf scorch. All species names were checked for synonymy and misspellings using Taxonome (Kluyver & Osborne, 2013). The resulting dataset consisted of 649 taxa.

Species level "leaf economic spectrum" traits (specific leaf area (SLA), foliar N content and foliar C/N ratio) were obtained from a dataset of ~350 grass species (Jardine, 2017) that had been derived from herbarium specimens. A principal components analysis (using the "princomp" function; R Core team, 2013) collapsed the variance in these three leaf traits into two axes. Together these two axes accounted for 98.7% of total variation (69.1% on dimension 1; 29.6% on dimension 2; Figure S2). Foliar N content and C/N ratio loaded most heavily on dimension 1, with N content being positively correlated (R^2=0.88; P<0.001) and C/N ratio being negatively correlated (R^2=0.82, P<0.001) with this dimension. SLA loaded heavily in dimension 2, and was positively correlated with this dimension (R^2=0.69, P<0.001; Figure S2).

PHYLOGENY

To account for evolutionary relationships among grass species, we used a published grass phylogeny (Spriggs, Christin & Edwards, 2014), that incorporated 3595 taxa and was constructed from 14 separate phylogenies (each representing a main grass lineage) combined with a well-resolved backbone phylogeny (GPWG II, 2012; see Spriggs, Christin & Edwards, 2014 for details). This phylogeny was subsetted for each trait analysis to include only the species for which there were data.

DATA ANALYSIS

Several variables were log-transformed (fire intensity, maximum culm height, mean precipitation, seasonal drought length and seasonal drought intensity) and fire return interval was box-cox transformed (using the "MASS" R package; Venables & Ripley, 2002) to improve normality. The relationships between fire characteristics (frequency and intensity) and plant traits and precipitation variables were analysed using phylogenetic generalised least squares with the ‘pgls’ function (“caper” package; Orme et al., 2012). The mode of post-fire persistence is predicted to interact with the seasonal drought variables (Pausas et al., 2016), so interaction terms between this trait and seasonal drought length or seasonal drought intensity were included in the models. However, the interactions with seasonal drought intensity were not significant for either fire characteristic, and were therefore removed to simplify the model. Two sets of models were constructed to account for the substantial differences in sampling for different traits. Specifically, sample size was limited by the availability of leaf economic spectrum trait data. The ‘reduced’ dataset excluded leaf economic spectrum traits, and so contained fewer traits but more species, while the ‘complete’ dataset included all traits but fewer species.

To investigate the relationship between trait values and evolutionary history, we tested for the presence of phylogenetic signals in plant traits, fire and seasonal drought characteristics and presence/absence in ‘fire-exposed’ environments. For continuous data, Pagel’s λ was estimated using the ‘pgls’ function （“caper” package; Orme et al., 2012). For categorical or binary data (photosynthetic pathway, mode of post fire persistence and presence/absence in fire-prone environments), we estimated the phylogenetic D statistic using the ‘phylo.d’ function in the “caper” package.

Results

FIRE SHAPES GRASS TRAITS

The reduced model (excluding leaf economic spectrum data) shows that fire frequency is strongly associated with certain grass functional traits, particularly photosynthetic type and mode of post-fire persistence (Table 2). High fire frequency is significantly linked with C_4 ($P=0.007$; Fig. 1), resprouter ($P<0.001$) grass species. Resprouters experience more frequent fire, with a median fire interval 2.3 months shorter than seeders (7.33 years vs 7.52 years); likewise C_4 species experience a much higher fire frequency than C_3 species (5.79 years vs 11.87 years fire return period). Plant height is not significantly related with fire frequency.

Fire intensity also influences grass traits, specifically plant height and mode of post-fire persistence, with high fire intensities associated with shorter ($P<0.001$), resprouting ($P<0.001$) species. Resprouters experience on average 40% higher fire intensity than seeders (111.4 vs 80.5 MW km^{-2}).

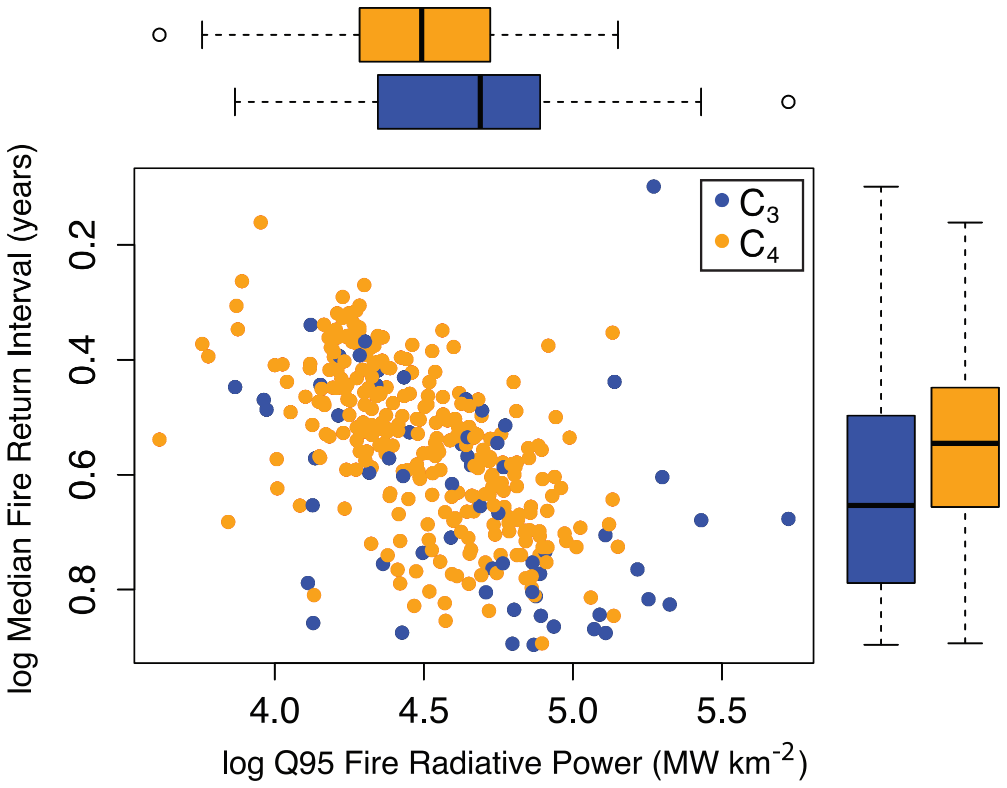

Figure 1. Fire characteristics influence grass photosynthetic pathway. C_4 species are associated with significantly higher fire frequencies compared to C_3 species ($P=0.007$). Fire intensity (fire radiative power) did not significantly filter grass species by photosynthetic type.

Table 2. Multiple linear regression (excluding leaf economic spectrum trait PCA axes) to determine the contributions of plant traits and environmental variables to fire characteristics. Together these significantly predicted the fire frequency ($F_{7,136}$=17.57, P<0.001, R^2=0.45) and intensity ($F_{7,324}$=21.47, P<0.001, R^2=0.30) experienced by each grass species. Fire frequency is characterized as the reciprocal of the median fire return interval, whereas fire intensity is the 95th quantile of fire radiative power values at locations where each species has been recorded. Models were implemented using phylogenetic generalized least squares. Significant model terms (P<0.05) are in bold. SE, standard error; '*', P<0.05; '**', P<0.01; '***', P<0.001

	Fire frequency		Fire intensity	
	Estimate ± SE	t value	Estimate ± SE	t value
Maximum culm height	-0.016 ± 0.016	-1.00	**-0.103 ± 0.029**	**-3.51 *****
Photosynthetic type	**0.081 ± 0.029**	**2.71 ****	-0.066 ± 0.053	-0.21
Mode of postfire persistance	**-0.464 ± 0.155**	**-3.00 ****	**0.325 ± 0.074**	**4.37 *****
Mean annual precipitation	**0.150 ± 0.025**	**6.13 *****	-0.005 ± 0.050	-0.10
Drought length	-0.129 ± 0.073	-1.76	**0.071 ± 0.010**	**6.95 *****
Drought intensity	**-0.028 ± 0.008**	**-3.26 ****	0.027 ± 0.014	1.90
Drought length * Mode of postfire persistance	**0.203 ± 0.068**	**2.98 ****	**-0.028 ± 0.009**	**-3.09 ****
Intercept	-0.168 ± 0.287	-0.59	**4.388 ± 0.388**	**11.32*****

The interaction term between mode of post-fire persistence and seasonal drought length is significant in the models for fire frequency (P=0.003) and intensity (P=0.002; Fig. 2; Table 2). Resprouters experience higher intensity and more frequent fires across the range of drought length than seeders, with this difference between modes of post-fire persistence lessening as drought length increases (Fig. 2).

Adding leaf economic spectrum traits into the fire frequency and intensity models greatly reduces the sample size of both, which in turn affects the significance of variables, as can be seen between a comparison of Table 2 and S1 (where sample size was reduced to the same number of species in the full dataset). Photosynthetic type was heavily skewed towards C_4 species (30/32) in the complete dataset and so was removed from the model. Fire frequency is significantly negatively associated with PCA axis 1, but is not associated with axis 2 (Table 3). This shows that frequent fires are associated with low leaf N content and high leaf C/N ratio (P=0.006), but that SLA is unaffected. Neither PCA axis nor any plant trait was associated to fire intensity (Table 3).

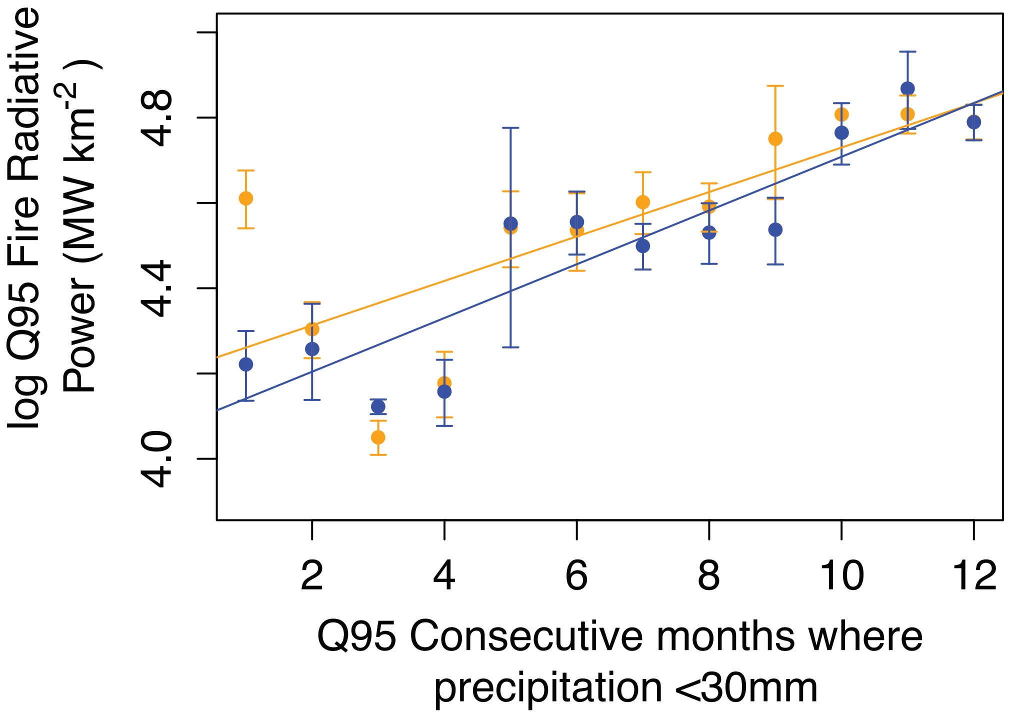

Figure 2. The effect of mode of post-fire persistence on the relationship between fire intensity and seasonal drought length. Points represent mean values per month and 95% confidence intervals for resprouters (orange) and seeders (blue). The relationships between the two variables differed significantly between resprouters and seeders such that resprouters experienced higher fire intensities across the range of drought lengths in comparison to seeders (P=0.002)

Table 3. Multiple linear regression (including leaf economic spectrum trait PCA axes) to determine the contribution of plant traits and environmental variables to fire characteristics. Together these significantly predicted fire frequency ($F_{8,30}$=6.451, P<0.001, R^2=0.53) and intensity ($F_{8,30}$=4.29, P<0.001, R^2=0.35) experienced by each grass species. Fire frequency is characterized as the reciprocal of median fire return interval, whereas fire intensity is the 95th quantile of fire radiative power values at locations where each species has been recorded. Models were implemented using phylogenetic generalized least squares. (P<0.05). SE, standard error; '*', P<0.05; '**', P<0.01; '***', P<0.001

	Fire frequency		Fire intensity	
	Estimate ± SE	t value	Estimate ± SE	t value
Maximum culm height	0.051 ± 0.029	1.78	-0.070 ± 0.069	-1.01
Mode of postfire persistance	-0.462 ± 0.874	-0.53	**1.217 ± 0.571**	**2.13 ***
PCA axis 1	**-0.032 ± 0.011**	**-2.98 *****	-0.017 ± 0.035	-0.50
PCA axis 2	-0.014 ± 0.022	-0.64	0.001 ± 0.054	0.01
Mean annual precipitation	**0.144 ± 0.053**	**2.72 ***	**-0.436 ± 0.135**	**-3.21 *****
Drought length	-0.076 ± 0.361	-0.21	0.464 ± 0.273	1.69
Drought intensity	**-0.035 ± 0.015**	**-2.34 ***	0.025 ± 0.034	0.74
Drought length * Mode of postfire persistance	0.169 ± 0.357	0.47	-0.490 ± 0.264	-1.86
Intercept	-0.509 ± 1.039	-0.49	6.471 ± 1.164	5.56 ***

FIRE CHARACTERISTICS

The species-level median fire-return interval is 5.2 years, which matches closely with the estimated mean value for tropical grasslands and savannas (5.5 years for the period 1900-2000; Mouillot & Field, 2005). Fire intensity values ranged from 22 to 350 MW per 1 km pixel. These values are considered 'low' to 'medium' fire intensities (category 1 or 2 *sensu* Ichoku et al., 2008), which is consistent with what is expected from grass-fuelled surface fires (Archibald et al., 2013).

As predicted, fire frequency and intensity are inversely correlated, such that places that burn frequently do so at a lower intensity ($F_{1,436}$=186.4, $P<0.001$; R^2=0.30; Fig. 2 and 3). The two fire characteristics also correlate with precipitation, such that high mean annual precipitation is associated with frequent ($F_{1,435}$=127.2, $P<0.001$, R^2=0.23) and low-intensity ($F_{1,435}$=309.5, $P<0.001$, R^2=0.41) fires (see Fig. S3). Likewise, fire intensity and frequency are correlated with seasonal drought characteristics, although less variation is accounted for (Fig. S4). Fire is most frequent in places that experience short ($F_{1,436}$=18.86, $P<0.001$, R^2=0.04) and intense ($F_{1,436}$=37.06, $P<0.001$, R^2=0.07) dry seasons, presumably because a long wet season allows a large fuel load to accumulate, which then dries in the short, intense dry season. On the other hand, highly-intense fires occur where seasonal droughts are long ($F_{1,436}$=82.4, $P<0.001$, R^2=0.16) and less intense ($F_{1,436}$=16.84, $P<0.001$, R^2=0.03).

The significance of rainfall and drought effects on fire characteristics is apparent from the multiple regressions. Mean annual precipitation is the strongest contributor to fire frequency ($P<0.001$; Table 2) but is unrelated to fire intensity. Seasonal drought intensity ($P=0.002$) is more important in determining fire frequency than drought length ($P=0.08$). Conversely, seasonal drought length ($P<0.001$) is a significant contributor to fire intensity, but seasonal drought intensity only marginally so ($P=0.06$).

PHYLOGENETIC SIGNAL

There is a phylogenetic signal in all of the plant traits, fire and drought characteristics (Table S2), with closely related grass species possessing more similar traits and residing in similar fire and seasonal drought regimes than more distantly related species. Whether a species resided in a fire-exposed environment or not also has a strong phylogenetic signal (Table S2).

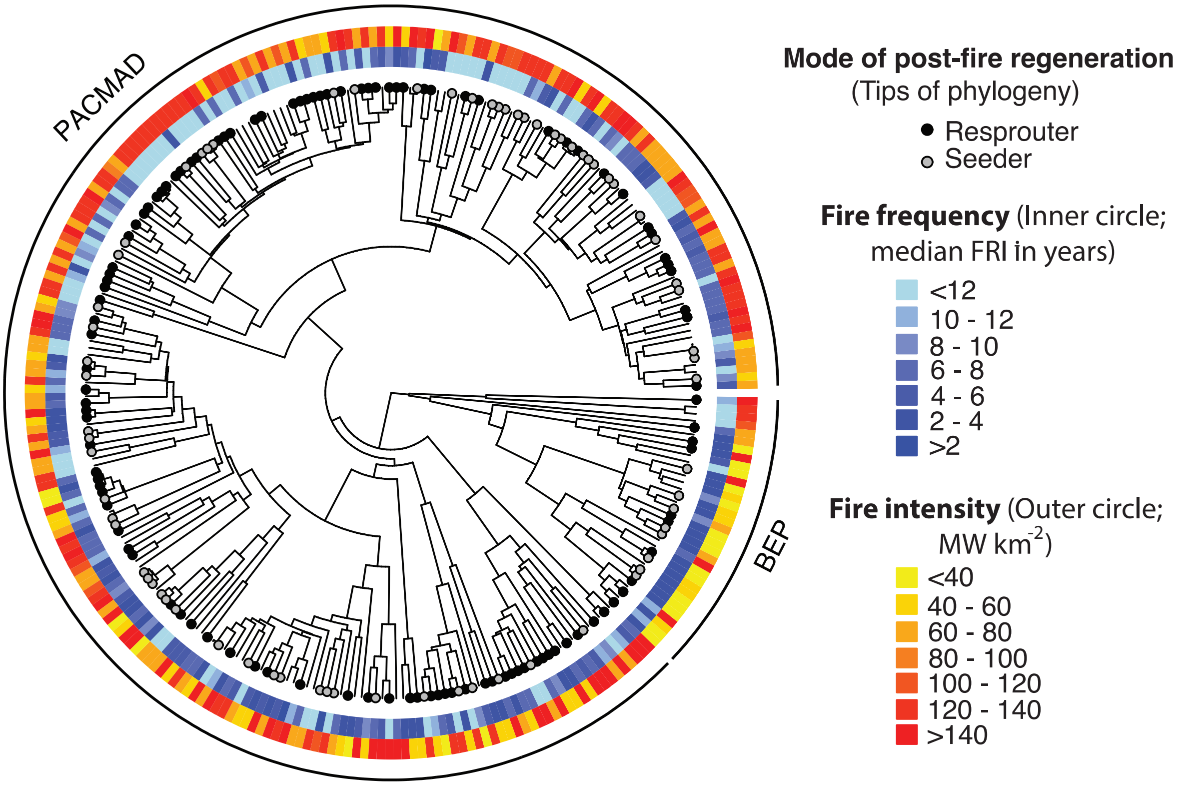

Figure 3. Fire characteristics and mode of post-fire persistence across the grass phylogeny. From the centre, the black tips of the phylogeny indicate resprouter species, and the grey tips indicate seeder species. The inner circle represents fire frequency values, measured as the median fire return interval (FRI) in years. The outer circle shows values of fire intensity, measured as fire radiative power (MW km^{-2}). Species shown are those with data for both fire frequency and intensity. PACMAD and BEP are the two major clades of Poaceae (grasses)

Discussion

Grasses are an important, fire-prone plant family that fuel the majority of fires on Earth (Mouillot & Field, 2005), and thus provide an opportunity to explore the influence of fire on functional diversity in a globally distributed herbaceous group. We found evidence that fire frequency and intensity shape the functional diversity of grass floras globally by acting as a strong driving force in determining species assemblages based on their traits. However, the plant-fire relationship is bidirectional, with plants also influencing fire through their flammability (Bond & van Wilgen, 1996; Beckage, Platt & Gross, 2009; Archibald et al., 2017). Disentangling effect and response traits in grass-fire interactions still therefore requires further work.

We found that C_4 species had shorter fire return times than C_3 species, suggesting that the C_4 pathway provides advantages over the C_3 type in fire-prone environments (Tix & Charvat, 2005; Ripley et al., 2010; Ratnam et al., 2011). C_4 species in tropical grasslands and savannas are highly productive, as afforded by their photorespiration-limiting CO_2 concentrating mechanism, which may result in faster seedling establishment and growth (Ripley et al., 2010; Atkinson et al., 2016), or the accumulation of large stored resources for post-fire resprouting (Knapp & Medina, 1999; Sage, 2004). Combined with this are the high water- and nitrogen-use efficiencies of C_4 species, which may be crucial in the high light, nitrogen-limited post-fire environment (Long, 1999; Keeley & Rundel, 2005). While experimental work using a small set of species found that phylogenetic lineage was more important than photosynthetic type in determining post-fire recovery (Ripley et al., 2015), our data showed that fire frequency is strongly related to photosynthetic pathway. This difference probably reflects the fact that our dataset sampled an order of magnitude more species and included a larger number of grass lineages than the previous work. However, the dependence of fire regime upon fuel quantity and quality adds complexity to this finding, as C_4 grasses typically produce large, dry fuel loads that in turn promotes intense, frequent fire (Ripley et al., 2015; Simpson et al., 2016). The C_4 photosynthetic pathway is associated with frequent fires because it provides advantages in tolerating fire as well as it results in flammable plants. An alternative explanation is that the environments occupied by C_4 grasses are more fire-prone than those occupied by C_3 grasses, meaning the relationship is not causal, but mediated via correlations with a third factor.

Grass leaf traits are associated with fire frequency at a global scale in a way that is consistent with observations at a community scale in grasses (Forrestel, Donoghue & Smith, 2014) and other fire-prone taxa (Cavender-Bares & Reich, 2012). Fire filters for traits related to living in the post-fire environment, which is characterised by nitrogen-limitation (Reich et al., 2001; Hernández & Hobbie, 2008; but see Coetsee, February & Bond, 2008), high light and frequently high competition (Vogl, 1974; Vitousek & Howarth, 1991). Our findings are consistent with grasses adopting a high nitrogen-use efficiency (low leaf nitrogen concentration) in places that burn frequently (Wedin & Tilman, 1990; Reich et al., 2001). High fire frequency was also associated with high leaf C/N ratios, which is associated with slow foliar decomposition (Aerts, 1997). High C/N ratios therefore facilitate fuel accumulation which, in turn, is associated with higher combustion rates and longer fire residence times (Simpson et al., 2016).

We predicted that plant height would correlate positively with fire frequency, with taller plants, that are more competitive and tolerant of disturbance, being associated with frequent fire (Weiher et al., 1999; Keith et al., 2007). However, we actually found that plant height was unrelated to fire frequency. Similarly, Forrestel and colleages (2014) found no difference in plant height between grass communities burnt at different frequencies, which suggests that this trait is not influenced by fire frequency. In contrast, studies investigating the effects of fire frequency on plant height in woody species found that plants were taller under low fire frequencies (Cavender-Bares & Reich, 2012; Cianciaruso et al., 2012; Shryock et al., 2014), with heightened competition in infrequently burnt sites suggested as the mechanism. The divergence in height response to fire between woody and herbaceous taxa emphasises the importance of investigating both plant groups and the danger of extrapolating findings across diverse, fire-prone taxa. The strong phylogenetic signals in grass maximum culm height and fire frequency may result in closely related species, of similar heights, living in similar fire regimes, as implied by global mapping (Lehmann et al., in prep).

Interestingly, there was a significant negative association between plant height and fire intensity, such that grass species experiencing high intensity fires are shorter. This could be for reasons independent of fire, such as, for example, a consequence of shorter grasses being associated with places with longer dry seasons (which tend to burn with higher intensities). On the other hand, this relationship between fire intensity and grass height may instead be a consequence of fuel structure driving differences in landscape flammability. For grasses, biomass quantity and density are instrumental to combustibility (i.e. the rate of heat release during fire; Simpson et al., 2016), a component of flammability that relates to fire intensity. Grass density is positively correlated with combustion rate (Simpson et al., 2016); therefore short grasses that form dense fuel beds may be associated with high intensity fires.

We find evidence that post-fire persistence traits sort along gradients of fire frequency and intensity, with resprouters associated with both higher fire frequencies and intensities. This is consistent with the finding that fire-prone grasslands globally tend to be dominated by resprouting species (Allan & Southgate, 2002; Bond, Midgley & Woodward, 2003; Overbeck & Pfadenhauer, 2007). The association between resprouters and frequent fire may not just be a product of filtering because rapidly regenerating resprouters may also provide fuel loads that fuel frequent fires (Archibald et al., 2013). Fire intensities tend to be relatively low in grass-dominated ecosystems (Archibald et al., 2013), potentially explaining why we found less correlation between plant traits and fire intensity, than with frequency (Trollope, Trollope & Hartnett, 2002; Uys, Bond & Everson, 2004; Peláez et al., 2013).

Both fire characteristics and species absence/presence within fire-prone environments showed strong phylogenetic signals, showing that closely related grass species experience more similar fire regimes than more distantly related ones. This is consistent with previous findings that grass clades sort along fire gradients (e.g Uys, Bond & Everson, 2004; Visser et al., 2012; Forrestel, Donoghue & Smith, 2014). Phylogenetic signals in seasonal drought also suggest grass species distributions are influenced by both fire and climate, and that these factors co-vary (Archibald et al., 2013). All plant traits investigated here showed phylogenetic signals; therefore the filtering of phylogenetically-conserved traits by fire may result in phylogenetically as well as functionally clustered communities (Webb et al., 2002). Indeed, fire has been shown to produce such patterns in plant communities (Verdú & Pausas, 2007; Ojeda, Pausas & Verdú, 2010; but see Silva & Batalha, 2010; Cianciaruso et al., 2012), including grassland communities (Forrestel, Donoghue & Smith, 2014). However, caution must be exercised when discussing analyses of phylogenetic signal when a small proportion of the total number of species are sampled (1-14% of ~12,000 recognised grass species, depending upon the trait) due to low taxonomic coverage and statistical power.

Fire-prone environments tend to be seasonally dry, with fires occuring during the dry season when fuel is dry and ignitions more likely to be successful. Seasonal drought is thus also likely to influence plant traits in fire-prone environments, particularly the mode of post-fire persistence. We found that seeder species are associated with more intense and longer seasonal droughts than resprouters. Resprouters tend to be dehydration avoiders, that regulate moisture levels via stomatal control and leaf shedding (Pausas et al., 2016). However carbon deficits may arise from prolonged stomatal closure, leading to an inability to maintain the costs of an extensive root system (Plaut et al., 2012). Resprouters are therefore associated with shorter droughts (Pausas & Bradstock, 2007; Pausas et al., 2016), as found here.

With both fire regimes and rainfall patterns anticipated to alter considerably in the coming decades (Settele et al., 2014), accurate predictions of vegetation responses to environmental change are required. Understanding how grass communities will respond to these changes is fundamental to protecting the functional diversity of this important plant group. Here we have identified global patterns in grass community assembly based on their traits, and find evidence that fire characteristics closely relate to post-fire persistence traits and flammability traits.

Acknowledgements

Research support was provided by a Natural Environment Research Council studentship to KJS, a Project Sunshine studentship to ECJ, and a Royal Society University Research Fellowship

UF120016 to GHT.

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Supporting Information

Figures and tables

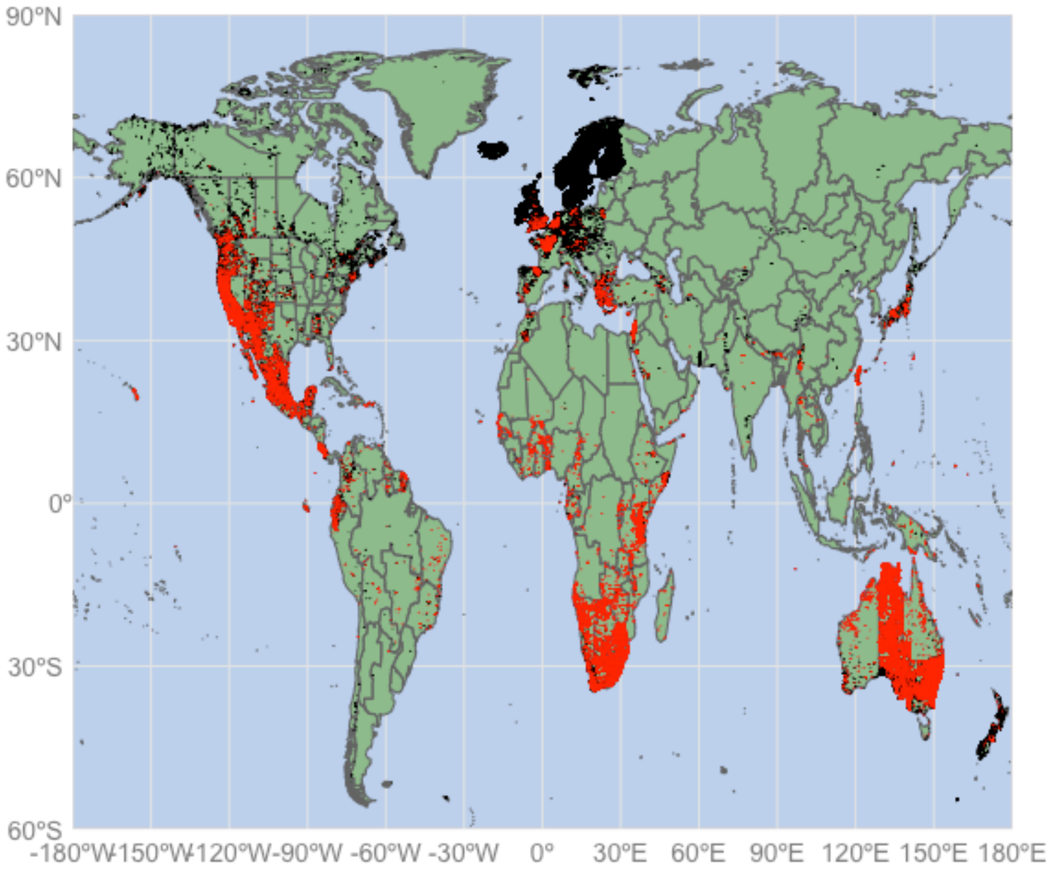

Figure S1. The distribution of occurrence data acquired from GBIF. Red points represent the occurrence of a 'fire exposed' species, whilst black dots are 'fire absent' species.

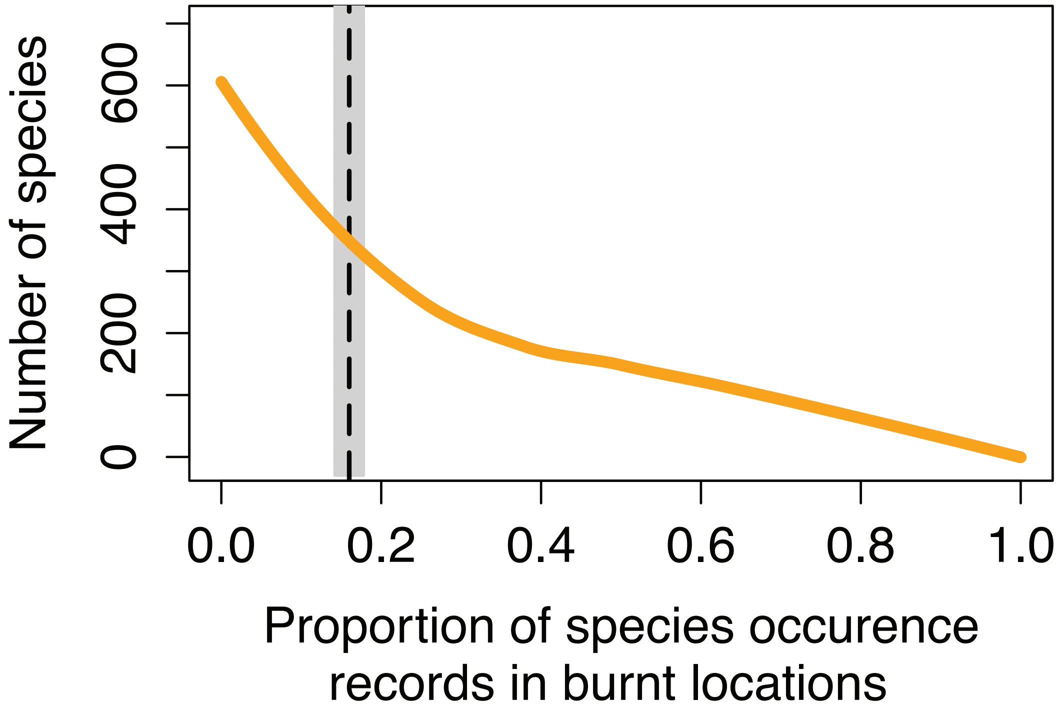

Figure S2. Proportion of occurrence records that fell within pixels that burnt (relative to the total number of records) against species frequency. The estimated breakpoint (0.16; used to distinguish whether species are ‘fire exposed’ or ‘fire absent’) is shown by the dashed line (and 95% confidence intervals in grey).

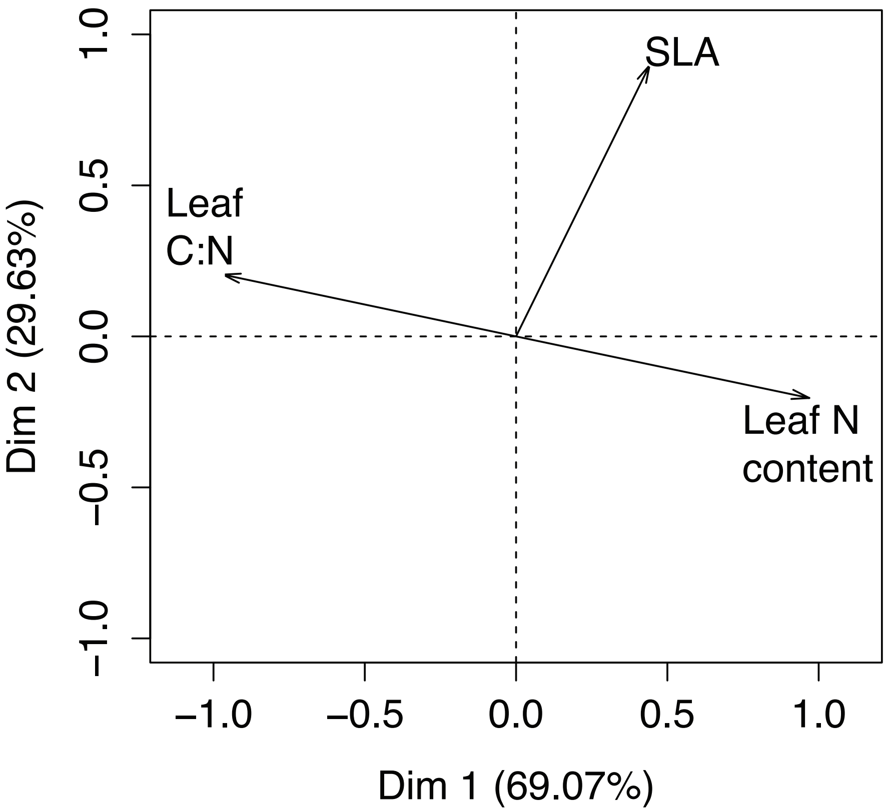

Figure S3. Principal components analysis biplot for leaf economic spectrum traits. All parameters were log-transformed to improve normality. SLA, specific leaf area; N, nitrogen; C, carbon.

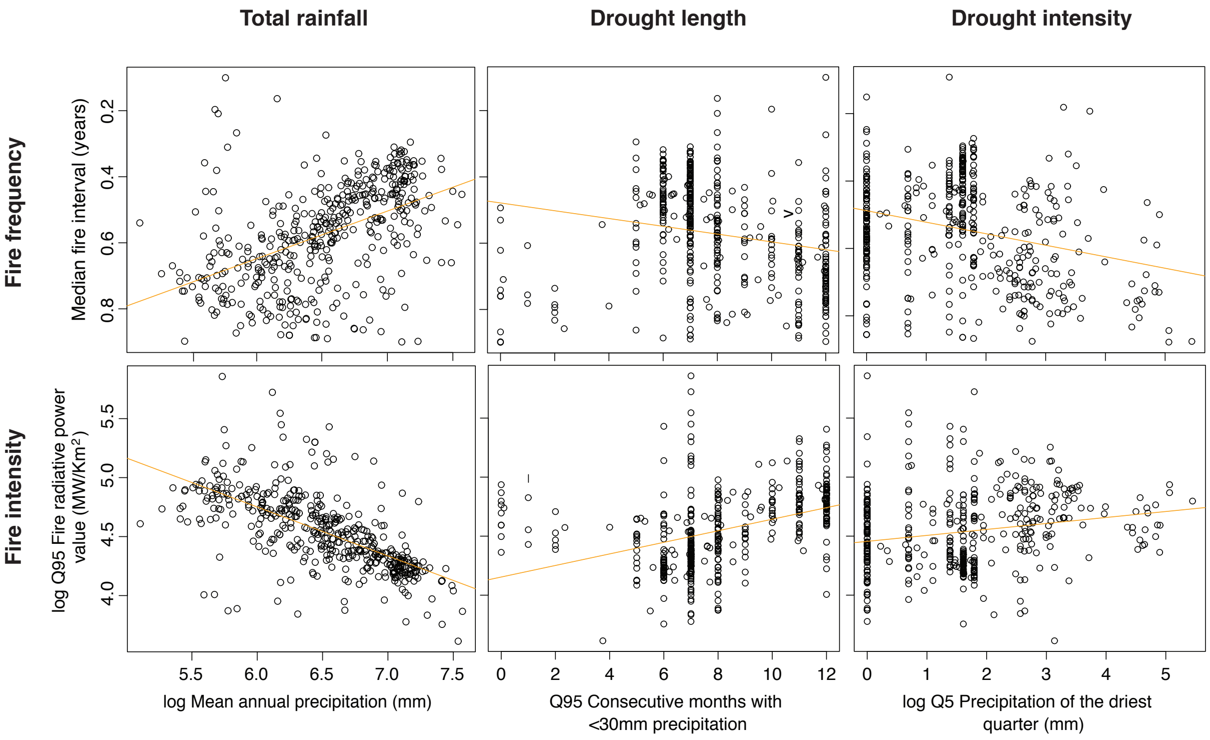

Figure S4. The relationships between fire and drought/rainfall characteristics. Orange lines represent statistically significant ($P<0.05$) linear models fitted to the data.

Table S1. Multiple linear regression (excluding leaf economic spectrum trait PCA axes) to determine the contributions of plant traits and environmental variables to fire characteristics with the sample size reduced to 39 species (i.e. the same as the 'complete' model). The 39 species were randomly selected. The variables significantly predicted the fire frequency ($F_{7,31}$=7.88, P<0.001, R^2=0.56) but not intensity ($F_{7,31}$=1.51, P=0.2, R^2=0.08) experienced by each grass species. Fire frequency is characterized as the reciprocal of the median fire return interval, whereas fire intensity is the 95th quantile of fire radiative power values at locations where each species has been recorded. Models were implemented using phylogenetic generalized least squares. Significant model terms (P<0.05) are in bold. SE, standard error; '*', P<0.05; '**', P<0.01; '***', P<0.001

	Fire frequency		Fire intensity	
	Estimate ± SE	t value	Estimate ± SE	t value
Maximum culm height	-0.011 ± 0.034	-0.34	-0.156 ± 0.088	-1.78
Photosynthetic type	**0.110 ± 0.052**	**2.13 ***	-0.189 ± 0.108	-1.75
Mode of postfire persistance	0.017 ± 0.400	0.04	0.473 ± 0.267	1.77
Mean annual precipitation	**0.253 ± 0.053**	**4.76 *****	0.025 ± 0.158	0.16
Drought length	0.160 ± 0.192	0.83	0.067 ± 0.039	1.70
Drought intensity	-0.014 ± 0.018	-0.74	0.020 ± 0.042	0.47
Drought length * Mode of postfire persistance	-0.024 ± 0.179	-0.14	-0.039 ± 0.032	-1.19
Intercept	**-1.594 ± 0.685**	**-2.33 ***	**4.538 ± 1.127**	**3.53****

Table S2. Phylogenetic signals for grass traits and fire/drought characteristics

Trait	N	Lambda (95% CI)	P value of lower bound	P value of upper bound
Maximum culm height	1634	0.833 (0.776 –0.861)	<0.001	<0.001
Mode of post-fire persistence*	393	0.377	<0.001	<0.001
Photosynthetic pathway*	1649	0.612	<0.001	0.9
PCA Dimension 1	123	0.547 (0.162-0.831)	0.001	<0.001
PCA Dimension 2	123	0.466 (0.175 – 0.722)	<0.001	<0.001
Fire frequency	218	0.395 (0.177 – 0.647)	<0.001	<0.001
Fire intensity	637	0.431 (0.253 – 0.602)	<0.001	<0.001
Presence in fire prone environments*	503	0.544	<0.001	<0.001
Mean annual precipitation	624	0.824 (0.733 – 0.888)	<0.001	<0.001
Drought length	640	0.633 (0.488 – 0.748)	<0.001	<0.001
Drought intensity	640	0.758 (0.647 – 0.838)	<0.001	<0.001

* Phylogenetic signals for binary traits were calculated using the phylogenetic D statistic. Values represent the inverse of the estimated D value.

Chapter 6

Chapter 6

General Discussion

This thesis adds to our understanding of how fire influences the functional traits of grasses. In this chapter, I summarise and evaluate my findings from the four data chapters within the context of the wider literature. As well as answering some questions, my work throws up many more. These questions, consisting of both follow-on questions and those not addressed in this thesis, are summarised in Figure 1 (and expanded upon in the text of this chapter). The interaction between fire and plants is two-way and complex, however this thesis largely focusses on the one-way influence of fire behaviour on grass traits. Therefore, many questions about the reverse interaction (the effect of plant traits on fire behaviour) emerge (Fig. 1). Importantly, both plants and fire are influenced by climate and human activity, and so increasing our understanding of how these interactions may be impacted in a changing world is vital.

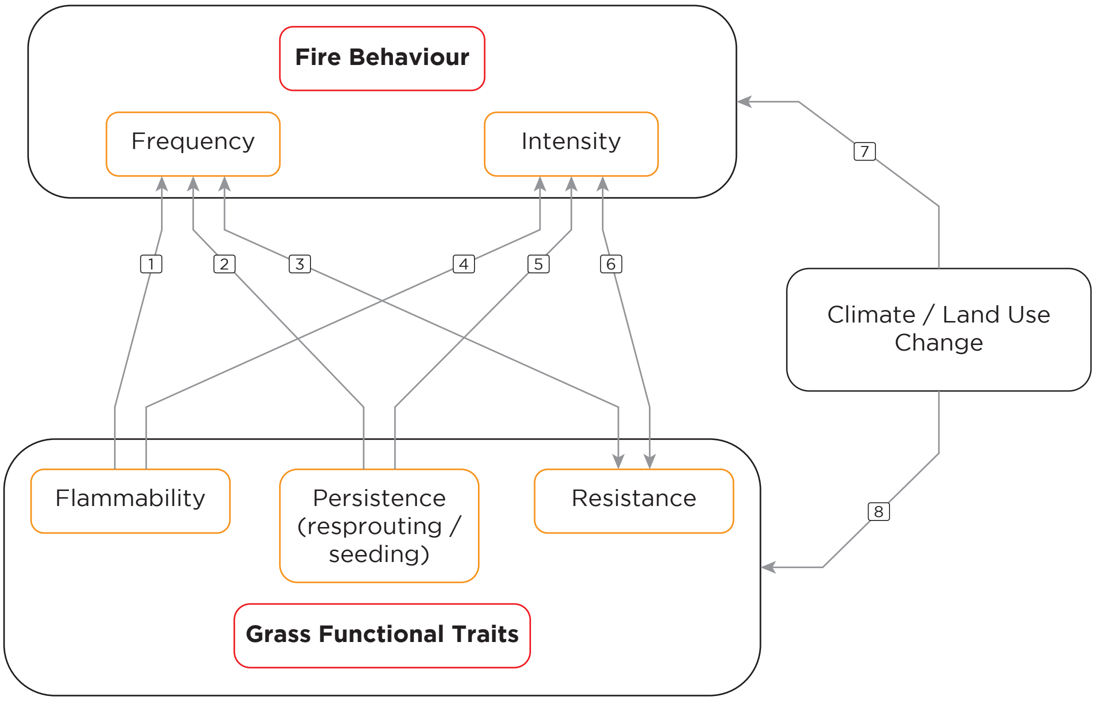

Figure 1. Schematic diagram demonstrating the relationships between fire, grass traits and climate that should be addressed in future work. The numbered arrows are referred to throughout the text in this chapter.

1. FIRE IS A DISTURBANCE SHAPING GRASS TRAITS

"*It is now unambiguous that fire has had a major role in shaping plant traits in fire-prone environments*" (p. 9, Archibald et al., 2017)

The perception of fire as an evolutionary and ecological driver has come a long way in the last few decades, from being largely overlooked to having a central role in shaping plant traits (Keeley et al., 2012, Archibald et al., 2017). Recent research has uncovered the long history of plant-fire interactions, and the powerful role fire has in shaping plant traits and floral communities over millennia (Glasspool, Edwards & Axe, 2004; Bond, Woodward & Midgley, 2005). On one hand, fire is now firmly accepted as a powerful selective filter for functional traits related to plant persistence, recovery and recruitment (Emerson & Gillespie 2008), with different fire regimes leading to the assembly of distinct communities that are functionally clustered for diverse traits (Pausas & Bradstock, 2007; Verdu & Pausas, 2007; Silva & Batalha, 2010; Forrestel, Donoghue & Smith, 2014). On the other hand, there is growing evidence of fire being a selective agent driving adaptation, with different fire regimes creating intraspecific variation in functional traits that is genetically determined (Parchman et al., 2012; Budde et al., 2014; Moreira, Castellanos & Pausas, 2014).

Despite the progress in establishing fire as a disturbance that shapes woody plant traits and communities (Keeley et al., 2011), few studies have focussed on fire effects on herbaceous fire-prone species. There are several potential reasons for this disparity. Serotiny is a particularly well-studied trait (e.g. Schwilk & Ackerly, 2001; He et al., 2012; Hernández-Serrano et al., 2014), probably due to its clear adaptive value for the successful recruitment of fire-killed species (Keeley et al., 2011), but is absent in herbaceous-dominated ecosystems (due to their short fire return times; Lamont et al., 2013). The lack of obvious, easily-measurable resistance traits (such as bark thickness; e.g. Pausas, 2015; Schubert et al., 2016) in herbaceous species may be a deterrent, as well as the presumed lower fitness benefits of fire adaptation to resprouting species (of which, many are herbaceous) compared to woody obligate seeders. The work in this thesis attempts to redress the balance by focussing on how fire influences the traits of a fire-prone, non-woody plant group, the grasses. The global, geologically long relationship between grass and fire (Bond et al., 2005; Keeley & Rundel, 2005; van der Werf et al., 2006; Scheiter et al., 2012; Linder et al., 2017), and the frequency of this disturbance in grassy ecosystems (Mouillot & Field 2005), makes this group an ideal study system for fire adaptations in herbaceous species.

The work done here establishes fire as a driver of grass functional traits. In Chapter 5, I show that fire assembles grass communities based on traits relating to the leaf economic spectrum, height, photosynthetic type and mode of post-fire persistence. Grass species are selectively allowed into fire-prone communities depending upon the possession of certain traits, and the effects of the association between grass traits and both fire frequency and fire intensity can be seen at a global scale. Untangling the two-way interaction between plant traits and fire behaviour to establish cause and effect (e.g. determining how mode of persistence drives fire frequency and intensity and not just considering the filtering of these traits by fire; arrows 2 and 5 of Fig. 1) requires further work.

In Chapter 4, I found that populations of resprouting grasses exposed to differing experimental fire regimes demonstrated phenotypic divergence in traits related to flowering and below-ground investment. Interestingly, these differences continued to exist after plants had been removed from the fire regime and grown in a common environment, providing the first evidence of plants having a 'memory' for fire, as has been found for other disturbances (Bruce et al., 2007; Robertson & Wolf, 2012; Walter et al., 2013; Scholes & Paige, 2015; Wang et al., 2017). These trait differences were not underpinned by genetic variation, suggesting that phenotypic plasticity plays an important role in adapting individuals to specific fire regimes. This differs from what has been found for obligate seeder species (Parchman et al., 2012; Budde et al., 2014; Moreira, Castellanos & Pausas, 2014), in which both phenotypic and genetic differences suggest selection is acting. These differences may result from the alternative modes of persistence through fire, whereby fire acts as a stronger selective force for obligate seeders than resprouters due to their greater cost of being burnt.

The most relevant grass traits to living with fire were studied here, but there are other traits potentially beneficial in fire-prone environments. Grass fire resistance traits, in the form of insulating, tightly packed leaf bases or the positioning of meristems (at or below ground level), have not been studied here (or elsewhere as far as I am aware) but may be adaptive (arrows 3 and 6; Figure 1). The protection afforded by insulating leaf bases and/or soil may be a pre-requisite for entry into areas subject to highly-intense fires. Likewise, heat- and smoke-stimulated germination are not studied here (due to the relative lack of data available, although some exists e.g. Sarmiento, 1992; Baxter et al., 1994; Verboom, Stock & Linder, 2002; Ghebrehiwot et al., 2012) that could be adaptive in fire-prone environments by providing a competitive advantage over other seedlings or resprouting individuals. Additionally, the study of trait syndromes, or correlated groups of traits used to define ecological strategies, could be relevant for grasses, as it has for pines (He et al., 2012). Energetic constraints mean that trade-offs exist amongst traits, resulting in individuals only displaying limited combinations of traits. Grass trait syndromes adaptive in fire-prone environments may exist, such as a highly flammability, resprouter strategy verses a heat/smoke stimulated germination, inflammable seeder strategy. Future work could establish whether such syndromes exist.

2. GRASSES ARE HETEROGENOUS FUELS TO FIRE

The finding of substantial differences in all components of grass flammability (Chapter 2) puts to rest the historic assumption that grasses vary little in their flammability. The co-occuring species studied showed significant variation in size-standardised flammability components, which are greatly emphasized when scaled up to the level of the individual. The key traits that underlie differences in grass flammability largely relate to the amount and the moisture content of above-ground biomass, as has been found in other fuel types (Byram 1959; Pyne 1984; Rothermel 1972; Nelson 2001). However, inconsistencies between the findings here and studies investigating flammability in other taxonomic groups stress the caution of extrapolating results across fuel types. For example, chemical biomass properties were not important to plant-scale flammability for grasses, but are for other plant groups that possess highly flammable compounds (Cornwell et al., 2015; Pausas et al., 2016). In addition, low grass biomass density caused a reduction in combustion rates through low fuel connectivity, whilst low densities in other fuel types cause high combustion through increased aeration (Schwilk & Ackerly, 2001).

Grass fuel quantity and moisture content were key to plant-scale flammability, but how these relationships scale up to the ecosystem-level remains unclear (arrows 1 and 4; Figure 1). There are many examples of invading grass species, that have different moisture contents to native species, drastically altering fire regimes in grassy ecosystems (Davies & Nafus, 2012; McGranahan et al., 2013; Livingston & Varner, 2016). Cheatgrass (*Bromus tectorum*) decreases the moisture content of the grassy fuel bed it has invaded in Western USA, resulting in a prolonged fire season (Davies & Nafus, 2012), whilst the invasion of *Festuca arundinacea* (a C_3 grass) into a C_4-dominated tallgrass prairie has conversely resulted in a decrease in fire intensity and spread (McGranahan et al., 2013). When comparing moisture contents of co-occuring savanna grasses in Chapter 2, it was the sole invasive species, *Cenchrus setaceus*, that had a substantially higher (up to 10 times greater) moisture content than native species, and was predicted to reduce fire intensity and spread. The cause of these interspecific differences in biomass moisture content between native and invasive species may be due to an asynchronous phenology (McGranahan et al., 2013) or the possession of different drought survival strategies (drought avoidance vs. drought tolerance).

The key role of biomass quantity in grass flammability (Chapter 2) is similarly supported by invasion-driven fire regime changes, where invading species increase the amount and connectivity of fuel beds (D’Antonio, 2000; Platt & Gottschalk, 2001; Davies & Nafus, 2012). For example, the invasion by Gamba grass (*Androgogon gayanus*) into northern Australian savannas has significantly altered fuel loads by replacing the native grass fuel bed with a much higher fuel load (<6 t ha^{-1} vs. <30 t ha^{-1}; Setterfield et al., 2010). The impact of this invasion on fire regime is an elevated fire intensity (1-3 MW m^{-1} in native grass fires vs. up to 16 MW m^{-1} in *A. gayanus*-fuelled fires; Setterfield et al., 2010) and an elongated severe fire season (Setterfield et al., 2013).

Whilst invasions provide extreme examples of how alien grass species with novel traits can change fire regimes, how more subtle changes in native community composition alter fire regimes are less known and require further research. However, if composition changes should alter the amount and moisture content of grass fuels, then fire behaviour is expected to change concurrently. As fire tends to show threshold behaviour, responding to biological and geophysical factors in non-linear ways (Archibald et al., 2017), even small changes in the flammability of a system can have large implications on fire characteristics (Cox & Durrett, 1988; Archibald, Staver & Levin, 2012). When the extent of grass fuelled fires is considered (>80% of annual burnt area is grassy; van der Werf et al., 2006), changes in the trait composition of grass communities could have considerable impacts upon fire regimes at a global scale.

3. IS ENHANCED FLAMMABILITY ADAPTIVE TO FIRE-PRONE GRASSES?

The establishment of the functional traits that drive grass flammability (Chapter 2) allows the exploration of this as an adaptive trait in frequently-burnt environments. Fire-prone grasses are predicted to benefit from being fast-flammable (Pausas, Keeley & Schwilk, 2017). By igniting easily, the removal of above-ground biomass can be achieved which, for basally-growing grasses, stimulates new growth and reduces self-shading (Zimmerman et al., 2010). By burning rapidly but briefly, plants suffer lower mortality as less heat is transferred to the soil and therefore to basal meristems ('pyrogenecity as protection' hypothesis; Gagnon et al., 2010). However, this hypothesis has not been tested in resprouting grass species. The finding that higher fuel loads both increased combustion rate and fire residence time (Chapter 2) throws this hypothesis somewhat into doubt. However, it is not only combustion duration that's importance to soil heating, but also the proximity of fuels to the soil surface. Grass species tend to hold their leaves (both dead and alive) off the ground to varying degrees, which may influence soil heating during a fire. Empirical evidence is needed to determine how grass traits, including plant architecture, influence fire residence time and plant mortality.

The 'kill-thy-neighbour' hypothesis (Bond & Midgley, 1995) also provides a framework for understanding the potential benefits of enhanced flammability to fire-prone grasses. The positive association found here between flammability and regrowth rate after fire (Chapter 3) suggests that if fire-tolerant individuals can increase neighbour mortality, and regrow quickly, they can make most of the resource-rich environment after fire for regeneration, recruitment and clonal spread. However, the flammability-enhancing traits explored here are likely determined by factors other than fire, and so cannot be explored in relation to evolutionary hypotheses. Whilst the 'kill-thy-neighbour' hypothesis is most relevant to obligate seeders (species following a "hot-flammable" strategy; Pausas, Keeley & Schwilk, 2017), it could also apply to resprouting grasses. However, species of fire-prone grasslands produce relatively cool, fast moving fires, as opposed to hotter, longer duration crown fires, and are often able to survive and resprout afterwards, throwing into doubt the relevance of the hypothesis to this group. However, heat-induced mortality in grasses has been documented, and can reach high rates (Zimmerman et al., 2010). The finding of considerable variation in grass flammability suggests that mortality may be variable and dependent upon the flammability of the ecosystem. Another question related to this hypothesis is how the traits of individual grasses influence the local fire regime, as the heat produced through their flammability must be experienced by plants in the immediate vicinity (and therefore influence their mortality). Evidence from a number of fuel types suggests that the most flammable members of a community may have a disproportionate effect on the flammability of the ecosystem (de Magalhaes & Schwilk, 2012; Van Altena et al., 2012; Blauw et al., 2015; Varner et al., 2017). If this is the case for grass flammability, the positive link between flammability and recovery after fire suggests that high flammability may be a ‘niche construction’ trait for some grass species (Schwilk, 2003). However, how the flammability of a grass species scales up to ecosystem flammability has not been established and should be addressed in future work.

The evolution of flammability is a subject that has been much contested in the literature since it was first suggested by Mutch in 1970. Over the years, a body of work on this topic has steadily grown, including theoretical frameworks, models and empirical studies. These studies both support and oppose the idea of flammability being subject to selection, but a recent analysis of papers published in the last 40 years on this topic found the majority of studies were in support (Archibald et al., 2017). Much of this work has focussed on serotinous species, demonstrating the correlated evolution of flammability and serotiny (Schwilk & Ackerly, 2001; He et al., 2012; Burger & Bond, 2015). In addition, strong evidence comes from work done on the obligate seeder shrub, *Ulex parviflorus*. Intra-specific variation in this species was associated with fire regime (Pausas et al., 2012), and had a genetic component (Moreira, Castellanos & Pausas., 2014).

As far as I am aware, there is no empirical evidence for the evolution of flammability in resprouting species. Why evidence exists for flammability being subject to selection in seeder species but not for resprouting species, may be due to a bias in flammability studies towards seeder species. However, in addition, selection will likely be harder to detect in resprouting species due to their longer, overlapping generation times and the lower cost to them of being burnt. Grasses offer a good study system for the evolution of flammability in a resprouting species due to the potential benefits of enhanced flammability to these species. The work done in this thesis should act as a starting point for future research uncovering whether flammability is a trait under selection in resprouter species.

4. THE FUTURE OF GRASS-FIRE ASSOCIATIONS

Grass-fire interactions are influenced by climatic conditions, and so are vulnerable to anthropogenic climate change. Fire-prone grasslands and savannas, being relatively dry and seasonal, are susceptible to shifts in climatic regime (Dubinin, Luschekina & Radeloff, 2011; Pausas & Fernández-Muñoz, 2012). They are expected to experience (and in many areas, are already experiencing) increased temperatures and changing precipitation patterns, in addition to the continuing rise in CO_2 concentrations. In addition, fire regimes are being altered across the globe by human activity, through the ignition, and more significantly, the suppression of fire (Andela et al., 2017). How these multiple, changing factors will combine to impact grassland communities and their fire regimes is unclear (Settele et al., 2014; arrows 7 and 8 of Fig. 1).

By drying out grass fuels, elevated temperatures will increase the flammability of these ecosystems, by providing more opportunities for burning, and drier fuels will result in more intense fires. Increases in temperature would also benefit C_4 grasses over C_3 species (due to increased photorespiration in the latter; Epstein et al., 2002), and result in the production of large, highly flammable fuel loads (Scheiter & Higgins, 2009).

Ecosystem productivity is largely driven by the availability of moisture, and so changes in rainfall will alter the amount of biomass produced. Changes in rainfall and variability are expected to differ regionally, but with an overall drying trend that will likely result in a reduction in cover and productivity of grasslands and savannas (Woodward & Lomas 2004). This reduced productivity will limit grass fuel loads, and lead to a decrease in fire frequency and spread.

Atmospheric CO_2 levels, amongst other factors, determine the relative benefits of C_3 and C_4 photosynthesis through its impacts on photorespiration rates. Heightened CO_2 levels have a ‘fertilising’ effect on C_3 species, including woody species, and are hypothesized to be causing an increase in woody density across grasslands worldwide (Ainsworth & Long, 2005; Buitenwerf et al., 2011; Higgins & Scheiter, 2012; Donohue et al., 2013). This transition from open to closed ecosystems will have considerable impacts upon community composition, with the exclusion of shade-intolerant species, and grass productivity, due to increased competition with woody species. Fire regimes will change concurrently with this decrease in ecosystem flammability.

Aside from their impacts on the climate, humans drastically alter the natural fire regimes of ecosystems through land-use change. Grasslands and savannnas are particularly vulnerable, with huge areas converted to arable and pastoral land. The planting of irrigated crops and the reduction in fuel load by grazers, both massively decrease local flammabilities and suppress fire. This impact can be seen at a global scale with the total burned area declining by 24% over the past two decades (Andela et al., 2017).

The vast extent and frequency of burning that occurs in grassy ecosystems (Moillot & Field, 2005; van der Werf et al., 2006) may mean that climate- and land use-driven changes to fire regimes, through alterations in community composition, function and flammability, will have considerable implications for Earth system processes, but these have not yet been adequately addressed. I hope the information provided in this thesis will add to the growing body of empirical and theoretical work that drives forward our understanding of the role of plant-fire interactions in the Earth system.

5. CONCLUSIONS

Grasses have a historical and contemporary close association with fire, making this group an ideal study system for trait selection and filtering by fire in herbaceous species.

Grasses are not homogenous fuels to fire but instead species differ in easily measurable functional traits, namely biomass quantity and moisture content, that in turn demonstrably influence flammability. The finding here of a positive link between flammability-enhancing traits and post-fire success in fire-prone grasses adds to the growing body of evidence that enhanced flammability may be advantageous in fire-prone environments. Grass species may be altering local fire conditions in order to optimise their fitness, by increasing their resprouting success.

The development of resprouting grasses is influenced by prior exposure to fire, independent of the current environmental conditions, suggesting a ‘memory’ for fire. This developmental priming of traits relating to flowering and below-ground investment, may improve the fitness of individual plants when exposed to fires in future.

Fire characteristics influence grass functional diversity at a global scale, with the possession of certain traits related to photosynthetic type, height, mode of post-fire persistence and leaf economic spectrum traits, key to entering fire-prone communities. Understanding how fire influences the structure of grassy ecosystems is vital for understanding the impacts of anthropogenic changes in the fire regime.

This work establishes fire as a force shaping the functional traits of fire-prone grasses, the effect of which can be seen at multiple biological scales.

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Appendix 1

100 YEARS Journal of Ecology British Ecological Society

Journal of Ecology doi: 10.1111/1365-2745.12503

Determinants of flammability in savanna grass species

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Summary

1. Tropical grasses fuel the majority of fires on Earth. In fire-prone landscapes, enhanced flammability may be adaptive for grasses via the maintenance of an open canopy and an increase in spatiotemporal opportunities for recruitment and regeneration. In addition, by burning intensely but briefly, high flammability may protect resprouting buds from lethal temperatures. Despite these potential benefits of high flammability to fire-prone grasses, variation in flammability among grass species, and how trait differences underpin this variation, remains unknown.

2. By burning leaves and plant parts, we experimentally determined how five plant traits (biomass quantity, biomass density, biomass moisture content, leaf surface-area-to-volume ratio and leaf effective heat of combustion) combined to determine the three components of flammability (ignitability, sustainability and combustibility) at the leaf and plant scales in 25 grass species of fire-prone South African grasslands at a time of peak fire occurrence. The influence of evolutionary history on flammability was assessed based on a phylogeny built here for the study species.

3. Grass species differed significantly in all components of flammability. Accounting for evolutionary history helped to explain patterns in leaf-scale combustibility and sustainability. The five measured plant traits predicted components of flammability, particularly leaf ignitability and plant combustibility in which 70% and 58% of variation, respectively, could be explained by a combination of the traits. Total above-ground biomass was a key driver of combustibility and sustainability with high biomass species burning more intensely and for longer, and producing the highest predicted fire spread rates. Moisture content was the main influence on ignitability, where species with higher moisture contents took longer to ignite and once alight burnt at a slower rate. Biomass density, leaf surface-area-to-volume ratio and leaf effective heat of combustion were weaker predictors of flammability components.

4. *Synthesis*. We demonstrate that grass flammability is predicted from easily measurable plant functional traits and is influenced by evolutionary history with some components showing phylogenetic signal. Grasses are not homogenous fuels to fire. Rather, species differ in functional traits that in turn demonstrably influence flammability. This diversity is consistent with the idea that flammability may be an adaptive trait for grasses of fire-prone ecosystems.

Key-words: biomass moisture content, biomass quantity, determinants of plant community diversity and structure, fire regime, functional traits, phylogeny, poaceae, resprouting

Introduction

Fire is a disturbance that has shaped plant traits and floral communities for over 420 million years (Glasspool, Edwards & Axe 2004; Bond, Woodward & Midgley 2005) and acts as a powerful selective filter for functional traits related to plant persistence, recovery and recruitment (Emerson & Gillespie 2008). Fire is also multidimensional and its effects on vegetation depend on the characteristics of the local fire regime (Keeley *et al.* 2011), which can vary considerably in frequency, intensity, size and season (Archibald *et al.* 2013). Different fire regimes can lead to the assembly of distinct populations and communities that are functionally clustered for diverse traits (Pausas & Bradstock 2007; Verdú & Pausas 2007; Silva & Batalha 2010; Forrestel, Donoghue & Smith

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2014). For example, resprouting species are favoured in frequent, low-intensity fire regimes, and obligate seeders that persist via seedling recruitment are favoured in infrequent, high-intensity fire regimes (Pausas & Bradstock 2007; Pausas & Keeley 2014).

Plant flammability may both influence and be influenced by fire regime (He, Lamont & Downes 2011; Pausas *et al.* 2012) but species variation in flammability has received relatively little attention (but see Scarff & Westoby 2006; Murray, Hardstaff & Phillips 2013; Grootemaat *et al.* 2015). Flammability is an emergent property of a plant's chemical and physical traits. However, the identification of these traits in several fire-prone taxa, particularly herbaceous species, has not been achieved. Flammability as a vegetation property consists of several interdependent components (Anderson 1970) that can each be quantified. Ignitability (the ease of ignition), combustibility (the intensity of combustion) and sustainability (the maintenance of burning over time) are flammability components and can be measured at multiple scales. For example, ignitability is often measured as ignition delay at the leaf or plant scale, while the rate of fire spread is a measure of ignitability that operates at the community scale (Gill & Zylstra 2005).

Plant flammability is a key determinant of fire behaviour (Bond & van Wilgen 1996; Beckage, Platt & Gross 2009). In woody plants, flammability varies considerably between and within species (e.g. Fonda 2001; Saura-Mas *et al.* 2010; Pausas *et al.* 2012; Cornwell *et al.* 2015), and minor changes in vegetation composition have repeatedly demonstrated significant alterations in vegetation flammability and fire regime (Rossiter *et al.* 2003; Brooks *et al.* 2004; Belcher *et al.* 2010). Flammability may act as a means by which plants modify fire regimes to engender favourable conditions (Schwilk 2003). For example, slow-growing, woody, obligate seeder species, such as *Pinus* species, require infrequent intense fire to complete their life cycle. High-temperature crown fires are vital for releasing stored seeds from the retained mature cones of these serotinous species and enhancing recruitment opportunities of seedlings via mortality of neighbouring trees (Lamont *et al.* 1991; Keeley *et al.* 2011). In contrast, resprouting perennial grasses, which dominate grasslands and savannas (Uys 2000; Allan & Southgate 2002; Overbeck & Pfadenhauer 2007), may benefit from very frequent fire (Archibald *et al.* 2013). These shade-intolerant species require the regular removal of standing dead biomass (Everson, Everson & Tainton 1988) and woody growth (Bond 2008), which may be aided by high plant flammability. Surface fires in grassy systems are characterized by rapid combustion and spread, low fire residence times and cool burn temperatures (Bradstock & Auld 1995; Archibald *et al.* 2013). Such fire characteristics are advantageous to resprouting grass species, protecting basal meristems from excessive heat through biomass that burns rapidly (Gagnon *et al.* 2010). In addition, high flammability, if linked to efficient post-fire recovery, may provide enhanced regeneration opportunities for these species by killing neighbouring plants and reducing post-fire competition (Bond & Midgley 1995).

Despite these predicted benefits of frequent fire to fire-prone grasses, interspecific variation in the flammability of such species has been little explored (Ripley *et al.* 2010), in contrast to knowledge about interspecific variation in post-fire response among grass species (Ripley *et al.* 2015). A historical belief persists that grasses and other herbaceous plants vary little in their flammability, which has led to the diversity of herbaceous fuels being reduced to one or few fuel classes in fire behaviour modelling (e.g. Anderson 1982). Given the considerable known variation in the flammability of woody species (Schwilk 2003; Scarff & Westoby 2006; Pausas *et al.* 2012; Murray, Hardstaff & Phillips 2013), such presumptions are unfounded. Substantial changes in grassland community flammability resulting from invasion by non-native grasses provide evidence to suggest considerable interspecific variation in grass flammability (Hughes, Vitousek & Tunison 1991; Rossiter *et al.* 2003). In addition, recent evidence shows that grass traits relating to post-fire recovery are shaped by fire regime (Forrestel, Donoghue & Smith 2014; Ripley *et al.* 2015), suggesting that traits relating to flammability may be responding in similar ways, resulting in intra- and interspecific variation in flammability.

Physical and chemical traits influencing some or all components of flammability relate to the quantity, quality, moisture content and aeration of biomass (Bond & van Wilgen 1996; Gill & Moore 1996). Biomass quantity is critical to combustibility and fire spread rate because it directly influences fire energy output rate (Byram 1959; Rothermel 1972). Biomass moisture content determines the extent to which fuels absorb heat energy, with high values associated with delayed ignition and low combustion and fire spread rates (Pyne 1984; Nelson 2001). Biomass surface-area-to-volume (SA/V) ratio influences curing and reaction rates within fires (Papio & Trabaud 1991; Gill & Moore 1996), with high values linked to rapid ignition, and rapid rates of combustion and fire spread. Increasing biomass density, defined as the mass of biomass per unit volume of fuel bed, raises fuel connectivity, therefore enhancing combustibility and fire spread rate. This relationship applies up to a certain threshold beyond which poor ventilation will limit drying and combustion rates (Rothermel 1972). Intrinsic properties of plant material, such as heat of combustion, affect combustibility and fire spread rate through the amount of heat energy released during complete combustion. Sustainability is often inversely related to combustibility and ignitability (e.g. de Magalhães & Schwilk 2012). Therefore, plant traits likely to enhance combustion and spread rate may indirectly reduce flaming duration. In contrast, high biomass quantity increases combustion and spread, but is also likely to enhance sustainability, as more fuel takes longer to burn. Plant traits important to flammability have been identified in a number of fire-prone taxa (e.g. Ganteaume *et al.* 2013; Schwilk & Caprio 2011). However, the traits that influence grass flammability, and more generally the flammability of herbaceous species, have not been empirically established or explored.

We examined three components of flammability, at multiple scales, for 25 species common in fire-prone South African grasslands. Five structural and chemical plant traits, known to influence vegetation flammability, were measured and correlated with flammability trait values (see Table 1). We hypothesized that (i) there is significant interspecific variation in flammability among grass species and that (ii) the measured plant traits can explain this variation, with each trait contributing to flammability components in different ways (see Table 1 for specific predictions). We also expected that flammability and plant traits covary due to the interdependent relationships between flammability components and plant traits. The strong phylogenetic patterns in grass distributions across fire-frequency gradients (e.g. Visser *et al.* 2012; Forrestel, Donoghue & Smith 2014) led us to predict that (iii) flammability is influenced by evolutionary history and contains a phylogenetic signal.

Materials and methods

PLANT MATERIAL

Plants were collected during the natural fire season in July 2014 in grassland and Nama-Karoo habitats near Grahamstown in the Eastern Cape of South Africa (see Table S1 in Supporting Information for site details). Fire return times over the 2000–2006 period were 2.3 years for vegetation surrounding Grahamstown (Tansey *et al.* 2008).

Seven individuals of 25 species, representing 5 grass subfamilies, were collected for study (see Table S2). All species were native to the region except *Cenchrus setaceus*, a North African invasive species (Milton 2004). For each species, seven randomly selected, healthy-looking adult plants were dug up while keeping their shoot architecture intact. Plants were stored in sealed plastic bags at room temperature for a maximum of 48 h to minimize changes in moisture content. A specimen of each species was deposited at the Selmar Schonland Herbarium (Rhodes University).

STRUCTURAL AND CHEMICAL TRAITS

A section of each individual (approximately one-third of the entire plant), with its below-ground biomass and soil removed, was used to measure five structural and chemical plant traits. Biomass quantity, density and moisture content were measured at the plant scale, while effective heat of combustion (EHoC) and SA/V ratio were measured at the leaf scale.

For measurements of leaf SA/V ratio and EHoC, leaves were removed from a randomly selected tiller of each individual. Total leaf area was measured on digital images using the computer program WinDIAS (Delta-T Devices, Cambridge, U.K.) that determines leaf area by selecting pixels of a pre-defined colour range. Leaf thickness was measured, at the middle of the leaf and excluding the midrib, for three leaves per tiller using digital callipers (accurate to 0.01 mm), and an average value was calculated. Leaf SA/V ratio was calculated from the average leaf area and leaf thickness of each species.

The heat of combustion is the energy released as heat when biomass undergoes complete combustion with oxygen, which typically relates to C:N ratio, lignin content and the presence of flammable compounds (Philpot 1969; Bond & van Wilgen 1996). We measured the EHoC, which is the heat of combustion of pyrolysate vapours, and does not assume that all char is consumed. Compared to measurements that involve the full thermal decomposition of biomass (such as in bomb calorimetry), EHoC is a more realistic estimate of the energy released from a wildfire in which combustion is incomplete, and most of the energy is released from burning the pyrolysate vapours. Oven-dried leaf samples of known mass (5.0 ± 0.4 mg) were conditioned at room temperature and humidity before being analysed in a microscale combustion calorimeter following the manufacturer's guidelines (FAA Micro Calorimeter, Fire Testing Technology Ltd, East Grinstead, UK). Each sample was held in nitrogen and heated at a rate of 3 °C per second driving off the volatile gases that were ignited and completely oxidized, and heat release was quantified by oxygen depletion calorimetry (Tewarson 2002). Total heat release was divided by the sample mass to provide the EHoC (kJ g^{-1}). Due to the high repeatability of this trait measurement, material from three randomly chosen individuals per species was tested in duplicate, to give an average value per individual and per species.

For plant-scale traits, the height (maximum vertical distance from ground level to the tallest point) and width (maximum horizontal spread) of each clump was determined. Biomass density was measured using a novel method, which determined the vertical biomass distribution for each individual. For this, the biomass of each clump was divided at five or more equal intervals along its vertical height, so that intervals were 2.5, 5, 10 or 15 cm in length depending on the plant height, and started at ground level. Each clump was cut with scissors at the selected intervals. The fresh and dry biomass of each section were weighed to four decimal places, the latter after oven drying at 70 °C to a constant weight. Cumulative dry biomass was calculated at each vertical height interval from ground level. Linear models were fitted to the logged cumulative dry biomass and vertical height for each individual. The slope of this relationship was used as a proxy

Table 1. Matrix summarizing the predicted relationships between plant and flammability traits. Flammability traits were determined at different scales (L, leaf; P, plant; C, community) and represent three flammability components. Symbols reflect the direction of the relationship ('+': positive; '−': negative; '0': none; 'N/A': could not be tested). Influence is either direct or indirect (in parentheses)

			Plant trait				
Flammability trait	Flammability component	Scale	Biomass quantity (g)	Biomass density (g cm^{-1})	Biomass moisture content (g g^{-1})	Leaf SA/V ratio	Leaf effective heat of combustion (J g^{-1})
Time to ignition (s)	Ignitability	L	N/A	N/A	−	+	0
Predicted rate of fire spread (m s^{-1})	Ignitability	C	+	+	−	+	+
Flaming time (s)	Sustainability	L, P	+	(−)	(+)	(−)	(−)
Combustion rate (g s^{-1})	Combustibility	L, P	+	+	−	+	+

for biomass density, in g cm^{-1}, with high values indicating densely packed biomass. For each clump, dry biomass values were combined to give the total dry biomass, and moisture content was calculated by dividing the difference between fresh and dry biomass by the dry biomass.

FLAMMABILITY

Flammability was represented by three components: ignitability, combustibility and sustainability (Anderson 1970). All components were measured for each individual at the leaf scale via epiradiator tests. In addition, combustibility and sustainability were determined at the plant scale by burning partial plant canopies. Plant-scale measurement of ignitability was beyond the scope of this experiment; however, a community-level measure was obtained by estimating the rate of fire spread for each individual by parameterizing Rothermel's (1972) fire spread model with plant trait data. Leaf- and plant-scale flammability components were measured both on fresh and dry biomass to determine the effect of moisture content. The 'fresh' clump was kept in a sealed plastic bag at room temperature, and the 'dry' clump was first dried at 70 °C for a minimum of 48 h.

Leaf-scale ignitability, sustainability and combustibility were measured as time to ignition, flaming time and mass loss rate, respectively, using a Quartz infrared 500 W epiradiator (Helios, Italquartz, Milan, Italy) in a fume cupboard with a constant vertical windspeed of 0.1 m s^{-1}. As application of leaf material directly to the epiradiator's silica disc surface always caused instantaneous combustion, 2-mm wire mesh was positioned 1 cm above the epiradiator's surface. The background temperature at the mesh surface (without fuel), measured by a thermocouple connected to a data-logger, ranged between 370 and 400 °C. Samples of 0.2 g (±0.001 g) leaf material were cut into 2-cm segments to standardize between samples and applied to the centre of the mesh. The 0.2 g mass was used because preliminary studies found that smaller masses failed to ignite, while larger fuel masses increased the risk that other fuel properties, particularly fuel height, influenced flammability values. Smaller samples were used for *Aristida congesta* subsp. *barbicollis* due to the low leaf mass of this species. Each test was filmed at 25 frames s^{-1}, and (i) time to ignition (TTI; the time between sample application to the epiradiator and first flaming) and (ii) flaming time (FT; the time from ignition to flame extinction) were subsequently determined. As samples were completely combusted by applying them to the epiradiator, an average leaf combustion rate was obtained by dividing the mass of samples by FT. Species average values for TTI and FT were obtained for fresh and dry material. The influence of leaf moisture content on these flammability traits was determined as the difference in values between fresh and dry samples of each individual and averaged per species.

As canopy architecture influences grass flammability (Martin 2010), a method that measures plant-scale flammability traits was utilized. Fresh and dry plant material from each individual were clamped on a stand on a four-point balance (Mark 205A; Bel Engineering, Monza, Italy) and burnt in a fume cupboard with a constant 0.1 m s^{-1} vertical wind speed (see Figure S1 for diagram of the set-up used). Samples were ignited by directing a Bunsen burner flame to the side of the base of the clump at a 45° angle and a 5 cm distance for a maximum of 3 s (less if ignition happened earlier). This resulted in successful ignition in all individuals. Mass loss was logged at 0.2-s intervals and the sigmoidal relationship produced was fitted with a Boltzmann equation. Data were excluded if fitting the relationship was not possible due to noise around the curve ($n = 40/350$), which occurred if large pieces of plant material fell off the balance during a burn. The width parameter used to fit the Boltzmann curve reflects the time period in which mass was drastically reduced and was used as a plant-scale measurement of sustainability (flaming time). Three seconds of data either side of the inflection point were selected and a linear regression fitted. The slope of this regression represents the maximum combustion rate in g s^{-1}. As preliminary results found this combustibility trait to be strongly driven by the biomass of the sample, interspecific comparisons were standardized for mass. Therefore, maximum combustion rate was plotted against mass change for each species, and linear models were fitted to the fresh, dry and combined data sets. As there was no change in mass common to all 25 species, the y-intercept extracted from the model fitted to the combined data set was used to characterize the intrinsic combustibility of each species. The combined data set was used as the slopes of the models fitted to the fresh and dry data did not differ significantly for any species, and model fit was improved by combining the data sets. Any unpaired samples were excluded to ensure a balanced data set of fresh and dry samples. The y-intercept differed significantly between fresh and dry models for three species (*Panicum* sp., *Hyparrhenia hirta* and *Merxmuellera stricta*) and in these cases, the y-intercept was extracted from linear models fitted to the fresh data set.

Forward fire spread rate values, the community-scale measure of ignitability, were predicted for each individual using Rothermel's (1972) surface fire spread model as implemented using the ros() function in the *Rothermel* package (Vacchiano & Ascoli 2014) in R (R Core Team 2013). Fire behaviour was simulated for each individual by parameterizing the model with data for the following traits: leaf SA/V ratio, leaf EHoC, biomass moisture content, plant height and fuel load (biomass quantity divided by the estimated cover area). See Table S3 for a details of the procedure followed and model assumptions.

PHYLOGENETIC ANALYSIS

We constructed a phylogeny that was initially based on a previously generated data set for grasses composed of the plastid markers *trnKmatK*, *ndhF* and *rbcL* (Grass Phylogeny Working Group II 2012) and augmented here. For ten species not represented in this previous data set, a fragment of *trnKmatK* was PCR-amplified from genomic DNA, following protocols and primers described previously (Grass Phylogeny Working Group II 2012). The newly generated sequences have been submitted to NCBI database (Benson *et al.* 2012) under the accession numbers KP860326 to KP860336. The new markers were manually aligned to the data set, which consisted of 606 taxa and 5649 aligned bp. This initial data set was downsized to 70 species, including all the taxa studied here and representatives of all grass lineages. A time-calibrated phylogenetic tree was obtained through Bayesian inference as implemented in BEAST (Bayesian evolutionary analysis by sampling trees; Drummond & Rambaut 2007). A general time-reversible substitution model with a gamma-shape parameter and a proportion of invariants (GTR+G+I) were used. The log-normal relaxed clock was selected. The tree prior was modelled by a Yule process. The monophyly of the BEP-PACMAD clade was enforced, leaving *Puelia olyriformis* as the outgroup. The calibration prior for the age of the BEP-PACMAD crown was set to a normal distribution, with a mean of 51.2 and a standard deviation of 0.001 (mean based on Christin *et al.* 2014). Two independent runs were conducted for 10 000 000 generations, sampling a tree every 1000 generations. The convergence of the runs and the appropriateness of the burn-in period, set to 2 000 000 generations, were verified using

Tracer (Rambaut A, Drummond AJ (2007) Tracer v1.4, available at http://beast.bio.ed.ac.uk/Tracer). Median ages were mapped on the maximum-credibility tree. The relationships among the species studied here were extracted from this tree and used for comparative analyses.

DATA ANALYSIS

Statistical analyses were carried out in the R environment (R Core Team 2013). Data were log-transformed to improve normality and to meet model assumptions where necessary.

Analysis of variance (ANOVA) was used to determine whether plant and flammability traits differed significantly between species. The influence of species, and state ('fresh' or 'dry'), on leaf-scale flammability was determined by two-way ANOVA. As biomass quantity values for the plant-scale burns are not representative of the species (i.e. for each species, clumps were subsampled and a range of masses were burnt), a species effect on the relationship between maximum combustion rate and biomass quantity was tested using the R package MCMCglmm (Hadfield 2010). This approach implements Markov chain Monte Carlo routines for fitting generalized linear mixed models, while accounting for non-independence and correlated random effects arising from phylogenetic relationships (Hadfield 2010). We fitted flammability (maximum combustion rate) and biomass quantity as a bivariate normal response, and species as a random effect. Models were run for 500 000 iterations with a burn-in of 1000 iterations, a thinning interval of 500 and weakly-informative priors (V = diag(2), nu = 0.002). The 95% highest posterior densities (HPD) of within-species and across-species slopes and the difference between slopes were estimated while accounting for phylogeny and used to assess whether slopes differed among species.

To test the hypotheses put forward in Table 1 and to establish the strength and direction of plant trait contributions to flammability components, a MCMC multi-response generalized linear mixed model approach was used again. Traits were separated into leaf and plant scale to ensure appropriate comparisons, using the same prior and specifications as before. The fit of the models to data was established by fitting linear models between the observed flammability trait values and those predicted by the models. The contribution of plant traits to fire spread rate was tested to determine whether strong relationships occurred across species when accounting for phylogeny, while acknowledging that some circularity is involved because spread rate was predicted based on the values of these traits.

To explore the pattern of covariance among plant and flammability traits, principal component analyses were performed using the princomp function (R core team 2013). Linear regressions were also used to establish the relationships among plant and flammability traits, with the latter being split into leaf-scale and plant-scale traits for analyses to ensure an appropriate comparison. The relationships between flammability traits measured at different scales were also established using linear regressions.

The influence of evolutionary history was established for each plant and flammability trait by testing for the presence of a phylogenetic signal. This was done using the pgls function in the *caper* package (Orme *et al.* 2012) which estimated Pagel's λ.

Results

FLAMMABILITY VARIATION AMONG SPECIES

All flammability components varied considerably across species (Fig. 1; Table S4). At the leaf-scale, significant interspecific variation was found in ignitability ($F_{24,144} = 5.02$, $P < 0.001$), sustainability ($F_{24,144} = 3.02$, $P < 0.001$) and combustibility ($F_{24,144} = 2.97$, $P < 0.001$). Ignition delays ranged from 1.0 s (*H. hirta*) to 4.0 s (*C. setaceus*) with a mean across species of 1.7 s. The mean flaming duration across species was 6.3 s and ranged from 4.3 s (*A. congesta* subsp. *barbicollis*) to 7.6 s (*Eragrostis plana*). Connected to flaming duration was average combustion rate, with *E. plana* burning at the slowest rate (27 mg s^{-1}) and *A. congesta* subsp. *barbicollis* at the fastest (49 mg s^{-1}).

At the plant scale, intrinsic combustibility (for a given biomass) differed by <2.5-fold across species, ranging from 0.064 g s^{-1} (*Eustachys paspaloides*) to 0.163 g s^{-1} (*Themeda triandra*). When investigating the relationship between combustion rate and biomass, the bivariate mixed effects model revealed that within-species slopes (pooled mean = 0.594, HPD: 0.507 to 0.707) and across-species slopes (mean = 0.797, HPD: 0.067 to 1.385) did not differ significantly (mean slope difference (Δb) = 0.212, HPD: −0.521 to 0.683) when accounting for phylogeny (Fig. 2). This common relationship was extrapolated while taking into account intrinsic combustibility differences, allowing combustion values to be predicted for the species mean total biomass. These predicted values of whole-plant combustion rates varied >20-fold among species, ranging from 0.06 g s^{-1} (*A. congesta* subsp. *barbicollis*) to 1.28 g s^{-1} (*M. disticha*; Fig. 2).

Fuel models based on the traits of *C. setaceus* predicted no fire spread, because biomass moisture content values exceeded the moisture of extinction, defined as the fuel moisture content above which a steady rate of fire spread is not possible. Of the remaining species that spread fire, the estimated rate of spread differed substantially (25-fold; Table S4) and varied significantly between species (ANOVA: $F_{24,150} = 42.42$, $P < 0.001$).

Substantial interspecific variation was also found in the five traits measured as explanatory traits for flammability (Fig. 1; see Table S5). Biomass moisture content values of the non-native *C. setaceus* were substantially higher than the other species. However, species still differed significantly for this trait when *C. setaceus* was excluded (ANOVA: $F_{23,144} = 14.39$, $P < 0.001$). The measurement of biomass density (i.e. vertical biomass distribution) produced consistent values within species (Fig. S2; species average CV = 28%), but considerable differences among species with slope values ranging from 0.155 (*Eragrostis lehmanniana*) to 0.831 (*M. stricta*).

Collection site did not influence flammability traits. Of the plant traits, vertical biomass distribution (P = 0.008) and leaf EHoC (P = 0.046) were the only ones affected by collection site (see Table S7).

TRAIT CONTRIBUTIONS TO FLAMMABILITY

Measured plant traits significantly predicted the components of flammability, particularly ignitability and plant-scale combustibility, in which 70% and 58% of variation could be explained by the plant traits, respectively (Tables 2 and 3). Variation in sustainability could be explained to a lesser extent by plant traits at the leaf (47%) and plant scale (37%),

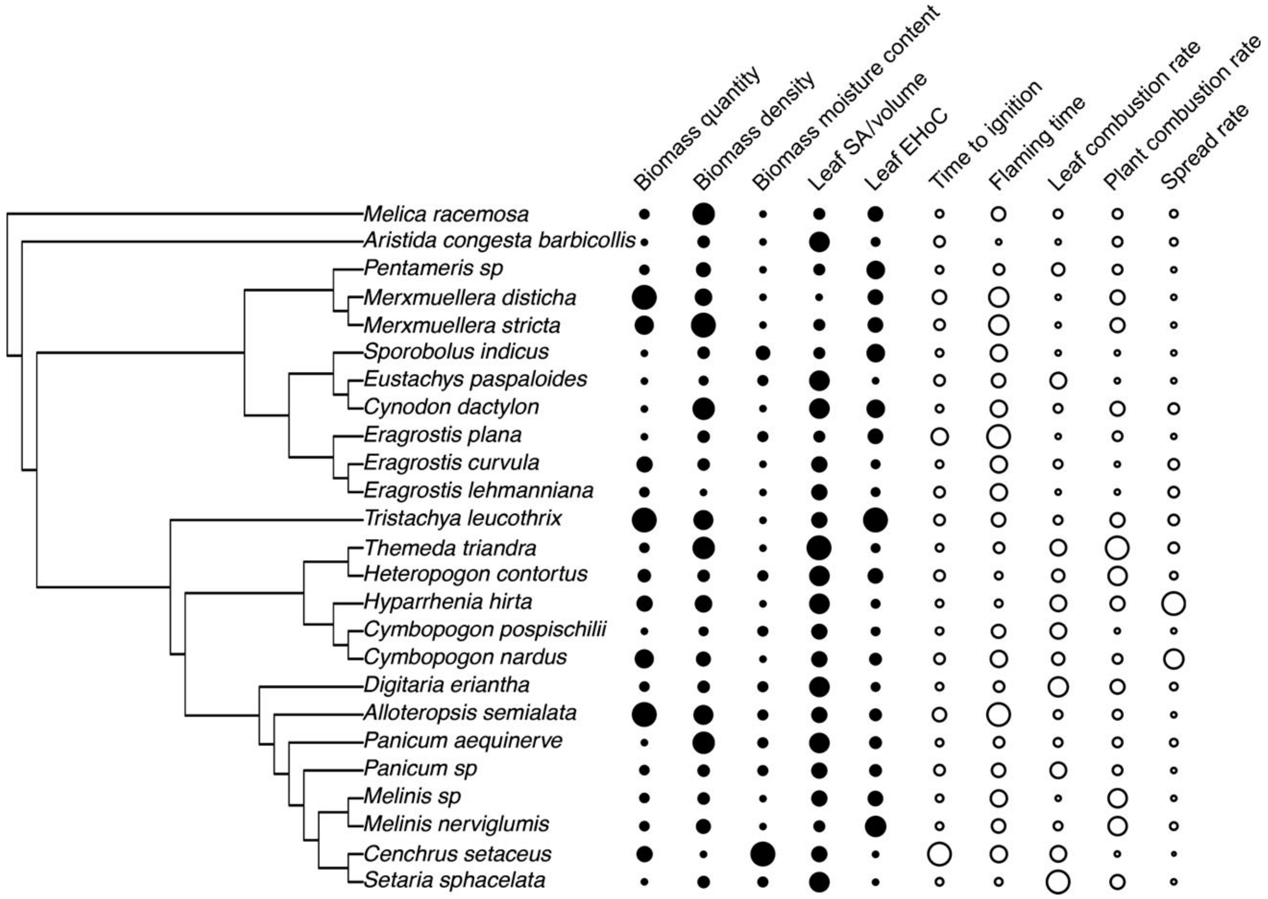


Fig. 1. The evolutionary relationships between species and average values of explanatory plant traits (solid circles) and flammability traits (open circles). Trait values are indicated by the size of the circles. A nonzero phylogenetic signal was found for leaf SA/V ratio (Pagel's $\lambda = 1$; $P = 1$ for $\lambda = 1$; $P < 0.001$ for $\lambda = 0$), leaf flaming time (Pagel's $\lambda = 0.45$; $P = 1.0$ for $\lambda = 1$; $P < 0.001$ for $\lambda = 0$) and leaf combustion rate (Pagel's $\lambda = 0.99$; $P = 0.93$ for $\lambda = 1$; $P = 0.037$ for $\lambda = 0$).

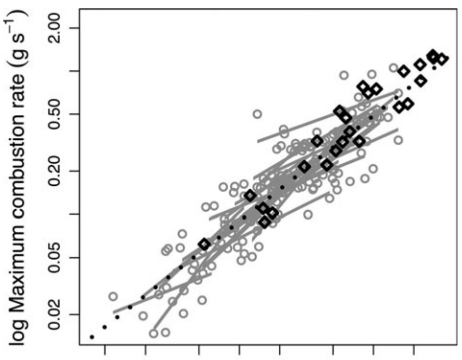


Fig. 2. Relationships between biomass quantity and maximum combustion rate across 25 grass species. The mean slopes of within-species relationships (grey lines) and across-species relationships (black dotted line) for maximum combustion rate with biomass burned do not differ significantly when phylogeny is accounted for. Data points are shown as grey circles. Estimates of whole-plant combustion rates (black diamonds) showed substantial variation (>20-fold). These values were calculated by extrapolating the common across-species relationship (black dashed line) to species mean total biomass values while taking into account the intrinsic combustibility differences among species.

as well as variation in leaf-scale combustibility (39%). The direction of relationships between plant and flammability traits is consistent with those predicted in Table 1, but there are exceptions. Both biomass density and leaf SA/V ratio were expected to correlate positively with predicted spread rate, but instead correlated negatively (Table 3).

Moisture content was key in determining leaf-scale flammability components (Table 2; Table S6). Ignitability was particularly influenced by moisture content, with fresh leaf material taking 42% longer to ignite on average than dry leaf material across species, with a maximum increase of 288% seen for *C. setaceus* (1.0 s dry vs. 4.0 s fresh). Once alight, fresh leaf material also burned on average for 7% longer at a 3% lower combustion rate compared to dry leaf material across species. Leaf SA/V ratio significantly influenced sustainability, with high values associated with low flaming duration. The EHoC of leaf material alone contributed little to overall leaf-scale flammability when compared to moisture or SA/V ratio (Table 2).

At the plant scale, biomass quantity was by far the strongest driver of sustainability and combustibility (Table 3). Plants with greater biomass burnt at a faster rate and for longer. Biomass density and moisture content significantly contributed to plant-scale combustibility, such that plants with high density and low moisture content combusted most rapidly (Table 3). The EHoC of leaf material significantly contributed to sustainability with high values associated with short flaming times (Table 3). Leaf SA/V ratio did not significantly contribute to plant-scale combustibility or sustainability.

Biomass load, moisture content, density and leaf SA/V ratio all contributed highly to predicted fire spread rate when taking phylogeny into account (Table 3). Fuel load contributed directly to reaction intensity and indirectly to the propagating flux ratio, via bulk density. Biomass moisture content contributed to spread rate by increasing the heat required for ignition and damping the reaction intensity (see Fig. S2). Leaf SA/V ratio influenced reaction intensity and the proportion of this reaching adjacent fuel (propagating flux ratio), as well as the proportion of fuel raised to ignition temperature (effective heating number; Fig. S2). Leaf EHoC contributed to the reaction intensity but played a small part in determining the overall predicted rate of spread (Table 3; Fig. S2).

TRAIT COVARIANCE

Principal components analysis (PCA) and linear regressions were used to explore patterns of covariance among the plant and flammability trait variables, with the latter being split into leaf-scale and plant-scale traits (Fig. 3). For the plant traits, the first two principal components accounted for 67.6% of the total variance. The first axis related to the chemical properties of biomass and how it is arranged spatially (leaf EHoC, biomass moisture content and density had the highest axis loadings). Leaf SA/V ratio loaded most heavily on the second axis, followed by biomass moisture content and density. Only biomass quantity did not fall as clearly on the first two principal components, which we believe is due to the high variation within the data (CV = 89.0%). For the leaf-scale flammability traits, the first two principal components accounted for 95.1%

Table 2. The contribution of plant traits to leaf-scale flammability components as determined by MCMC phylogenetic generalized linear mixed models. Values represent posterior mean estimates of the slopes, the upper and lower 95% confidence intervals and *P* values (those in bold are significant at $P = 0.05$). In combination, species mean trait values of leaf moisture content, SA/V ratio and effective heat of combustion (EHoC) significantly predicted ignitability ($F_{1,166} = 398.3$, $P < 0.001$, $R^2 = 0.70$), sustainability ($F_{1,166} = 147.5$, $P < 0.001$, $R^2 = 0.47$) and combustibility ($F_{1,166} = 105.4$ $P < 0.001$, $R^2 = 0.39$)

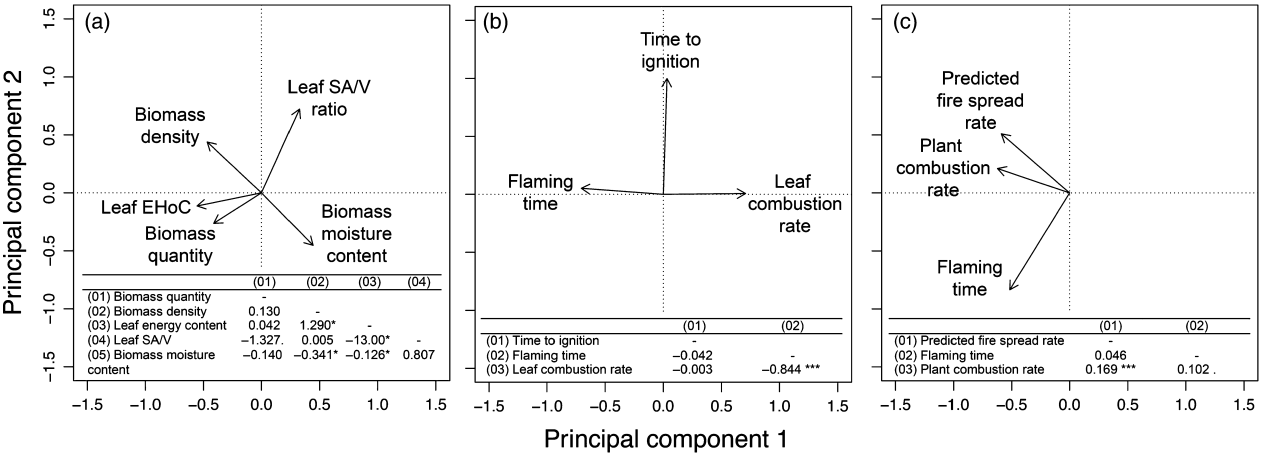
		Leaf moisture content*	Leaf SA/V ratio	log Leaf EHoC
Ignitability (time to ignition)	Estimate	0.691	−0.174e-3	−0.135e-4
	(95% CI)	(0.620 to 0.760)	(−0.420e-3 to 0.872 e-5)	(−0.527e-4 to 0.290e-4)
	P value	**<0.001**	0.17	0.49
Sustainability (flaming time)	Estimate	0.492	−0.876e-3	0.159e-4
	(95% CI)	(0.421 to 0.567)	(−0.142e-2 to -0.359 e-4)	(−0.626e-4 to 0.113e-3)
	P value	**<0.001**	**0.002**	0.741
Combustibility (combustion rate)	Estimate	−0.303e-2	0.522e-5	−0.227e-6
	(95% CI)	(−0.406e-2 to −0.170e-2)	(−0.547e-5 to 0.164e-4)	(−0.254e-5 to 0.193e-5)
	P value	**<0.001**	0.36	0.86

*Parameter characterized as: the species mean difference in ignition delay (for ignitability) or flaming duration (for sustainability and combustibility) between fresh and dry leaf material for each individual.

Table 3. The contribution of plant traits to plant-scale flammability components as determined by MCMC phylogenetic generalized linear mixed models. Values represent posterior mean estimates of the slopes, the upper and lower 95% confidence intervals and *P* values (those in bold are significant at $P = 0.05$). Values represent posterior mean estimates of the slopes, the upper and lower 95% confidence intervals and *P* values (those in bold are significant at $P = 0.05$). In combination, the five plant traits significantly predicted sustainability ($F_{1,151} = 90.07$, $P < 0.001$, $R^2 = 0.37$), combustibility ($F_{1,151} = 210.8$, $P < 0.001$, $R^2 = 0.58$) and ignitability ($F_{1,173} = 184.2$, $P < 0.001$, $R^2 = 0.51$)

		log Biomass quantity	log Biomass density	log Biomass moisture content	Leaf SA/V ratio	log Leaf EHoC*
Sustainability (flaming time)	Estimate	0.434	−0.614	1.036	−0.050	−0.012
	(95% CI)	(0.350 to 0.517)	(−2.162 to 0.889)	(−0.688 to 2.753)	(−0.162 to 0.055)	(−0.023 to 0.001)
	P value	**<0.001**	0.443	0.252	0.363	0.060
Combustibility (maximum combustion rate)	Estimate	0.035	0.149	−0.108	0.105e-2	−0.580e-4
	(95% CI)	(0.028 to 0.041)	(0.021 to 0.277)	(−0.250 to 0.027)	(−0.858e-2 to 0.012)	(−0.101e-2 to 0.103e-2)
	P value	**<0.001**	**0.024**	0.116	0.910	0.826
Ignitability (predicted spread rate)	Estimate	2.002	−0.061	−0.034	0.128e-2	0.121e-3
	(95% CI)	(0.951 to 3.015)	(−0.094 to −0.033)	(−0.044 to −0.025)	(0.789e3 to 0.169e-2)	(−0.993e-4 to 0.360e-3)
	P value	**<0.001**	**<0.001**	**<0.001**	**<0.001**	0.309

*Species mean values.



	(01)	(02)	(03)	(04)
(01) Biomass quantity	-			
(02) Biomass density	0.130	-		
(03) Leaf energy content	0.042	1.290*	-	
(04) Leaf SA/V	−1.327.	0.005	−13.00*	-
(05) Biomass moisture content	−0.140	−0.341*	−0.126*	0.807

	(01)	(02)
(01) Time to ignition	-	
(02) Flaming time	−0.042	-
(03) Leaf combustion rate	−0.003	−0.844 ***

	(01)	(02)
(01) Predicted fire spread rate	-	
(02) Flaming time	0.046	-
(03) Plant combustion rate	0.169 ***	0.102 .

Fig. 3. Principal components analysis biplots of explanatory plant traits (a) and flammability traits at the leaf scale (b) and plant scale (c). The tables within each plot indicate the slope and significance of linear regressions between each pair of variables. Data for all traits were log-transformed to improve normality except leaf SA/V ratio. EHoC is the leaf effective heat of combustion. $P < 0.1$; *, $P < 0.05$; ***, $P < 0.001$.

of the total variance. Leaf flaming time and combustion rate were negatively correlated ($P < 0.001$), and fell in opposing directions on the first PCA axis (Fig. 3), which reflects how combustion rate was derived from flaming time. Time to ignition was unrelated to flaming time and combustion rate and was orthogonal to both in the PCA (Fig. 3). For plant-scale flammability traits, 71.8% of total variance is accounted for by the first two principal components. Traits did not separate on the first axis, but did on the second axis which related to burning intensity. High rates of plant combustion were associated with rapid predicted fire spread rates ($P < 0.001$) and marginally with longer flaming times ($P = 0.071$; Fig. 3).

The relationships between flammability traits measured at different scales were variable, with a significantly positive correlation found for ignitability (leaf time to ignition vs. predicted rate of spread; $P = 0.025$), but no significant correlation for combustibility (leaf-scale combustion rate vs. plant-scale combustion rate; $P = 0.29$).

INFLUENCE OF EVOLUTIONARY HISTORY ON FLAMMABILITY

Support for a phylogenetic signal was found for leaf-scale combustibility (Pagel's $\lambda = 0.99$; $P = 0.93$ for likelihood ratio test against $\lambda = 1$; $P = 0.037$ against $\lambda = 0$) and sustainability (Pagel's $\lambda = 0.45$; $P = 0.67$ against $\lambda = 1$; $P = 0.011$ against $\lambda = 0$), but not for the other flammability traits. Of the plant traits, there was a strong phylogenetic signal for leaf SA/V ratio (Pagel's $\lambda = 1.00$; $P = 1.00$ against $\lambda = 1$; $P < 0.001$ against $\lambda = 0$), with closely related species tending to have similar values of leaf SA/V ratio. No phylogenetic signal was found in the other plant traits.

Discussion

This large comparative study of grass flammability provides strong support for the hypothesis that grass species vary significantly in multiple components of flammability. This finding suggests that static classifications of grassy and herbaceous vegetation as homogenous fuels mask considerable interspecific and community variation in flammability. Consequently, fire behaviour predictions based on such fuel models may lose accuracy when community composition is not accounted for.

A substantial proportion of variation in ignitability and combustibility (70% and 58%, respectively) can be explained by a combination of the five plant traits measured here. For sustainability, a smaller proportion of variation was accounted for (37%), perhaps because this component is not only driven by plant traits, but is also directly influenced by combustibility. Additionally, some variation in sustainability could be accounted for by traits relating to leaf chemistry, such as nitrogen, phosphorus and tannin concentrations (Grootemaat *et al.* 2015) that were not measured in this study. Biomass quantity was the key trait influencing plant-scale flammability components and also determined the influence of an individual plant on local fire characteristics. The importance of biomass quantity for combustibility, sustainability and fire spread rates in the field is illustrated by the elevated flammability of landscapes caused by the raised fuel load production of non-native grasses (Hughes, Vitousek & Tunison 1991; D'Antonio & Vitousek 1992; Rossiter *et al.* 2003). While making a relatively small contribution to flammability components once alight, biomass moisture content was key to ignitability, with higher moisture contents requiring more energy to dry and heat biomass to the point of ignition (Trollope 1978; Gill & Moore 1996; Alessio *et al.* 2008; Plucinski & Anderson 2008). By influencing ignitability, and therefore the likelihood of fire occurring in the first place, moisture content exerts a strong influence on vegetation flammability. Our finding of high interspecific variation in EHoC (effective heat of combustion) also conflicts with the notion that grass energy content is an almost constant value (Trollope 1984). However, EHoC contributed little to leaf-scale flammability components, supporting the idea that this intrinsic property is less important in determining flammability than fuel mass, structure and moisture content (Bond & van Wilgen 1996). Despite this small importance overall, the EHoC marginally contributed to plant-scale flaming time.

The inconsistent relationships between components of flammability, and within flammability components measured at different scales, suggest that descriptions of flammability should incorporate all suitable components and should be taken at an appropriate scale. The mixed covariance between flammability components found here suggests that one cannot always be used as a proxy for the others. Therefore, studies that consider one or even two components of flammability may mask the complexity of vegetation flammability (Anderson 1970). Similar to the findings of Martin (2010), we find support for the importance of incorporating plant architecture into measurements of grass flammability. Inconsistencies between combustibility at the leaf- and plant-scale highlight that other factors (such as biomass quantity and density) are key determinants of combustibility at the plant scale. Bench-scale measurements of flammability have been criticized as not being representative of flammability in the field (Fernandes & Cruz 2012), and our findings emphasize the need for caution when extrapolating flammability traits between different scales. In comparison with leaf-scale studies, the flammability component values obtained here are more representative of flammability in the field because they are measured at the plant scale and on field-state plants that are at the phenological stage most appropriate to fire occurrence.

The phylogenetic signal found in some flammability components (leaf-scale combustibility and sustainability) suggests that evolutionary history may partially explain patterns of grass flammability and the strong sorting of grass lineages across fire-frequency gradients (Uys, Bond & Everson 2004; Visser *et al.* 2012; Forrestel, Donoghue & Smith 2014). However, conclusions on phylogenetic signal derived from a small phylogeny must remain cautious due to low statistical power (Boettiger, Coop & Ralph 2012).

Through their flammability, plants may modify the fire regime they experience in order to increase their fitness in fire-prone environments (Schwilk 2003). Resprouting grasses are likely to benefit from frequent fires that remove standing biomass and maintain an open canopy, because they are typically intolerant of shading (Everson, Everson & Tainton 1988; Bond 2008). The grasses studied here showed high ignitability, combustibility and predicted fire spread rates, when compared to woody vegetation fuels (e.g. Pausas *et al.* 2012; Ganteaume *et al.* 2013). Furthermore, grasses are able to regrow quickly after fire. This combination of high flammability and rapid regrowth drives a fire regime characterized by high fire frequency (Grigulis *et al.* 2005). Plant-scale combustion rate was marginally positively related to flaming time, with high biomass plants burning at a faster rate and for longer. This finding is in contrast with other studies (e.g. de Magalhães & Schwilk 2012) that found a negative relationship between the two. It also does conflicts with the idea of high flammability providing resprouting plants protection against lethal temperatures (Gagnon *et al.* 2010), as for grasses that have higher fuel loads, rapid combustion is not associated with lowered burning durations and a subsequent reduction in heat transfer to the soil and below-ground plant parts. The interspecific variation in flammability components observed across a set of species that commonly coexist in the field further suggests a role for interspecific competition in promoting flammability as an adaptive trait. Potentially, enhanced plant flammability can increase the mortality of neighbouring, less fire-tolerant individuals and thereby reduce post-fire competition (Bond & Midgley 1995). Furthermore, some evidence provides intriguing support for a link between high flammability and ecological success in fire-prone grassland species (Ripley *et al.* 2015). The influence of flammability at the species level on grassland community-level flammability has not been determined. However, findings from other vegetation fuel types show that flammability tends to be driven by the most flammable species of a community, such that fuel loads are non-additive (van Altena *et al.* 2012; de Magalhães & Schwilk 2012). The knowledge gained in this study can be used in further work to determine whether high flammability is an adaptation to life in frequently burnt environments for grasses and has thus been a fundamental trait in grass evolution. In addition, the knowledge of interspecific variation in grass flammability obtained here can lead to a better understanding of wildfire behaviour, particularly in grassland ecosystems. This could potentially contribute to an improvement of global carbon modelling and lead to new insights about ecosystem feedback to fire.

Acknowledgements

Research support was provided by a Natural Environment Research Council studentship to K.J.S., Royal Society University Research Fellowship URF120119 to P.A.C. and URF120016 to G.H.T. and a European Research Council Starter Grant ERC-2013-StG-335891-ECOFLAM to C.M.B. Author contributions: K.J.S., G.H.T., B.S.R., C.M.B., C.E.R.L. and C.P.O. designed the study. K.J.S., B.S.R. and P.A.C. generated the data. K.J.S., P.A.C., B.S.R., G.H.T. and C.P.O. analysed the data. K.J.S. wrote the manuscript with the help of all the authors. We thank Tony Palmer, Claire Adams and Nosipho Plaatjie for their support in the laboratory and field, Albert Phillimore for assistance with the MCMCglmm analyses and James Simpson for his help with graphics. We also thank Hans Cornelissen and two anonymous referees for their constructive comments on the manuscript.

Data accessibility

Trait data: Species average values uploaded as online supporting information; raw data available in DRYAD entry doi: 10.5061/dryad.2c506.

Sequence data: GenBank accession numbers available as online supporting information.

Phylogeny: Nexus file available in DRYAD entry doi: 10.5061/dryad.2c506.

MCMCglmm R Script: Available in DRYAD entry doi: 10.5061/dryad.2c506.

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Received 19 March 2015; accepted 26 October 2015
Handling Editor: Hans Cornelissen

Supporting Information

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

Figure S1. Schematic drawing of the set-up used to measure plant-scale combustibility and sustainability.

Figure S2. Cumulative dry biomass over vertical plant height for the grass species.

Figure S3. The influence of plant traits on components of Rothermel's (1972) fire spread rate model.

Table S1. Climate data from plant collection sites.

Table S2. Grass species names, collection site and GenBank accession details.

Table S3. Plant traits values used to model the forward rate of fire spread (m min^{-1}).

Table S4. Species mean flammability component values.

Table S5. Species mean plant trait values.

Table S6. Results of analysis of variance (two-way ANOVA with interaction) of leaf-scale flammability by species and state (fresh or dry).

Table S7. Mean plant trait values for the three collection sites.