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Determinants of flammability in savanna grass species

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Running headline: Determinants of flammability in savanna grass species

27 *Summary*

- 28 1. Tropical grasses fuel the majority of fires on Earth. In fire-prone landscapes, enhanced
29 flammability may be adaptive for grasses via the maintenance of an open canopy and
30 an increase in spatio-temporal opportunities for recruitment and regeneration. In
31 addition, by burning intensely but briefly, high flammability may protect resprouting
32 buds from lethal temperatures. Despite these potential benefits of high flammability to
33 fire-prone grasses, variation in flammability among grass species, and how trait
34 differences underpin this variation, remain unknown.
- 35 2. By burning leaves and plant-parts, we experimentally determined how five plant traits
36 (biomass quantity, biomass density, biomass moisture content; leaf surface-area-to-
37 volume ratio and leaf effective heat of combustion) combined to determine three
38 components of flammability (ignitability, sustainability and combustibility) at the leaf
39 and plant scales in 25 grass species of fire-prone South African grasslands at a time of
40 peak fire occurrence. The influence of evolutionary history on flammability was
41 assessed based on a phylogeny built here for the study species.
- 42 3. Grass species differed significantly in all components of flammability. Accounting for
43 evolutionary history helped to explain patterns in leaf-scale combustibility and
44 sustainability. The five measured plant traits predicted components of flammability,
45 particularly leaf ignitability and plant combustibility in which 70% and 58% of
46 variation respectively could be explained by a combination of the traits. Total above-
47 ground biomass was a key driver of combustibility and sustainability with high-
48 biomass species burning more intensely and for longer, and producing the highest
49 predicted fire spread rates. Moisture content was the main influence on ignitability,
50 where species with higher moisture contents took longer to ignite and once alight burnt
51 at a slower rate. Biomass density, leaf surface-area-to-volume ratio and leaf effective
52 heat of combustion were weaker predictors of flammability components.

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

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

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79 **Introduction**

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

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

103 Plant flammability is a key determinant of fire behaviour (Bond & van Wilgen 1996;
104 Beckage *et al.* 2009). In woody plants, flammability varies considerably between and within

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

125 Despite these predicted benefits of frequent fire to fire-prone grasses, inter-specific
126 variation in the flammability of such species has been little explored (Ripley *et al.* 2010), in
127 contrast to knowledge about interspecific variation in post- fire response among grass species
128 (Ripley *et al.* 2015). A historical belief persists that grasses and other herbaceous plants vary
129 little in their flammability, which has led to the diversity of herbaceous fuels being reduced to
130 one or few fuel classes in fire behaviour modelling (e.g Anderson 1982). Given the

131 considerable known variation in the flammability of woody species (Schwilk 2003; Scarff &
132 Westoby 2006; Pausas *et al.*, 2012; Murray, Hardstaff & Phillips 2013), such presumptions are
133 unfounded. Substantial changes in grassland community flammability resulting from invasion
134 by non-native grasses provide evidence to suggest considerable inter-specific variation in grass
135 flammability (Hughes, Vitousek & Tunison 1991; Rossiter *et al.* 2003). In addition, recent
136 evidence shows that grass traits relating to post-fire recovery are shaped by fire regime
137 (Forrestel *et al.* 2014; Ripley *et al.* 2015), suggesting that traits relating to flammability may be
138 responding in similar ways, resulting in intra- and inter-specific variation in flammability.

139 Physical and chemical traits influencing some or all components of flammability relate
140 to the quantity, quality, moisture content and aeration of biomass (Gill & Moore 1996; Bond &
141 van Wilgen 1996). Biomass quantity is critical to combustibility and fire spread rate because it
142 directly influences fire energy output rate (Byram 1959; Rothermel 1972). Biomass moisture
143 content determines the extent to which fuels absorb heat energy, with high values associated
144 with delayed ignition and low combustion and fire spread rates (Pyne 1984, Nelson 2001).
145 Biomass surface-area-to-volume (SA/V) ratio influences curing and reaction rates within fires
146 (Papio & Trabaud 1990, Gill & Moore 1996), with high values linked to rapid ignition, and
147 rapid rates of combustion and fire spread. Increasing biomass density, defined as the mass of
148 biomass per unit volume of fuel bed, raises fuel connectivity, therefore enhancing
149 combustibility and fire spread rate. This relationship applies up to a certain threshold beyond
150 which poor ventilation will limit drying and combustion rates (Rothermel 1972). Intrinsic
151 properties of plant material, such as heat of combustion, affect combustibility and fire spread
152 rate through the amount of heat energy released during complete combustion. Sustainability is
153 often inversely related to combustibility and ignitability (e.g. de Magalhães & Schwilk 2012).
154 Therefore plant traits likely to enhance combustion and spread rate may indirectly reduce
155 flaming duration. In contrast, high biomass quantity increases combustion and spread, but is
156 also likely to enhance sustainability, as more fuel takes longer to burn. Plant traits important to

157 flammability have been identified in a number of fire-prone taxa (e.g. Ganteaume *et al.* 2009;
158 Schwilk & Caprio 2011). However, the traits that influence grass flammability, and more
159 generally the flammability of herbaceous species, have not been empirically established or
160 explored.

161 We examined three components of flammability, at multiple scales, for 25 species
162 common in fire-prone South African grasslands. Five structural and chemical plant traits,
163 known to influence vegetation flammability, were measured and correlated with flammability
164 trait values (see Table 1). We hypothesized that 1) there is significant inter-specific variation in
165 flammability among grass species, and that 2) the measured plant traits can explain this
166 variation, with each trait contributing to flammability components in different ways (see Table
167 1 for specific predictions). We also expected that flammability and plant traits covary due to
168 the interdependent relationships between flammability components and plant traits. The strong
169 phylogenetic patterns in grass distributions across fire frequency gradients (e.g Visser *et al.*
170 2012, Forrestel *et al.* 2014) led us to predict that 3) flammability is influenced by evolutionary
171 history and contains a phylogenetic signal.

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183 ***Materials and methods***

184 Plant material

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

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

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197 Structural and chemical traits

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

202 For measurements of leaf SA/V ratio and EHoC, leaves were removed from a randomly
203 selected tiller of each individual. Total leaf area was measured on digital images using the
204 computer program WinDIAS (Delta-T Devices, Cambridge, U.K.) that determines leaf area by
205 selecting pixels of a pre-defined colour range. Leaf thickness was measured, at the middle of
206 the leaf and excluding the midrib, for three leaves per tiller using digital callipers (accurate to
207 0.01mm), and an average value was calculated. Leaf SA/V ratio was calculated from the
208 average leaf area and leaf thickness of each species.

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

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

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

239

240 Flammability

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

251 Leaf-scale ignitability, sustainability and combustibility were measured as time to
252 ignition, flaming time and mass loss rate respectively using a Quartz infrared 500 W
253 epiradiator (Helios, Italquartz, Milan, Italy) in a fume cupboard with a constant vertical
254 windspeed of 0.1 m s^{-1} . As application of leaf material directly to the epiradiator's silica disk
255 surface always caused instantaneous combustion, 2mm wire mesh was positioned 1cm above
256 the epiradiator's surface. The background temperature at the mesh surface (without fuel),
257 measured by a thermocouple connected to a data-logger, ranged between 370 and 400°C .
258 Samples of 0.2 g ($\pm 0.001 \text{ g}$) leaf material were cut into 2 cm segments to standardise between
259 samples, and applied to the centre of the mesh. The 0.2 g mass was used because preliminary
260 studies found that smaller masses failed to ignite, whilst larger fuel masses increased the risk

261 that other fuel properties, particularly fuel height, influenced flammability values. Smaller
262 samples were used for *Aristida congesta* subsp. *barbicollis* due to the low leaf mass of this
263 species. Each test was filmed at 25 frames s⁻¹, and i) time to ignition (TTI; the time between
264 sample application to the epiradiator and first flaming) and ii) flaming time (FT; the time from
265 ignition to flame extinction) were subsequently determined. As samples were completely
266 combusted by applying them to the epiradiator, an average leaf combustion rate was obtained
267 by dividing the mass of samples by FT. Species average values for TTI and FT were obtained
268 for fresh and dry material. The influence of leaf moisture content on these flammability traits
269 was determined as the difference in values between fresh and dry samples of each individual,
270 and averaged per species.

271 As canopy architecture influences grass flammability (Martin 2010), a method that
272 measures plant-scale flammability traits was utilised. Fresh and dry plant material from each
273 individual was clamped on a stand on a four-point balance (Mark 205A; Bel Engineering,
274 Monza, Italy) and burnt in a fume cupboard with a constant 0.1 m s⁻¹ vertical wind speed (see
275 Figure S1 for diagram of the set-up used). Samples were ignited by directing a Bunsen burner
276 flame to the side of the base of the clump at a 45° angle and a 5cm distance for a maximum of
277 3 seconds (less if ignition happened earlier). This resulted in successful ignition in all
278 individuals. Mass loss was logged at 0.2-second intervals and the sigmoidal relationship
279 produced was fitted with a Boltzmann equation. Data were excluded if fitting the relationship
280 was not possible due to noise around the curve (n = 40/350), which occurred if large pieces of
281 plant material fell off the balance during a burn. The width parameter used to fit the Boltzmann
282 curve reflects the time period in which mass was drastically reduced and was used as a plant-
283 scale measurement of sustainability (flaming time). Three seconds of data either side of the
284 inflection point were selected and a linear regression fitted. The slope of this regression
285 represents the maximum combustion rate in g s⁻¹. As preliminary results found this
286 combustibility trait to be strongly driven by the biomass of the sample, interspecific

287 comparisons were standardised for mass. Therefore, maximum combustion rate was plotted
288 against mass change for each species, and linear models were fitted to the fresh, dry and
289 combined datasets. As there was no change in mass common to all 25 species, the y-intercept
290 extracted from the model fitted to the combined dataset was used to characterise the intrinsic
291 combustibility of each species. The combined dataset was used as the slopes of the models
292 fitted to the fresh and dry data did not differ significantly for any species, and model fit was
293 improved by combining the datasets. Any unpaired samples were excluded to ensure a
294 balanced dataset of fresh and dry samples. The y-intercept differed significantly between fresh
295 and dry models for three species (*Panicum* sp., *Hyparrhenia hirta* and *Merxmuellera stricta*)
296 and in these cases, the y-intercept was extracted from linear models fitted to the fresh dataset.

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

304

305 Phylogenetic analysis

306 We constructed a phylogeny that was initially based on a previously generated dataset for
307 grasses composed of the plastid markers *trnKmatK*, *ndhF*, and *rbcL* (Grass Phylogeny
308 Working Group II 2012), and augmented here. For ten species not represented in this previous
309 dataset, a fragment of *trnKmatK* was PCR-amplified from genomic DNA, following protocols
310 and primers described previously (Grass Phylogeny Working Group II 2012). The newly
311 generated sequences have been submitted to NCBI database (Benson *et al.* 2012) under the
312 accession numbers KP860326 to KP860336. The new markers were manually aligned to the

313 dataset, which consisted of 606 taxa and 5649 aligned bp. This initial dataset was downsized to
314 70 species, including all the taxa studied here and representatives of all grass lineages. A time-
315 calibrated phylogenetic tree was obtained through Bayesian inference as implemented in
316 BEAST (Bayesian evolutionary analysis by sampling trees; Drummond & Rambaut 2007). A
317 general time-reversible substitution model with a gamma-shape parameter and a proportion of
318 invariants (GTR+G+I) was used. The log-normal relaxed clock was selected. The tree prior
319 was modelled by a Yule process. The monophyly of the BEP-PACMAD clade was enforced,
320 leaving *Puelia olyrifomis* as the outgroup. The calibration prior for the age of the BEP-
321 PACMAD crown was set to a normal distribution, with a mean of 51.2 and a standard
322 deviation of 0.001 (mean based on Christin *et al.* 2014). Two independent runs were conducted
323 for 10,000,000 generations, sampling a tree every 1,000 generations. The convergence of the
324 runs and the appropriateness of the burn-in period, set to 2,000,000 generations, were verified
325 using Tracer (Rambaut A, Drummond AJ (2007) Tracer v1.4, available at
326 <http://beast.bio.ed.ac.uk/Tracer>). Median ages were mapped on the maximum-credibility tree.
327 The relationships among the species studied here were extracted from this tree, and used for
328 comparative analyses.

329

330 Data analysis

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

333 Analysis of variance (ANOVA) was used to determine if plant and flammability traits
334 differed significantly between species. The influence of species, and state ('fresh' or 'dry'), on
335 leaf-scale flammability was determined by two-way ANOVA. As biomass quantity values for
336 the plant-scale burns are not representative of the species (i.e for each species, clumps were
337 sub-sampled and a range of masses were burnt), a species effect on the relationship between
338 maximum combustion rate and biomass quantity was tested using the R package MCMCglmm

339 (Hadfield 2010). This approach implements Markov chain Monte Carlo routines for fitting
340 generalized linear mixed models, whilst accounting for non-independence and correlated
341 random effects arising from phylogenetic relationships (Hadfield 2010). We fitted flammability
342 (maximum combustion rate) and biomass quantity as a bivariate normal response, and species
343 as a random effect. Models were run for 500,000 iterations with a burn-in of 1,000 iterations, a
344 thinning interval of 500 and weakly-informative priors ($V=\text{diag}(2)$, $\nu=0.002$). The 95%
345 highest posterior densities (HPD) of within-species and across-species slopes and the
346 difference between slopes were estimated whilst accounting for phylogeny and used to assess
347 whether slopes differed among species.

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

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

363 The influence of evolutionary history was established for each plant and flammability
364 trait by testing for the presence of a phylogenetic signal. This was done using the pgls function

365 in the *caper* package (Orme *et al.* 2012) which estimated Pagel's λ .

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391 **Results**

392 Flammability variation among species

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

401 At the plant-scale, intrinsic combustibility (for a given biomass) differed by < 2.5-fold
402 across species, ranging from 0.064 g s⁻¹ (*Eustachys paspaloides*) to 0.163 g s⁻¹ (*Themeda*
403 *triandra*). When investigating the relationship between combustion rate and biomass, the
404 bivariate mixed effects model revealed that within-species slopes (pooled mean = 0.594, HPD:
405 0.507 to 0.707) and across-species slopes (mean = 0.797, HPD: 0.067 to 1.385) did not differ
406 significantly (mean slope difference (Δb) = 0.212, HPD: -0.521 to 0.683) when accounting for
407 phylogeny (Fig. 2). This common relationship was extrapolated whilst taking into account
408 intrinsic combustibility differences, allowing combustion values to be predicted for the species
409 mean total biomass. These predicted values of whole-plant combustion rates varied >20-fold
410 among species, ranging from 0.06 g s⁻¹ (*A. congesta* subsp. *barbicollis*) to 1.28 g s⁻¹ (*M.*
411 *disticha*; Fig. 2).

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

417 Substantial inter-specific variation was also found in the five traits measured as
418 explanatory traits for flammability (Fig. 1; see Table S5). Biomass moisture content values of
419 the non-native *C. setaceus* were substantially higher than the other species. However, species
420 still differed significantly for this trait when *C. setaceus* was excluded (ANOVA: $F_{23,144}=14.39$,
421 $P<0.001$). The measurement of biomass density (i.e. vertical biomass distribution) produced
422 consistent values within species (Fig. S2; species average CV= 28%), but considerable
423 differences among species with slope values ranging from 0.155 (*Eragrostis lehmanniana*) to
424 0.831 (*M. stricta*).

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

428

429 Trait contributions to flammability

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

438 Moisture content was key in determining leaf-scale flammability components (Table 2;
439 Table S6). Ignitability was particularly influenced by moisture content, with fresh leaf material
440 taking 42% longer to ignite on average than dry leaf material across species, with a maximum
441 increase of 288% seen for *C. setaceus* (1.0 s dry vs 4.0 s fresh). Once alight, fresh leaf material
442 also burned on average for 7% longer at a 3% lower combustion rate compared to dry leaf

443 material across species. Leaf SA/V ratio significantly influenced sustainability, with high
444 values associated with low flaming duration. The EHoC of leaf material alone contributed little
445 to overall leaf-scale flammability when compared to moisture or SA/V ratio (Table 2).

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

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

462

463 Trait covariance

464 PCA and linear regressions were used to explore patterns of covariance among the plant and
465 flammability trait variables, with the latter being split into leaf-scale and plant-scale traits (Fig.
466 3). For the plant traits, the first two principal components accounted for 67.6% of the total
467 variance. The first axis related to the chemical properties of biomass and how it is arranged
468 spatially (leaf EHoC, biomass moisture content and density had the highest axis loadings). Leaf

469 SA/V ratio loaded most heavily on the second axis, followed by biomass moisture content and
470 density. Only biomass quantity did not fall as clearly on the first two principal components,
471 which we believe is due to the high variation within the data (CV = 89.0%). For the leaf-scale
472 flammability traits, the first two principal components accounted for 95.1% of the total
473 variance. Leaf flaming time and combustion rate were negatively correlated ($P < 0.001$), and fell
474 in opposing directions on the first PCA axis (Fig. 3), which reflects how combustion rate was
475 derived from flaming time. Time to ignition was unrelated to flaming time and combustion
476 rate, and was orthogonal to both in the PCA (Fig. 3). For plant-scale flammability traits, 71.8%
477 of total variance is accounted for by the first two principal components. Traits did not separate
478 on the first axis, but did on the second axis which related to burning intensity. High rates of
479 plant combustion were associated with rapid predicted fire spread rates ($P < 0.001$) and
480 marginally with longer flaming times ($P = 0.071$; Fig. 3).

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

485
486 Influence of evolutionary history on flammability
487 Support for a phylogenetic signal was found for leaf-scale combustibility (Pagel's $\lambda = 0.99$;
488 $P = 0.93$ for likelihood ratio test against $\lambda = 1$; $P = 0.037$ against $\lambda = 0$) and sustainability (Pagel's λ
489 $= 0.45$; $P = 0.67$ against $\lambda = 1$; $P = 0.011$ against $\lambda = 0$), but not for the other flammability traits. Of
490 the plant traits, there was a strong phylogenetic signal for leaf SA/V ratio (Pagel's $\lambda = 1.00$;
491 $P = 1.00$ against $\lambda = 1$; $P < 0.001$ against $\lambda = 0$), with closely related species tending to have similar
492 values of leaf SA/V ratio. No phylogenetic signal was found in the other plant traits.

493

494

495 ***Discussion***

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

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

521 However, EHoC contributed little to leaf-scale flammability components, supporting the idea
522 that this intrinsic property is less important in determining flammability than fuel mass,
523 structure and moisture content (Bond & van Wilgen 1996). Despite this small importance
524 overall, the EHoC marginally contributed to plant-scale flaming time.

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

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

547 Through their flammability, plants may modify the fire regime they experience in order
548 to increase their fitness in fire-prone environments (Schwilk 2003). Resprouting grasses are
549 likely to benefit from frequent fires that remove standing biomass and maintain an open
550 canopy, because they are typically intolerant of shading (Everson *et al.* 1988; Bond 2008). The
551 grasses studied here showed high ignitability, combustibility and predicted fire-spread rates,
552 when compared to woody vegetation fuels (e.g Pausas *et al.* 2012; Ganteaume *et al.* 2013).
553 Furthermore, grasses are able to regrow quickly after fire. This combination of high
554 flammability and rapid regrowth drives a fire regime characterised by high fire frequency
555 (Grigulis *et al.* 2005). Plant-scale combustion rate was marginally positively related to flaming
556 time, with high biomass plants burning at a faster rate and for longer. This finding is in contrast
557 with other studies (e.g. de Magalhães & Schwilk 2012) that found a negative relationship
558 between the two. It also does conflicts with the idea of high flammability providing resprouting
559 plants protection against lethal temperatures (Gagnon *et al.* 2010), as for grasses that have
560 higher fuel loads, rapid combustion is not associated with lowered burning durations and a
561 subsequent reduction in heat transfer to the soil and below-ground plant parts. The inter-
562 specific variation in flammability components observed across a set of species that commonly
563 coexist in the field further suggests a role for inter-specific competition in promoting
564 flammability as an adaptive trait. Potentially, enhanced plant flammability can increase the
565 mortality of neighbouring, less fire-tolerant individuals, and thereby reduce post-fire
566 competition (Bond & Midgley 1995). Furthermore, some evidence provides intriguing support
567 for a link between high flammability and ecological success in fire-prone grassland species
568 (Ripley *et al.* 2015). The influence of flammability at the species level on grassland
569 community-level flammability has not been determined. However findings from other
570 vegetation fuel types show that flammability tends to be driven by the most flammable species
571 of a community, such that fuel loads are non-additive (de Magalhães & Schwilk 2012; van
572 Altena *et al.* 2012). The knowledge gained in this study can be used in further work to

573 determine whether high flammability is an adaptation to life in frequently burnt environments
574 for grasses, and has thus been a fundamental trait in grass evolution. In addition, the
575 knowledge of inter-specific variation in grass flammability obtained here can lead to a better
576 understanding of wildfire behaviour, particularly in grassland ecosystems. This could
577 potentially contribute to an improvement of global carbon modelling, and lead to new insights
578 about ecosystem feedback to fire.

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610

611 *Data Accessibility*

- 612 - Trait data: Species average values uploaded as online supporting information; raw data
613 available in DRYAD entry doi: 10.5061/dryad.2c506
- 614 - Sequence data: GenBank accession numbers available as online supporting information
- 615 - Phylogeny: Nexus file available in DRYAD entry doi: 10.5061/dryad.2c506
- 616 - MCMCglimm R Script: Available in DRYAD entry doi: 10.5061/dryad.2c506

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869 **SUPPORTING INFORMATION**

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

871 **Table S1** Climate data from plant collection sites.

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

873 **Table S3** Plant traits values used to model the forward rate of fire spread (m min⁻¹).

874 **Table S4** Species mean flammability component values.

875 **Table S5** Species mean plant trait values.

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

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

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

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

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

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904 Tables

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906 **Table 1.** Matrix summarising the predicted relationships between plant and flammability traits.

907 Flammability traits were determined at different scales (L, leaf; P, plant; C, community) and

908 represent three flammability components. Symbols reflect the direction of the relationship ('+':

909 positive; '-': negative; '0': none; 'N/A': could not be tested). Influence is either direct or

910 indirect (in parentheses)

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Flammability trait	Flammability component	Scale	Plant trait				
			Biomass quantity (g)	Biomass density (g cm ⁻¹)	Biomass content (g g ⁻¹)	Biomass moisture	Leaf SA/V ratio
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	+	+	-	+	+

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

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		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)
	<i>P</i> 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)
	<i>P</i> 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)
	<i>P</i> value	<0.001	0.36	0.86

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

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933 **Table 3.** The contribution of plant traits to plant-scale flammability components as determined
934 by MCMC phylogenetic generalized linear mixed models. Values represent posterior mean
935 estimates of the slopes, the upper and lower 95% confidence intervals and *P* values (those in
936 bold are significant at *P*=0.05). Values represent posterior mean estimates of the slopes, the
937 upper and lower 95% confidence intervals and *P* values (those in bold are significant at
938 *P*=0.05). In combination, the five plant traits significantly predicted sustainability ($F_{1,151}=90.07$,
939 $P<0.001$, $R^2=0.37$), combustibility ($F_{1,151}=210.8$, $P<0.001$, $R^2=0.58$) and ignitability
940 ($F_{1,173}=184.2$, $P<0.001$, $R^2=0.51$).

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		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)
	<i>P</i> 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)
	<i>P</i> 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)
	<i>P</i> value	<0.001	<0.001	<0.001	<0.001	0.309

942 §Species mean values

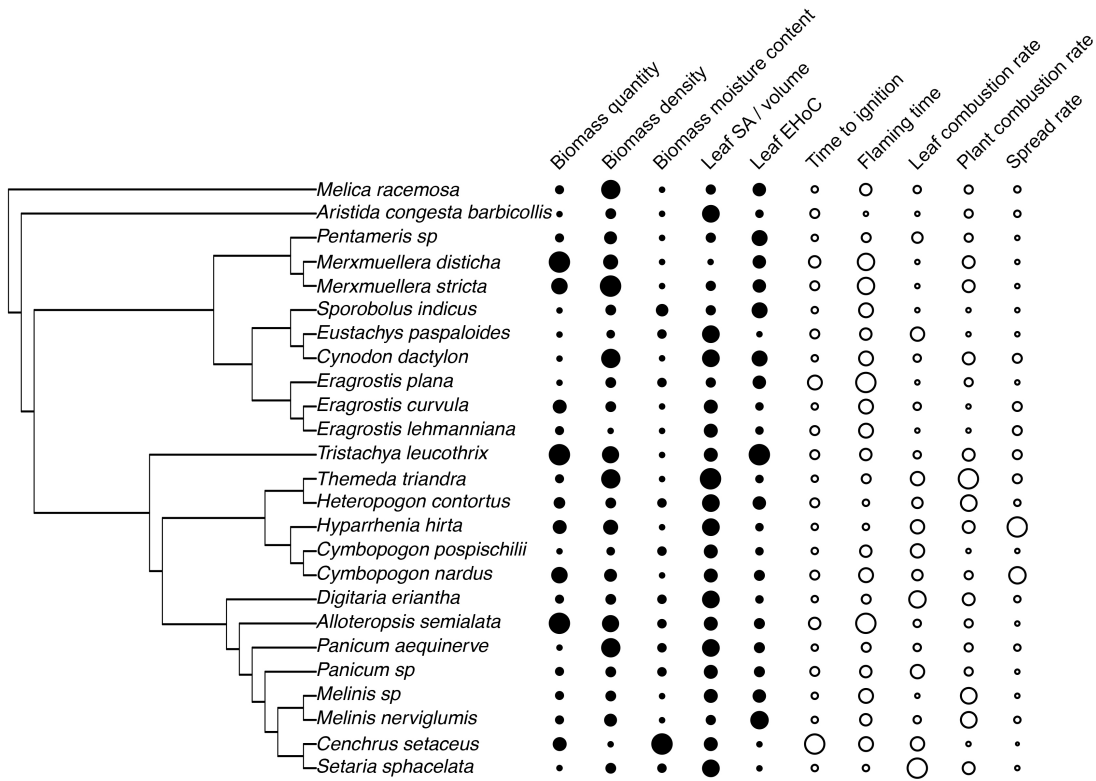
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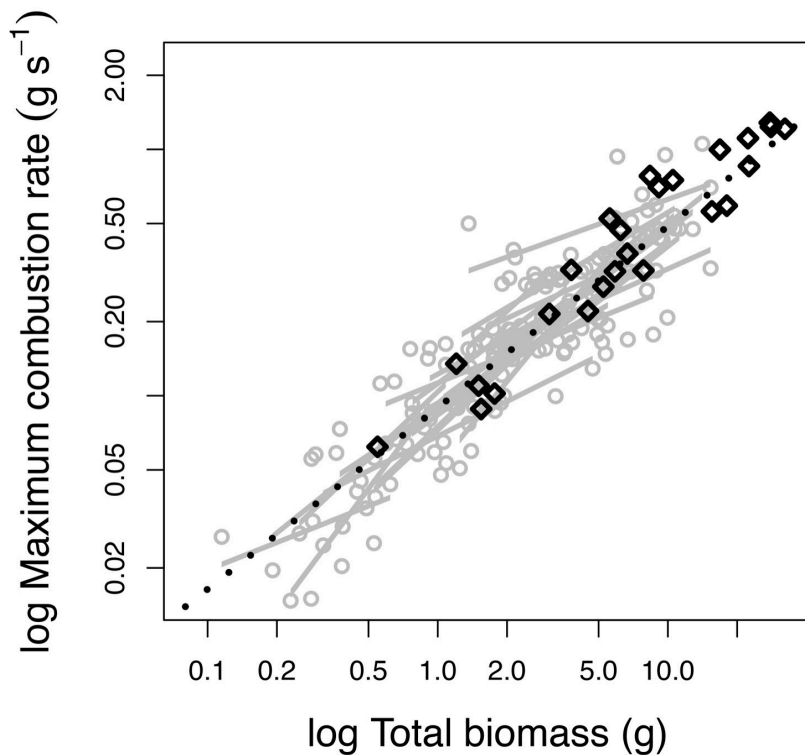
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 952 **Fig. 1.** The evolutionary relationships between species and average values of explanatory plant
 953 traits (solid circles) and flammability traits (open circles). Trait values are indicated by the size
 954 of the circles. A non-zero phylogenetic signal was found for leaf SA/V ratio (Pagel's $\lambda = 1$;
 955 $P=1$ for $\lambda=1$; $P<0.001$ for $\lambda=0$), leaf flaming time (Pagel's $\lambda = 0.45$; $P=1.0$ for $\lambda=1$; $P<0.001$
 956 for $\lambda=0$) and leaf combustion rate (Pagel's $\lambda =0.99$; $P=0.93$ for $\lambda=1$; $P=0.037$ for $\lambda=0$).

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 964 **Fig. 2.** Relationships between biomass quantity and maximum combustion rate across 25 grass
 965 species. The mean slopes of within-species relationships (grey lines) and across-species
 966 relationships (black dotted line) for maximum combustion rate with biomass burned do not
 967 differ significantly when phylogeny is accounted for. Data points are shown as grey circles.
 968 Estimates of whole-plant combustion rates (black diamonds) showed substantial variation
 969 (>20-fold). These values were calculated by extrapolating the common across-species
 970 relationship (black dashed line) to species mean total biomass values whilst taking into account
 971 the intrinsic combustibility differences among species.

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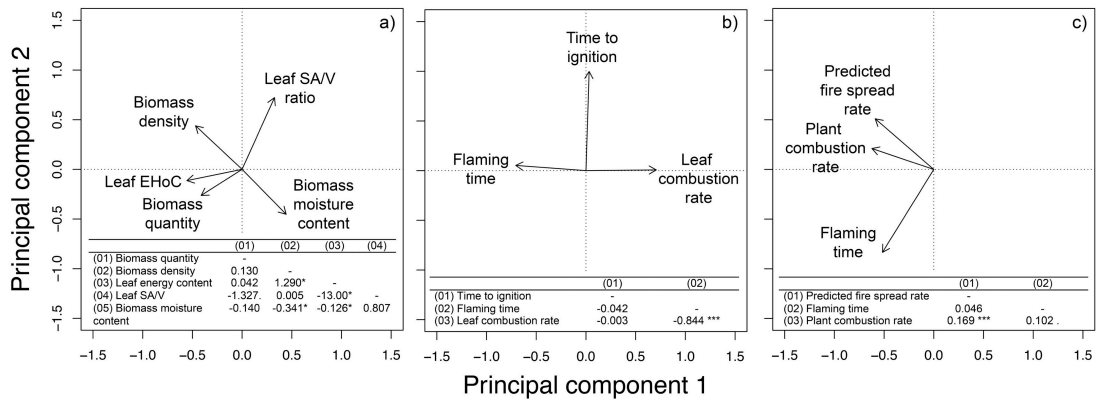
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 980 **Fig. 3.** Principal components analysis biplots of explanatory plant traits (a) and flammability
 981 traits at the leaf-scale (b) and plant-scale (c). The tables within each plot indicate the slope and
 982 significance of linear regressions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