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**African geoxyles evolved in response to fire; frost came later**

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**Items to be published as online appendix:**

1. Notes S1 Trait assignments in *Protea* phylogeny
2. Notes S2 Why are subshrub geoxylic proteas so short?
3. Fig. S1
4. Fig. S2
5. Table S1
6. Table S2

35

36 **Abstract**

37 It has been proposed in separate studies that fire or frost were the critical selective agents in the  
38 evolution of subshrub geoxyles (SGs) in African subtropical grasslands. We attempt to resolve this  
39 controversy by examining the evolution of SGs among the entire genus *Protea* that is widespread  
40 throughout southern/central Africa. We show that SGs are not confined to grasslands but occur in  
41 a wide range of non-forest types, including mediterranean shrublands. SG proteas arose 1–11  
42 million years ago but their multiple origins among other geoxyles, confounded by strong  
43 intraspecific variability among grassland species, makes it impossible to identify the ancestral  
44 growth form. We conclude that the evolutionary history of SG proteas has occurred under  
45 lightning-prone conditions that promoted fire and were essentially frost-free; exposure to frost has  
46 been limited to certain elevated locations in more recent times. This is supported by many SGs  
47 having pyrogenic flowering and lack of seed storage among grassland species.

48

49 **Keywords:** fire, frost, geoxyle, grassland, *Protea*, shrubland

50

51 **Subshrub geoxyles in Africa: the current controversy**

52 Identifying the agents of selection responsible for the evolution of critical adaptive traits is a  
53 key task in evolutionary ecology. There has been recent controversy about the origins of the  
54 resprouting subshrubs in central/southern (summer-rainfall) Africa. Maurin et al. (2014)  
55 examined the origin of these suffrutescent (hereafter, subshrub) geoxyles [i.e., plants with  
56 underground woody structures, sometimes supplemented by rhizomes, that enable resprouting  
57 after dieback, Lindman (1914) in Du Rietz (1931)] and contended that they arose in the late  
58 Cenozoic in response to frequent grassland fires. Finckh et al. (2016) responded that their  
59 evidence indicated frost instead was the key selective force as frost damage was common and  
60 recurrent fire was too recent. Davies et al. (2016) reiterated their previous interpretation but  
61 noted that much remained to be known about the evolutionary history of this growth form.  
62 Earlier, White (1977) believed that the distribution of geoxylic subshrubs was edaphically  
63 controlled, associated with nutrient-poor, seasonally waterlogged sands. Here, we address the  
64 stated need for more intensive sampling of lineages and their distribution (Frost 2012; Davies et  
65 al. 2016) by examining resprouting in the entire genus *Protea* that has a 28-million-year history  
66 (Valente et al. 2010). This genus is widespread in Africa, stretching from the SW tip of the  
67 continent to central Africa with outliers reaching Ethiopia in the NE and Guinea in the NW  
68 (Valente et al. 2010). We used the distribution and phylogenetic history of *Protea* to examine

69 the claims that a) subshrub geoxyles (SGs) in Africa are restricted to the savanna grasslands  
70 with a summer rainfall (White 1977; Maurin et al. 2014) since *Protea* is also widespread  
71 elsewhere, and b) that SGs are more likely an adaptive response to the ravages of frost rather  
72 than of fire (Finckh et al. 2016). The answers involve knowing: i) the distribution of proteas in  
73 relation to vegetation type, and incidence of fire and frost; ii) the morphological limits and  
74 genetic vs environmental controls of relevant growth forms; iii) the relative damage caused by  
75 fire vs frost; iv) whether fire or frost arose first as the key selective agent; and v) whether SGs  
76 are associated with other biological attributes that might give a clue to the critical selective  
77 agent.

78 Maurin et al. (2014) listed 23 proteas, which occur in the subtropical savanna grasslands  
79 of central Africa with a predominantly summer rainfall, that they considered to be subshrub  
80 geoxyles (SGs). We examined their morphological traits and compared them with the majority  
81 of proteas that occurs in the Cape shrublands, with a winter-(to uniform)-rainfall, to see if any  
82 SGs also occurred there. Finckh et al. (2016) pointed out that fires at their Angolan Plateau site  
83 were human- rather than lightning-caused, and therefore were too recent to have an  
84 evolutionary impact. The distributions of all SG proteas were therefore compared against  
85 selected temperature, lightning-strike and fire records in an attempt to identify limiting factors  
86 in common. This was supplemented by a comparison of species/lineage ages in each region that  
87 might provide a clue to the climates under which they arose.

88

### 89 **Morphology and habitat-type of subshrub geoxylic proteas**

90

91 Six of the 23 endemic grassland proteas listed by Maurin et al. (2014) as SGs were included in the  
92 Valente et al. (2010) phylogeny and we added two more from the Valente analysis (Table S1). The  
93 heights of these two species were not significantly different from the other 23 ( $P = 0.650$ ,  $t$ -test).  
94 We then identified 17 apparent SG species that are confined to the Cape shrublands (Table S1)  
95 among those used by Valente. Heights of the shrubland proteas were not significantly different  
96 from the grassland species though with a tendency to be lower and less variable (Table 1). All 42  
97 proteas resprout from rootstocks most of which are best described as lignotubers (Frost 2012;  
98 Lamont et al. 2013), i.e., swollen woody structures that can store buds anywhere on their upper  
99 surface, supported by a woody primary root of much narrower dimensions. Maurin et al. (2014)  
100 treated all the SG species they recorded among 22 families as arising from xylopodia, i.e., swollen  
101 woody structures with a few apical buds supported by swollen roots, but only recorded previously  
102 from Brazil where unrelated SGs abound (Rizzini and Heringer 1961; Simon et al. 2009).

103 However, the morphological descriptions/images for 30 SGs analysed by Maurin et al. (2014) that  
104 we examined were more likely to possess lignotubers and woody rhizomes with two having  
105 taproot tubers without any hard wood (data not provided here). Unlike the grassland geoxyles,  
106 most shrubland proteas have rhizomes arising from burls with fewer having a procumbent or erect  
107 habit, more similar to the Maurin et al. (2014) master list (Table 1). However, short, simple or  
108 sparsely divided branches, as in Maurin's list, characterize all shrubland and grassland species.  
109 Though SGs are often considered to be deciduous or with ephemeral branches (White 1977), this  
110 was only occasionally recorded in any of the three lists. Thus, a wide range of woody, bud-storing  
111 structures that support low, spreading shoot systems can be identified among SGs (xylopodia,  
112 lignotubers, woody rhizomes, taproot tubers, root suckers) so that no one resprouting mechanism  
113 accounts for their ability to survive disturbance.

114 The phylogeny of Valente et al. (2010) included 17 shrubland and 9 grassland proteas that  
115 we were able to assign to SGs (Fig. 1). It was of interest to know if subshrub geoxyles (SGs) are  
116 more likely to occur in one vegetation type rather than another. We constructed a dated phylogeny  
117 for *Protea* based on Valente et al. (2010) and Lamont et al. (2013). We assigned the growth forms,  
118 subshrub and shrub geoxyle and fire-surviving tree, and the vegetation type (grassland or  
119 shrubland) to all species from Table S1 and Rebelo (2001). Taking their phylogenetic position into  
120 account, we tested for any correlated shift of SGs between the habitat of grassland and shrubland  
121 (see Notes S1, Supplementary Material for details). There was no contingent association between  
122 presence of SG proteas and their location (shrubland vs grassland) ( $\log_e \text{BF} = -10.1$ , i.e.,  $P \gg$   
123  $0.05$ , see Notes S1, Supplementary Material). Thus, we conclude that SGs among proteas are not  
124 confined to subtropical grasslands (with a summer rainfall) but are also prominent in  
125 sclerophyllous shrublands predominantly under a mediterranean climate (with a summer drought  
126 and winter rainfall). This is also true for *Leucospermum* (Proteaceae) with one species listed by  
127 Maurin et al. (2014) but 11 SGs also in the Cape shrublands (Rebelo 2001). Similarly, a unique  
128 lineage of seven prostrate, serotinous banksias (Proteaceae), six resprouting via woody rhizomes  
129 (Witkowski and Lamont 1997), occurs in shrublands and woodlands of mediterranean  
130 southwestern Australia, while the single species in savanna is a fire-tolerant tree without seed  
131 storage (He et al. 2011). It is also worth noting that the original examples of 'dwarf-shrubs' and  
132 'herbaceous' geoxyles listed by Lindman (1914) [in Du Rietz (1931)] were from Europe, e.g.  
133 *Helianthemum chamaecistus* (with a root-crown). Other early researchers also noted the presence  
134 of SGs in non-grasslands: e.g. *Myrica elliptica* in the low shrublands of the Outeniqua Range, 350  
135 km east of Cape Town (Fig. 2, Burt Davey 1922), and the xylopodial-bearing *Pterocaulon*

136 *interruptum* on the east coast of Brazil “far away from the savanna” (Lindman 1900). Thus, SGs  
137 generally are not confined to grasslands but occur in a wide range of open vegetation types.  
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### 139 **Distribution of subshrub geoxylic proteas and environment**

140

141 For the nine subshrub geoxylic proteas in grasslands analysed by Valente *et al.* (2010), individual  
142 species ranged 15–78% of their populations on loam to 5–85% on sand while one occurred on  
143 clay and another on peat (collated from Rebelo 2009). Thus, edaphic constancy within and  
144 between SGs is negligible and soil type is unlikely to explain their distribution (contrast White  
145 1977), nor their evolution (supported by Lamont *et al.* 2013). SGs of the Cape occur within 120  
146 km of the coast but the SE African SGs occur up to 320 km inland near Polokwane (Pietersburg)  
147 (Fig. 2). Most shrubland and grassland *Protea* SGs occur in the area outside the mean winter  
148 isotherm of 10 °C, i.e., the area with 0–20 annual frost days (Finckh *et al.* 2016), including some  
149 species in the frost-free zone (<50 km from the coast). The 7 °C isotherm surrounds the area with  
150 35–70 annual frost days and includes about 20% of the grassland populations. It is clear that most  
151 SGs do not occur in particularly frost-prone areas but some do. Since the distribution of SG  
152 proteas strongly reflects the distribution of proteas generally (see map in Valente *et al.* 2010) we  
153 conclude that there is nothing especially frost-tolerant about them that can account for their  
154 belowground, bud-storage efficacy.

155 The symptoms of both fire and frost are to cause dieback of adult plants and death of  
156 young plants in particular. However, dieback of adults due to frost is never as severe as that due to  
157 fire because the bases of aerial stems survive and a few axillary buds remain intact (Holdo 2005,  
158 Fig. 2c in Finckh *et al.* 2016). This is attributable to the insulating effect of the highly flammable  
159 litter and living grass layers, and the fact that winter-dormant buds, as occur with grassland  
160 proteas (Smith and Granger 2017), are highly resistant to freezing (Ristic and Ashworth 1997).  
161 Receiving 60 frost-days annually in the Drakensberg Mountains, the non-SG species, *Protea*  
162 *roupelliae* subsp. *roupelliae*, had a 40% survival rate over eight years since establishment when  
163 unburnt, but only 4% survival when burnt annually (Smith and Granger 2017). Adult proteas are  
164 moderately resistant to frost (Rebelo 2009). Thus, *P. cynaroides*, experiences a 50% reduction in  
165 leaf chlorophyll fluorescence at 5.2°C (Bannister and Lord 2006) but complete recovery would  
166 occur from the unharmed axillary buds. The same species would lose all its aboveground mass if  
167 burnt but there is full recovery from fire via buds in the lignotuber (Rebelo 2001). Wakeling *et al.*  
168 (2012) showed that some dieback of acacia seedlings in the South African Highveld occurred from  
169 1350 m elevation (23 frost days during the trial) but mortality only exceeded the savanna sites

170 (located at < 1000 m) from 1650 m (37 frost days). So frost can kill young plants but there are vast  
171 areas of grassland (from 1000 to 1650 m) where frost is not severe enough to kill seedlings. Even  
172 young, fire-killed proteas show some frost-resistant traits (Prunier et al. 2012). Wakeling *et al.*  
173 (2012) concluded that the absence of trees in the grasslands was not due to frost *per se* but to slow  
174 growth rates that rendered the young plants more vulnerable to the frequent risk of incineration.  
175 That SG proteas do occur in grasslands must therefore depend on their rapid development of  
176 belowground bud-storing structures that are primarily resistant to fire.

177 In the absence of human intervention, savanna/grassland fires are initiated by lightning  
178 (Kennedy and Potgieter 2003). Finckh et al. (2016) noted that the incidence of lightning was low  
179 in Angolan grasslands during winter (dry season) when they were most likely to burn (that we also  
180 confirm, Fig. S1), reducing support for the SG as a fire-related trait. However, most current fires  
181 are deliberately lit, whereas prior to human occupation of the area, fires were historically most  
182 likely to occur at the start of the wet season (September–November) when lightning is abundant  
183 (Fig. S1; Kennedy and Potgieter 2003). The South African Highveld is fire-prone from March to  
184 November (Smith and Granger 2017) with abundant lightning in March–April and especially  
185 October–November with some in August–September that even now probably contributes to early  
186 dry and wet season fires (Fig. 3). Despite human intervention, there is still a close association  
187 between the incidence of lightning strikes and fire frequency (Manry and Knight 1986). Plotting  
188 lightning isolines on a vegetation map of South Africa shows that, in fact, the southeast grasslands  
189 are the most lightning-prone part of South Africa (Fig. S2; also see Keeley et al. 2012). The  
190 western Cape is in the range 100–250 lightning strikes/50 x 50 km<sup>2</sup>/annum increasing to 3500  
191 strikes at the eastern extreme. Most of the grasslands are in the range 1750–4400 strikes. Coupled  
192 with their high flammability, this renders the grasslands strongly fire-prone. While it is  
193 confounded with management fires, grasslands typically burn at 2–3-year intervals (Roques et al.  
194 2001) and many SGs may even burn annually (Medwecka Kornas 1980). Indeed, with the long  
195 human occupation of the area (300,000 years; Archibald *et al.* 2012) and the switch to winter fires  
196 further inhibiting recovery (Kennedy and Potgieter 2003), resprouting shrubs would not only be  
197 continually pruned back to the subshrub category but it is sufficient time for some ecological (e.g.,  
198 spatial redistribution) and evolutionary changes to have taken place. This variable incidence of fire  
199 might well explain why it has been possible to recognize so many subspecific ranks among  
200 woodland/grassland proteas.

201 Fires are less frequent in the Cape shrublands (Fig. 3A,B), typically at 10–20-year  
202 intervals (Rundel et al. 2016) and here peak incidence of lightning and fire activity coincide (Fig.  
203 3). Not only has the Cape had a shorter history of human occupation (165,000 years; Brown et al.

204 2009) but the timing conducive to human-lit fires (again the dry season) coincides with the  
205 occurrence of lightning (Fig. 3), thus reducing the impact of humans on the presence of SGs there.  
206 The abundance of fixed-form, lignotuberous-rhizomatous proteas in the Cape might be more  
207 attributed to the presence of a mediterranean climate, with its severe summer droughts and intense,  
208 moderate-interval fires (Lamont et al. 2013; Causley et al. 2016) with frosts rare except in the  
209 mountain ranges. In conclusion, while the incidence of lightning and frost varies greatly in the  
210 Cape, it is clear that any site where SG proteas occur was far more likely to be burnt by lightning-  
211 initiated fires than to experience frost by the late Quaternary.

212

### 213 **Age and evolution of subshrub geoxylic proteas**

214

215 *Protea* originated in the Cape 27.8 million years ago (Ma) under fire-prone conditions (Lamont et  
216 al. 2013; Fig. 1). Using continuous-time Markov model of trait evolution for discrete traits (Pagel  
217 and Meade 2006), we reconstructed the evolutionary trajectory of key traits in *Protea* (see Notes  
218 S1, Supplementary Material). The ancestral condition was nonsprouting (fire-killed) though a  
219 resprouting lineage appeared early, at 18.7 Ma. The rest of the clade remained nonsprouting until  
220 the resprouting grassland subclade emerged 12.7 Ma (). This did not diversify until a SG lineage  
221 arose 7.0 Ma with a sister lineage whose ancestral state is unclear as it is currently a mixture of  
222 resprouting SGs, shrubs and trees. Thus, SGs have arisen several times throughout the  
223 evolutionary history of the genus. Overall, shrubland SGs are twice the age of grassland SGs  
224 (Table 2) with *P. lorea* in the shrublands the oldest at 10.8 Ma. Similarly, shrubby resprouters  
225 (that we treat as geoxyles with a larger growth form, Table 3) are older in the Cape (by 3.7 My)  
226 with *P. cynaroides* oldest at 12.4 Ma. Thus, resprouting shrubs have a longer history than SGs in  
227 the Cape (by 1.6 My), though there is no indication that shrub geoxyles were the ancestors of the  
228 subshrub geoxyles. This contrasts with the grasslands where resprouting subshrubs, shrubs and  
229 trees have similar mean ages, in the range 3–2 Ma, again with no indication of any evolutionary  
230 sequence (Table 2). Such a mixture of phylogenetic relationships, confounded by strong  
231 intraspecific variability (Table S2), makes it impossible to identify the ancestral growth form  
232 among grassland proteas. Thus, we are not able to support the contention that SGs are necessarily  
233 derived from forest-dwelling relatives (Maurin et al. 2014). Mean ages of the 32 grassland SGs in  
234 21 families (from Maurin et al. 2014, excluding *Protea*) were not significantly different (3.6 Ma)  
235 than for *Protea* with 20 SGs (4.2 Ma, Table 2).

236

### 237 **Prevailing environment during early evolution of subshrub geoxylic proteas**

238

239 *Fire* – South Africa has a long history of fire that has recently been traced to the Upper Cretaceous  
240 (Muir et al. 2015; He et al. 2016) when fire also directed evolution of the reproductive biology of  
241 the proteoid Proteaceae (Lamont and He 2012). By 20–15 Ma many plant traits tied to the  
242 presence of fire were present (Fig. 4). Terrestrial orchids in both the shrublands and grasslands  
243 (Bytebier et al. 2011) and bloodroots (Haemodoraceae) in the shrublands (He et al. 2016) were  
244 already displaying fire-stimulated flowering. Speciation of Restionaceae at the generic level,  
245 whose soil-stored diaspores are stimulated to germinate by fire (essentially smoke), peaked in the  
246 period 35–5 Ma (He et al. 2016). Confirmation of fire-proneness at these times comes from  
247 charcoal records in the highly mixed vegetation (with 6 Proteaceae pollen types) of Saldanha Bay,  
248 100 km N of Cape Town, 25–20 Ma (Roberts et al. 2017) and the Namibian grasslands, 1600 km  
249 north of Cape Town, 9–3 Ma (Hoetzel et al. 2013). The association of fire with C4 grasslands is  
250 well-established (Scheiter et al. 2012) and these can be traced from 18 Ma in Africa, especially  
251 from 10 Ma (Edwards et al. 2010). The ancestral protea possessed on-plant seed storage (serotiny)  
252 where the key to its fitness advantage is fire-stimulated seed release and seedling recruitment in  
253 the post-fire environment (Causley et al. 2016). However, Lamont et al. (2013) showed that  
254 proteas were only able to invade the grasslands from the Cape once resprouting was combined  
255 with the loss of serotiny (and seed storage in general that is not only redundant but possibly  
256 maladaptive in an environment where fires are likely every year), which was achieved by 12.7 Ma.  
257 Thus, the first SGs arose in shrublands and grasslands that were both highly fire-prone but with  
258 quite different fire-properties (Fig. 3).

259

260 *Climate* – Diversification in *Protea* began just prior to the Mid-Miocene Climatic Optimum 15 Ma  
261 (Fig. 4), escalating from 6 Ma but declining markedly with the onset of glaciation 2.5 Ma in the  
262 Pleistocene (Fig. 1). Most extant resprouting species/lineages arose under conditions much  
263 warmer than currently, including the first SGs in shrubland and grassland (Fig. 4). The warmer the  
264 annual average temperatures, the less likely frosts will occur (Alexander et al. 2006). Utescher et  
265 al. (2009) estimated that annual ground frost days in northern Germany, with a temperature regime  
266 not unlike the mountain ranges where some SG proteas occur (Rebello 2001), were close to zero  
267 from 15 Ma (mean temperature of coldest month  $>10^{\circ}\text{C}$ ) and only began to rise substantially from  
268 4 Ma when  $> 50\%$  of SG lineages had already arisen (Table 2). Thus, Sciscio et al. (2016)  
269 determined a mean annual temperature of  $21^{\circ}\text{C}$  at 11.6 Ma in the Cape Peninsula (possessing  
270 several SGs) compared with a current temperature of  $17^{\circ}\text{C}$  that even now is frost-free. The mean  
271 age of shrubland SGs coincided with the Miocene–Pliocene boundary and the grassland SGs with



272 the Pliocene–Pleistocene boundary, so climates must have been less warm and only frost-prone  
273 during evolution of some upland grassland proteas (Fig. 4). Nevertheless, pollen records indicate  
274 that the vegetation was *Protea*-dominated savanna rather than grassland at this time, more akin to  
275 the current savanna to the north and west that is frost-free and has a history from the Pliocene  
276 (Vrba 1985; Hoetzel et al. 2013, Finckh et al. 2016). In fact, several SG proteas listed by Maurin  
277 *et al.* (2014) (*P. welwitschii* subsp. *hirta*, *P. wentzeliana*, *P. enervis*, *P. angolensis* var. *angolensis*,  
278 *P. inyanganiensis*) occur in this savanna-type (so cannot be mapped in our Fig. 2). We conclude  
279 that the evolutionary history of SG proteas has occurred under strongly fire-prone conditions that  
280 were essentially frost-free and that exposure to frost has been limited to certain elevated  
281 (Highveld) locations in more recent times.

### 282 283 **Covariation of the subshrub geoxylic habit with other reproductive traits**

284  
285 The incidence of fire-stimulated flowering in grassland savannas is exceptionally high among the  
286 world floras (Lamont and Downes 2011; Platt et al. 1988). This may be related to the abundance  
287 of herbaceous and geophytic species among which this trait is best represented. There can be no  
288 better proof of the effectiveness of fire as an agent of selection than pyrogenic flowering (He *et al.*  
289 2016). While it is historically poorly recorded, and it is difficult to locate plants unburnt for any  
290 length of time in grasslands, our lists of SGs, including that of Maurin et al. (2014) (Table 1),  
291 show levels (25–44 %) much higher than for fire-prone floras generally, e.g. 10 % in Australian  
292 heathlands (Lamont and Keith 2017). Though it is far from universal, this confirms that many  
293 geoxyles have had a long association with fire that has promoted evolutionary changes in their  
294 sexual reproductive phenology as well as in their vegetative recovery.

295         There is no fitness advantage in storing seeds in an ecosystem where germination is likely  
296 every year as fires that create suitable conditions for germination and establishment are likely  
297 every year (Gignoux et al. 2009). Indeed, if there is a ‘cost’ associated with storage, it might even  
298 be maladaptive. Inspection of the species used by Maurin et al. (2014) shows that almost all have  
299 succulent fruits (with non-dormant seeds) or require no pretreatment for germination (Table 1;  
300 Weiersbye and Witkowski 2002). Similarly, Lamont *et al.* (2013) showed that the only way  
301 proteas could invade the savanna grasslands from the Cape was to reverse the near-universal trait  
302 of canopy seed storage in the shrublands to universal non-storage. Dayrell et al. (2017) also  
303 demonstrated that there is little soil seed storage in the Brazilian savannas but attributed it to the  
304 reliable wet seasons of so-called OCBIL (old, climatically-buffered, infertile landscape) systems.  
305 This interpretation cannot be accepted, for such major OCBILS as the Cape and southwestern

306 Australia are characterized instead by their extremely high levels of seed storage (Enright et al.  
307 2007) – the difference in levels of seed storage between these regions can in fact be attributed to  
308 their contrasting fire regimes. If frost was the dominant constraint in grasslands then soil storage  
309 would have been favoured historically, for the seed store remaining allows a second chance at  
310 seedling recruitment following initial failure (the so-called bet-hedging advantage). Even so, our  
311 detection of SG proteas in both shrublands (with seed storage) and grasslands (without seed  
312 storage) means that the likelihood of seed storage is not relevant to understanding the general  
313 biology of SGs.

314

## 315 **Conclusions**

316

317 We followed up the pairwise comparisons of subshrub (suffrutescent) resprouters with  
318 their taller sisters in many families by Maurin et al. (2014) with a full analysis of an entire genus,  
319 *Protea*, to test ideas on the relative importance of fire and frost in their evolution. Treating the  
320 subshrub geoxyle at a strictly morphological level, we find that they are just as likely to occur in  
321 the mediterranean shrublands as in the savanna grasslands of southern/central Africa. Since the  
322 distribution of the SG growth form reflects the distribution of proteas generally, it is not an  
323 adaptation to a particular fire regime, as this may vary greatly in terms of seasonality, frequency  
324 and intensity throughout its range. This greatly reduces the likelihood of frost as the key selective  
325 agent but not of lightning-caused fires where they occur, both now and historically. We show that  
326 shrubland subshrub geoxyles appeared much earlier than grassland subshrub geoxyles, consistent  
327 with the delay in migration of proteas from the Cape north and east to the subtropical grasslands,  
328 but that their evolutionary longevity still matches with species examined by Maurin et al. (2014).  
329 Thus, the background of subshrub geoxylic proteas in African grasslands is somewhat different  
330 from those in South America, morphologically (no xylopodia) and historically, with a fire-prone  
331 rather than a rainforest past (Simon *et al.* 2009). This may not be true for some other clades in the  
332 shrublands that have non-fire-prone affinities (such as *Searsia*, *Euclea*, *Olea*, *Rapanea*, Richard  
333 Cowling, pers. comm.)

334 Subshrub geoxylic proteas arose ultimately from nonsprouting (fire-killed) serotinous  
335 shrub lineages, usually in parallel with the origins of resprouting shrubs and trees without any later  
336 reversals to the parent type. The SG growth form is part of a continuum of size under the general  
337 umbrella of geoxyles that is not always fixed at species rank but subject to the vagaries of fire that  
338 continually reduces stature and promotes lignotuber evolution and enlargement (Notes S2: Why  
339 are SG proteas so short?). Any occasional observed resistance by SGs to frost can be attributed to

340 their prior adaptation to ancestral fire. Above-average occurrence of pyrogenic flowering and  
341 universal absence of seed storage are correlated traits with resprouting that confirm the over-riding  
342 impact of fire. Frost can be considered a mild form of disturbance (compared with fire) in terms of  
343 its effects on plants so that there is no need for any morpho/physiological change in the  
344 underground bud-storing structures even in the presence of severe frost – SGs are already exapted  
345 to frost. However, while seedlings cannot adapt to fire, they may develop some frost-resistance in  
346 frost-prone populations (Prunier et al. 2012) so that mechanisms unrelated to resprouting may  
347 have enabled some frost-resistance to evolve more recently.

348

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350

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354

### 355 **References**

- 356 Alexander LV, Zhang X, Peterson TC, Caesar J, Gleason B, Klein Tank AMG, Tagipour A (2006)  
357 Global observed changes in daily climate extremes of temperature and precipitation. *J Geophys*  
358 *Res* 111: D05109, doi:10.1029/2005JD006290.
- 359 Archibald S, Staver AC, Levin SA (2012) Evolution of human-driven fire regimes in Africa. *Proc*  
360 *Natl Acad Sci* 109: 847-852.
- 361 Bannister P, Lord JM (2006) Comparative winter frost resistance of plant species from southern  
362 Africa, Australia, New Zealand, and South America grown in a common environment  
363 (Dunedin, New Zealand). *NZ J Bot* 44: 109-119.
- 364 Brown KS, Marean CW, Herries AIR, Jacobs Z, Tribolo C, Braun D, Roberts DL, Meyer MC,  
365 Bernatchez J (2009) Fire as an engineering tool of early modern humans. *Science* 325: 859-  
366 862.
- 367 Burt Davy J (1922) The suffrutescent habit as an adaptation to environment. *J Ecol* 10: 211-219.
- 368 Bytebier B, Antonelli A, Bellstedt DU, Linder HP (2011) Estimating the age of fire in the Cape  
369 flora of South Africa from an orchid phylogeny. *Proc R Soc B* 278: 188–195.
- 370 Causley CL, Fowler WM, Lamont BB, He T (2016) Fitness benefits of serotiny in fire- and  
371 drought-prone environments. *Plant Ecol* 217: 773-779.
- 372 Chisumpa SM, Brummitt RK (1987) Taxonomic notes on tropical African species of *Protea*. *Kew*  
373 *Bulletin* 42: 815-853.

374 Clarke PJ, Lawes MJ, Midgley JJ, Lamont BB, Ojeda F, Burrows GE, Enright NJ, Knox KJE  
375 (2013). Resprouting as a key functional trait: how buds, protection and reserves drive  
376 persistence after fire. *New Phytol* 197: 19-35.

377 Davies TJ, Daru BH, Bank M, Maurin O, Bond WJ (2016) Multiple routes underground? Frost  
378 alone cannot explain the evolution of underground trees. *New Phytol* 209: 910-912.

379 Dayrell RL, Garcia QS, Negreiros D, Baskin CC, Baskin JM, Silveira FA (2017) Phylogeny  
380 strongly drives seed dormancy and quality in a climatically buffered hotspot for plant  
381 endemism. *Ann Bot* 119: 267-277.

382 Edwards EJ, Osborne CP, Strömberg C, Smith, SA, C4 Grasses Consortium (2010) The origins of  
383 C4 grasslands: integrating evolutionary and ecosystem science. *Science* 328: 587-591.

384 Enright NJ, Mosner E, Miller BP, Johnson N, Lamont BB (2007) Soil versus canopy seed storage  
385 and plant species coexistence in species-rich shrublands of southwestern Australia. *Ecology*  
386 88: 2292-2304.

387 Finckh M, Revermann R, Aidar MP (2016) Climate refugees going underground—a response to  
388 Maurin et al. (2014). *New Phytol* 209: 904-909.

389 Frost PGH (2012) The responses and survival of organisms in fire-prone environments. In:  
390 Booysen, PDV, Tainton NM (Eds) *Ecological effects of fire in South African ecosystems*  
391 (Vol. 48). Springer, Berlin.

392 Gignoux J, Lahoreau G, Julliard R, Barot S (2009) Establishment and early persistence of tree  
393 seedlings in an annually burned savanna. *J Ecol* 97: 484-495.

394 He T, Lamont BB, Manning J (2016) A Cretaceous origin for fire adaptations in the Cape flora.  
395 *Scientific Reports* 6: 34880.

396 Hoetzel S, Dupont L, Schefuss E, Rommerskirchen F, Wefer G (2013) The role of fire in Miocene  
397 to Pliocene C4 grassland and ecosystem evolution. *Nature Geosci* 6: 1027–1030.

398 Hoffmann WA, Solbrig OT (2003) The role of topkill in the differential response of savanna  
399 woody species to fire. *Forest Ecol Manag* 180: 273-286.

400 Holdo RM (2005) Stem mortality following fire in Kalahari sand vegetation: effects of frost, prior  
401 damage, and tree neighbourhoods. *Plant Ecol* 180: 77-86.

402 Hyde MA, Wursten BT, Ballings P, Coates Palgrave M (2016) *Flora of Zimbabwe: Records of*  
403 *Protea welwitschii*. [http://www.zimbabweflora.co.zw/speciesdata/species-  
display.php?speciesid=120800](http://www.zimbabweflora.co.zw/speciesdata/species-<br/>404 display.php?speciesid=120800) Retrieved 6 Oct 2016.

405 Keeley JE, Bond WJ, Bradstock RA, Pausas JG, Rundel PW (2012) *Fire in Mediterranean*  
406 *Ecosystems: Ecology, Evolution and Management*. Cambridge University Press, Cambridge.

407 Kennedy AD, Potgieter ALF (2003) Fire season affects size and architecture of *Colophospermum*  
408 *mopane* in southern African savannas. *Plant Ecol* 167: 179-192.

409 Lamont BB, Downes KS (2011) Fire-stimulated flowering among resprouters and geophytes in  
410 Australia and South Africa. *Plant Ecol* 212: 2111-2125.

411 Lamont BB, Enright NJ (2000) Adaptive advantages of aerial seed banks. *Plant Species Biol* 15:  
412 157-166.

413 Lamont BB, He T (2012) Fire-adapted Gondwanan Angiosperm floras arose in the Cretaceous.  
414 *BMC Evol Biol* 12: 223.

415 Lamont BB, He T, Downes KS (2013) Adaptive responses to directional trait selection in the  
416 Miocene enabled Cape proteas to colonize the savanna grasslands. *Evol Ecol* 27: 1099-1115.

417 Lamont BB, Keith D (2017) Heathlands and associated shrublands. In: Keith D (ed) *Vegetation of*  
418 *Australia*. 3<sup>rd</sup> ed, Cambridge University Press, Cambridge (in press).

419 Lindman CAM (1900) Vegetationen i Rio Grande do Sul (Sydobrasilien). Nordin and Josephson,  
420 Stockholm, Sweden.

421 Manry DE, Knight RS (1986) Lightning density and burning frequency in South African  
422 vegetation. *Vegetatio* 66: 67-76.

423 Maurin O, Davies TJ, Burrows JE, Daru BH, Yessoufou K, Muasya AM, van der Bank M, Bond  
424 WJ (2014) Savanna fire and the origins of the ‘underground forests’ of Africa. *New Phytol*  
425 204: 201-214.

426 Medwecka Kornas A. (1980) *Gardenia subacaulis* a pyrophytic suffrutex of the African savanna.  
427 *Acta Botanica Academiae Scientiarum Hungaricae* 26: 131-138.

428 Mucina L, Rutherford MC (2006) The vegetation of South Africa, Lesotho and Swaziland. South  
429 African National Biodiversity Institute, Pretoria.

430 Muir RA, Bordy EM, Prevec R (2015) Lower Cretaceous deposit reveals first evidence of a post-  
431 wildfire debris flow in the Kirkwood Formation, Algoa Basin, Eastern Cape, South Africa.  
432 *Cretaceous Research* 56: 161-179.

433 Platt WJ, Evans GW, Davis MM (1988) Effects of fire season on flowering of forbs and shrubs in  
434 longleaf pine forests. *Oecologia* 76: 353-363.

435 Prunier R, Holsinger KE, Carlson JE (2012) The effect of historical legacy on adaptation: do  
436 closely related species respond to the environment in the same way? *J Evol Biol* 25: 1636-  
437 1649.

438 Rebelo AG (2001) A Field Guide to the Proteas of Southern Africa. Fernwood Press, South  
439 Africa.

440 Rebelo AG. 2009. Protea Atlas Project. South African National Biodiversity Institute,  
441 Kirstenbosch, South Africa. <http://www.proteaatlas.org.za>. Retrieved 26 Aug 2009.

442 Ristic Z, Ashworth EN. 1997. Mechanisms of freezing resistance of wood tissues: recent  
443 advancements. In Basra AS, Basra RK. (Eds) Mechanisms of Environmental Stress  
444 Resistance in Plants. Amsterdam, the Netherlands. pp. 123-136.

445 Rizzini C, Heringer E (1961) Underground organs of plants from southern Brazilian savannas,  
446 with special reference to the xylopodium. *Phyton* 17: 105-124.

447 Roques KG, O'Connor TG, Watkinson AR (2001) Dynamics of shrub encroachment in an African  
448 savanna: relative influences of fire, herbivory, rainfall and density dependence. *J Appl Ecol*  
449 38: 268-280.

450 Rundel PW, Arroyo MTK, Cowling RM, Keeley JE, Lamont BB, Vargas P (2016) Mediterranean  
451 biomes: evolution of their vegetation, floras and climate. *Ann Rev Ecol Evol Syst* 47: 383–  
452 407.

453 Scheiter S, Higgins SI, Osborne CP, Bradshaw C, Lunt D, Ripley BS, et al (2012) Fire and fire -  
454 adapted vegetation promoted C4 expansion in the Late Miocene. *New Phytol* 195: 653-666.

455 Sciscio L, Tsikos H, Roberts DL, Scott L, van Breugel Y, Damste JS, Schouten DR, Grocke DR.  
456 (2016) Miocene climate and vegetation changes in the Cape Peninsula, South Africa:  
457 Evidence from biogeochemistry and palynology. *Palaeogeography, Palaeoclimatology,*  
458 *Palaeoecology* 445: 124-137.

459 Simon MF, Grether R, de Queiroz LP, Skema C, Pennington RT, Hughes CE (2009) Recent  
460 assembly of the Cerrado, a neotropical plant diversity hotspot, by in situ evolution of  
461 adaptations to fire. *Proc Natl Acad Sci* 106: 20359-20364.

462 Smith FR, Granger JE (2017) Survival and life expectancy of the tree *Protea roupelliae* subsp.  
463 *roupelliae* in a montane grassland savanna: effects of fire regime and plant structure. *Austral*  
464 *Ecology* DOI: 10.1111/aec.12459

465 Utescher T, Mosbrugger V, Ivanov D, Dilcher DL (2009) Present-day climatic equivalents of  
466 European Cenozoic climates. *Earth Planet Sci Lett* 284: 544-552.

467 Valente LM, Reeves G, Schnitzler J, Mason IP, Fay MF, Rebelo TG, Chase MW, Barraclough TG  
468 (2010) Diversification of the African genus *Protea* (Proteaceae) in the Cape biodiversity  
469 hotspot and beyond: equal rates in different biomes. *Evolution* 64: 745–760.

470 Vrba ES (1985) Early hominids in southern Africa: updated observations on chronological and  
471 ecological background. In Tobias PV (Ed) *Hominid Evolution*. New York, Alan R. Liss. pp.  
472 195–200.

473 Weiersbye IM, Witkowski ETF. 2002. Seed fate and practical germination methods for 46

474 perennial species that colonize gold mine tailings and acid mine drainage-polluted soils in the  
 475 grassland biome. In: Seydack AHW Vorster T, Vermeulen WJ, van der Merwe IJ (Eds).  
 476 Multiple use management of natural forests and woodlands: policy refinements and scientific  
 477 progress. Proceedings of the Natural Forests and Savanna Woodlands Symposium III, KNP,  
 478 Department of Water Affairs and Forestry Indigenous Forest Management, Pretoria. pp. 221-  
 479 255.

480 Wakeling JL, Cramer MD, Bond WJ (2012) The savannah-grassland ‘treeline’: why don’t savanna  
 481 trees occur in upland grasslands? *J Ecol* 100: 381-391.

482 White F (1977) The underground forests of Africa: a preliminary review. *Gardens Bulletin*,  
 483 Singapore 29: 57-71.

484 Witkowski ETF, Lamont BB (1997) Does the rare *Banksia goodii* have different vegetative,  
 485 reproductive or ecological attributes from its widespread co-occurring relative *B. gardneri*? *J*  
 486 *Biogeog* 24: 469-482.

487 Zachos JC, Dickens GR, Zeebe RE (2008) An early Cenozoic perspective on greenhouse warming  
 488 and carbon-cycle dynamics. *Nature* 451: 279–283.

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**Table 1** Traits for subshrub geoxylic proteas (see Supplementary Table S1 for details) and all subshrub geoxyle species analysed by Maurin *et al.* (2014). All arose from rootstocks (usually lignotubers) sometimes with creeping stems or rhizomes. – means that data not supplied nor available in the literature.

Trait	Grassland/savanna proteas ( <i>n</i> = 17)	Shrubland proteas ( <i>n</i> = 25)	All species in Maurin ( <i>n</i> = 35)
Climate ☐ rainfall	summer	winter (uniform)	summer
Mean height (m)	0.66	0.41*	☐ (mostly rhizomatous so must be short)
Minimum height (m)	0.10	0.15	☐
Maximum height (m)	1.00	1.20	☐

Rhizomatous (%)	12	70.5	66.5
Creeping/decumbent (%)	28	6	10
Erect/suberect (%)	60	23.5	13.5
Branches undivided (%)	80	82	□
Sparsely branched (%)	20	17.5	□
Deciduous/stems ephemeral (%)	12	23.5	7? (poorly known)
Fire-stimulated flowering (%)	25? (poorly known)	35	44
Seed storage (plant or soil) (%)	0	100	0

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\* $P = 0.082$  (unequal variances)



499 **Table 2** Mean ages (plus max(imum) and min(imum) ages) in million years of subshrub and  
500 shrub geoxylic *Protea* species/lineages (defined in Fig. S4) in shrubland (s, winter-rainfall) and  
501 grassland (g, summer-rainfall) habitats derived from the chronogram in Fig. 1. t-test refers to a  
502 comparison between habitats; 1-t(ailed) tests applied when the directional hypothesis was  
503 supported numerically and 2-t(ailed) when they were not or not applicable. (un)equal refers to  
504 variances. Maurin = ages from Maurin *et al.* (2014) excluding proteas. Growth forms and habitats  
505 from Table S1 and Rebelo (2001).  
506

<i>Protea</i> growth form	Habitat	<i>n</i>	Mean	Max	Min	<i>P</i> (t-test)
Subshrub geoxyles	shrubland	14	4.9	10.8	1.5	0.0344 (1-t, unequal)
	grassland	6	2.5	6.9	1.0	
Shrub geoxyles	shrubland	5	7.8	12.4	2.5	0.0098 (1-t, equal)
	grassland	5	2.1	3.6	0.8	
a. Subshrub geoxyles	s + g	20	4.2	10.8	1.0	0.2637 (1-t, equal)
b. Shrub geoxyles	s + g	10	5.0	12.4	0.8	
c. Subshrub geoxyles (Maurin)	a vs c (g)	32	3.6	15.2	0.3	0.5386 (2-t, unequal)
1. Subshrub geoxyles	grassland	6	2.5	6.9	1.0	
2. Shrub geoxyles	1 vs 2 (g)	5	2.1	3.6	0.8	0.7272 (2-t, equal)
3. Trees	1 vs 3 (g)	4	3.0	5.6	0.9	0.7572 (2-t, equal)

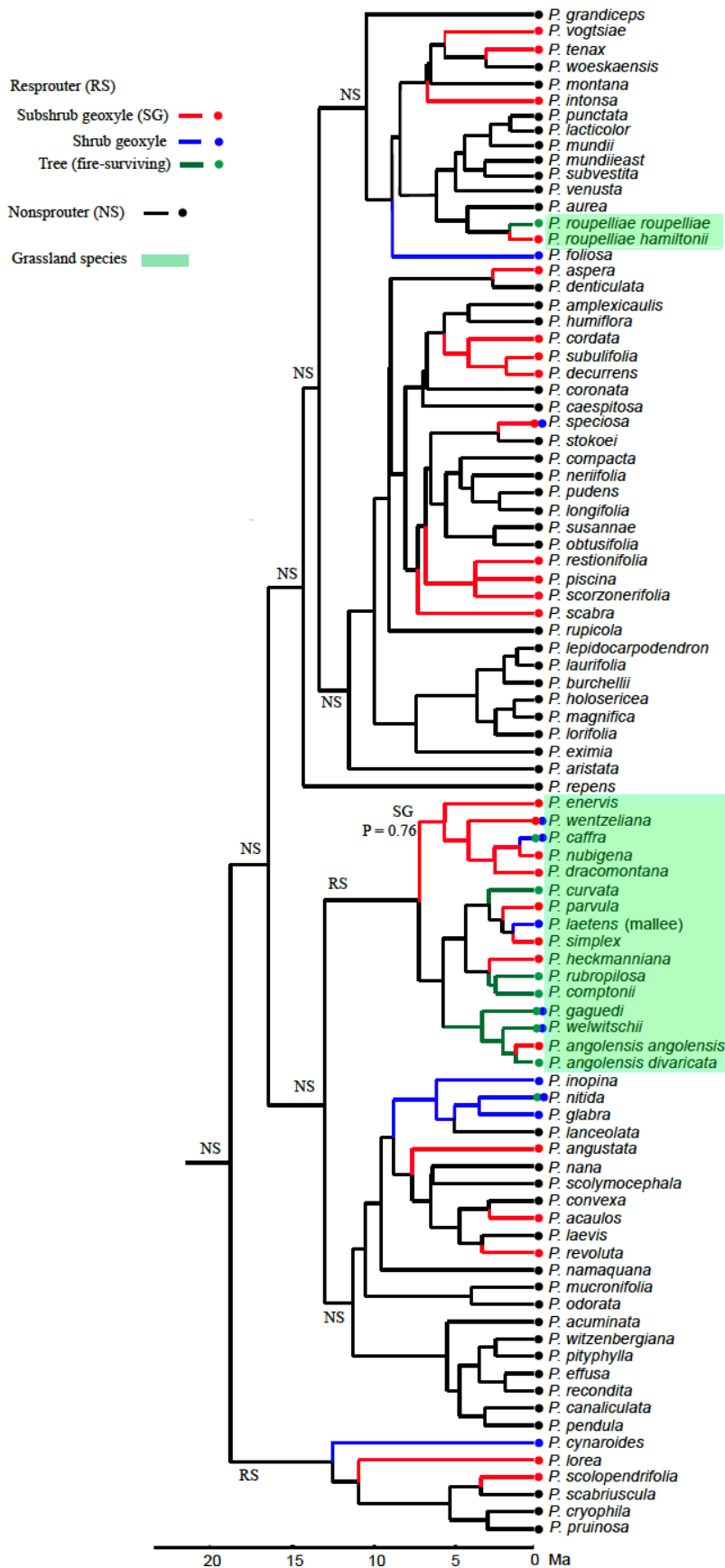
507 **Table 3** Key to resprouting types showing the four subdivisions of geoxyles and contrasting  
508 them with geophytes, aeroxyles and caudiciform plants. Note that some individual species may  
509 range from subshrubs to trees depending on growing conditions (Table S2).  
510

- 
1. Resprouter with non-woody underground parts that bear a few concealed buds (bulb, corm, primary rhizomes, swollen stems/roots), above-ground parts ephemeral or, if present, incinerated by fire – **geophyte**
  2. Resprouter with woody underground parts (lignotuber, xylopodium, secondary rhizomes/roots) that bear a few to many concealed buds, sometimes also above ground and many equisized stems – **geoxyle**
    1. *Subshrub* ( $\leq 1$  m tall) – all above-ground parts sparsely branched and ephemeral, or, if present or woody, incinerated by fire
    2. *Shrub* ( $> 1$ – $2.5$  m tall) – strongly branched stems woody but mostly incinerated by fire
    3. *Mallee* ( $> 2.5$  m tall) – strongly branched stems woody and survive fire
    4. *Tree (clonal)* ( $> 2.5$  m tall) – trunk and branches woody and survive fire
  3. Resprouter with woody trunk and strongly branched stems that bear many aerial concealed buds – *Tree (non-clonal)* ( $> 2.5$  m tall) – trunk and main branches survive fire – **aeroxyle**

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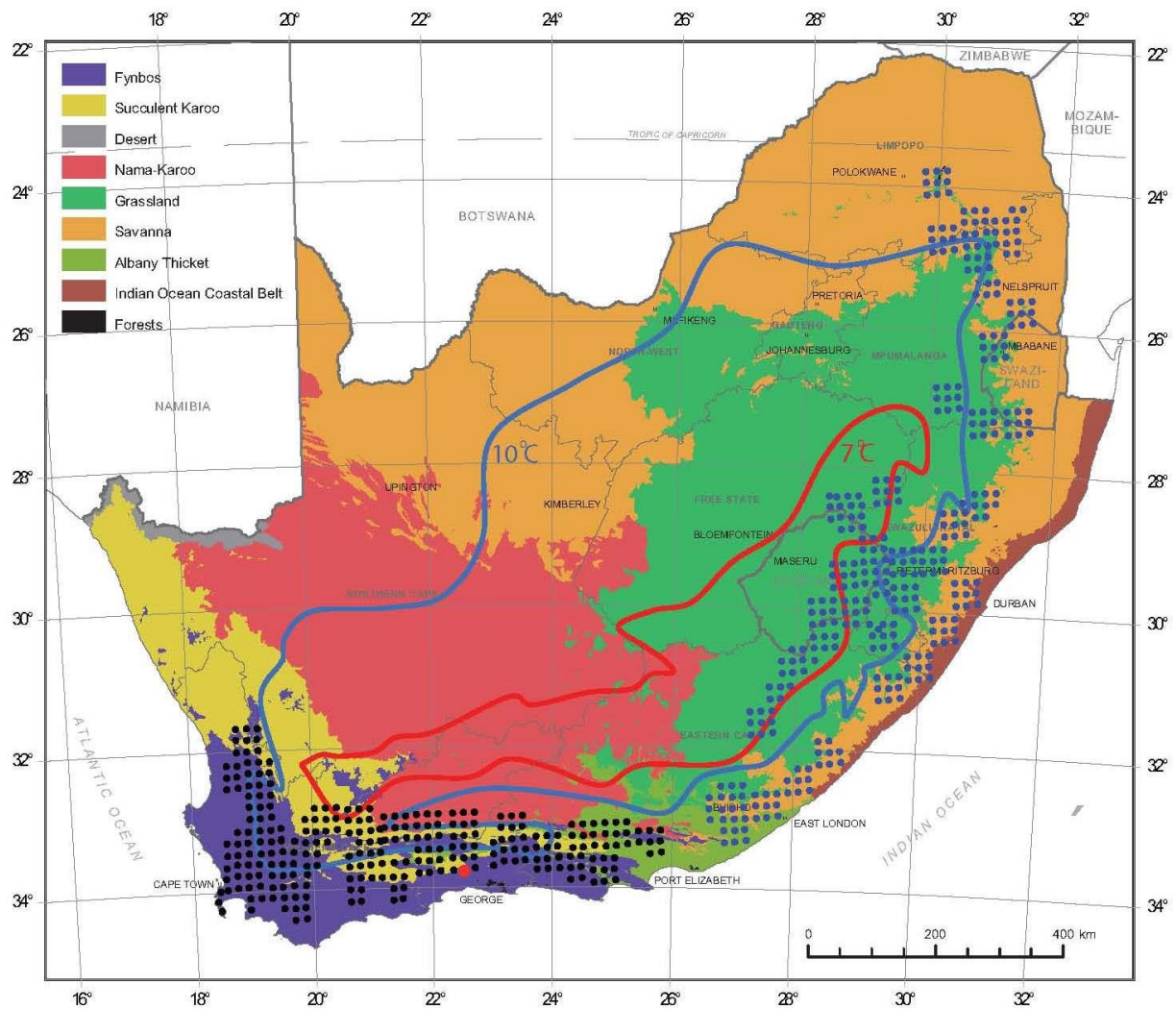
511 Note: **Caudiciform** plants whose non-woody trunks and apical buds survive fire and may  
512 exist for many years below ground before emerging (cycads, palms, grasstrees, treeferns,  
513 some aloes) have been omitted from this scheme  
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521 **Fig. 1.** Chronogram for *Protea* showing grassland (highlighted in green) and shrubland (not  
 522 highlighted) species, with lineages for nonsprouting shrubs (black), and resprouting subshrubs  
 523 (red), shrubs (blue) and trees (green) indicated.



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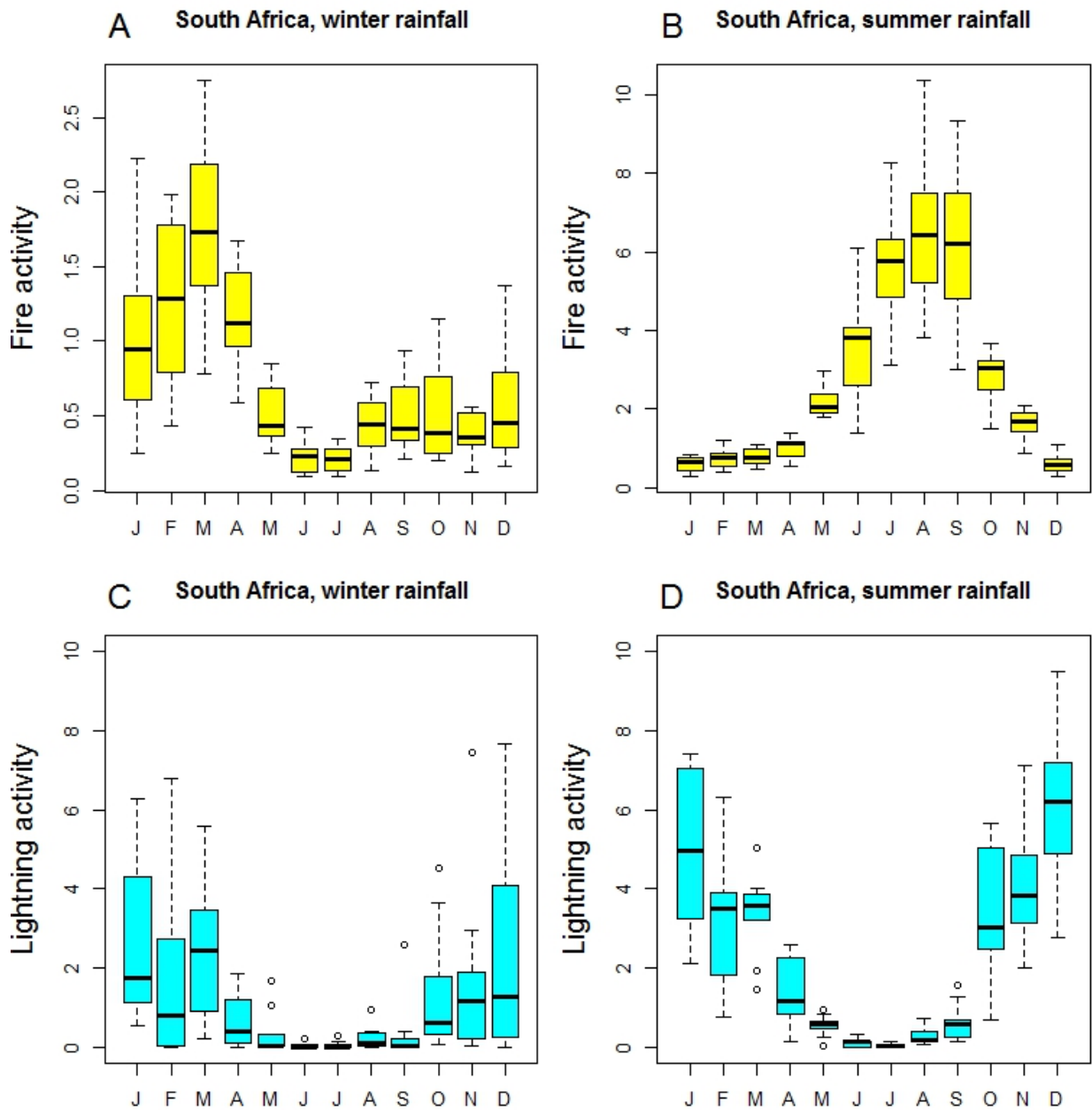
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**Fig. 2.** Biome map of South Africa (from Mucina and Rutherford 2006) to which has been added the distribution of subshrub geoxylic proteas in shrubland (black dots) and grassland (blue dots) (from Rebelo 2001). The orange dot is the location of a subshrub geoxyle, *Myrica elliptica* (Myricaceae), outside the grasslands as reported by Burt Davey (1922). Also added are two selected isotherms for mean winter temperatures (<http://www.south-africa-tours-and-travel.com/south-africa-climate.html>). Note that the coastal strip to 50 km inland is usually frost-free (Finckh et al. 2016).



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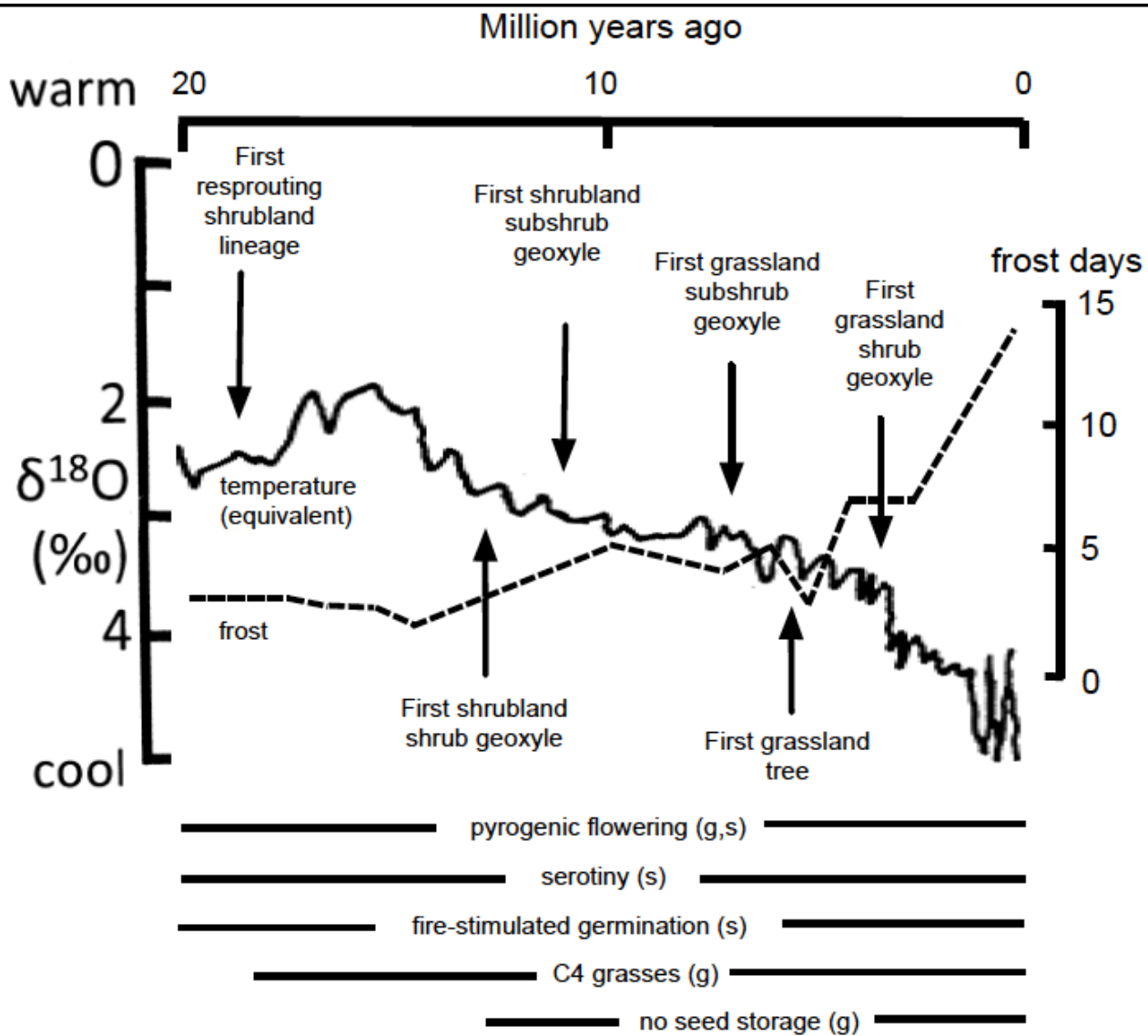
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**Fig. 3.** Monthly fire activity (A, B) and lightning activity (C, D) for the winter (A, C) and summer (B, D) rainfall regions of South Africa, estimated from remote sensing data. The Y-axis indicates relative activity and therefore has no units; variability about the mean refers to 15 and 9-year variability for fire and lightning, respectively (details in Notes S1, Supplementary Material). Equivalent information for the Angolan Plateau (as studied by Finckh et al. 2016) is shown in Fig. S1.



548

549 **Fig. 4** Key times in the early evolution of resprouting proteas (from Fig. 1) relative to isotopic  
 550 oxygen levels as a surrogate for mean world temperatures (adapted from Zachos et al. 2008) and  
 551 mean of the estimated frost days pa in northern Germany at the same time (Utescher et al. 2009).  
 552 The presence of surrogates for fire over the same period is indicated by the continual presence of  
 553 lineages in the African grasslands (g) or shrublands (s) with pyrogenic flowering (Bytebier et al.  
 554 2011; He et al. 2016), serotiny (Lamont and He 2012), fire-stimulated germination (Lamont and  
 555 He 2012, He et al. 2016), C4 grasses (Edwardes et al. 2010) and lack of seed storage (Lamont et  
 556 al. 2013).

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## 564 **Online Supplementary material**

565

### 566 **Notes S1: Trait assignments in *Protea* phylogeny**

567

568 We constructed a dated phylogeny for *Protea* based on Valente et al. (2010) and Lamont et al.  
569 (2013). We assigned the growth forms, subshrub and shrub geoxyle and fire-surviving tree, to all  
570 species from Table S1 and Rebelo (2001). We used a continuous-time Markov model of trait  
571 evolution for discrete traits, employing BayesTraits V2 (Pagel and Meade 2006). The analysis  
572 parameters used the MultiState module with exponential distributed priors, with 10 million  
573 Markov chain Monte Carlo (MCMC) iterations after the burn-in. Ancestral trait of a node was  
574 assigned as either of the three traits if the posterior probability of the particular trait was greater  
575 than an arbitrary criterion of 0.5, otherwise it was left unassigned. The method assumes that  
576 species traits remained unchanged until reaching the first sister node when the trait state is re-  
577 assigned (supported by all probabilities in fact exceeding 0.50), and that the most likely trait  
578 assigned to a node applies until the next node was reached. This is consistent with all previous  
579 work on the topic and enabled comparison with the results of Maurin et al. (2014) and Simon et  
580 al. (2009).

581 It was of interest to know if subshrub geoxyles (SGs) are more likely to occur in one  
582 vegetation type rather than another. Taking their phylogenetic position into account, we tested  
583 for any correlated shift of SGs between the habitat of grassland and shrubland using BayesTraits  
584 V2. The analysis parameters used the discrete module with exponential distributed priors, with  
585 10 million reversible-jump MCMC iterations after burn-in. The Discrete module compared trait  
586 models independent (no correlation among shifts) and dependent (correlation among shifts). A  
587 Bayes Factor was calculated from the harmonic means of the MCMC chains, with a  $\log_e \text{BF} > 5$   
588 indicating strong evidence of correlated evolution, and a  $\log_e \text{BF} < 2$  indicating no evidence of  
589 correlated evolution (Pagel 1994).

590

### 591 **Remotely sensed fires and lightning activity**

592

593 We estimated monthly fire and lightning activity for three regions of southern Africa, two of  
594 them based on the distribution of geoxylic *Protea* species (see Fig. 2 of main text) and the other  
595 based on the study area of Finckh et al. (2016):

- 596 1) Winter rainfall regions of South Africa, defined as that section of South Africa south of  
597 32°S and west of 26.5°E. This region is dominated by Mediterranean-type shrublands.

- 598 2) Summer rainfall region of South Africa, defined as the region south of 24°S and east of  
599 26.5°E; this includes Lesotho and Swaziland. This region is dominated by subtropical  
600 savannas and grasslands.
- 601 3) Angolan miombo, defined as the WWF ecoregion with the same name (code: AT0701)  
602 and corresponding to central Angola and extending into the Democratic Republic of  
603 Congo. This region is the focus of Finckh et al. (2016).

604

605 Lightning activity was estimated from the Lightning Image Sensor data set, downloaded from the  
606 Global Hydrology Resource Center (GHRC, NASA, <https://ghrc.nsstc.nasa.gov>) for the period  
607 1998-2006 (9 years). This data set provides the date and geolocation of lightnings around the  
608 world (resolution of 3 × 6 km). Fire activity was estimated from MODIS hotspots from the Terra  
609 satellite (Collection 5 Active Fire Products; Giglio 2013), as compiled in the Clima Modelling  
610 Grid at 0.5° resolution (MOD14CMH; dataset downloaded from the University of Maryland,  
611 USA) for the period 2001–2015 (15 years). This data set provides the date and geolocation of  
612 hotspots around the world. We selected lightnings and hotspots for each of the three target  
613 regions and aggregated them by each month of the year. The number of lightnings and hotspots  
614 were standardized by the size of each region (i.e., divided by the size in thousands of km<sup>2</sup>). We  
615 then plotted the values by months and showing the variability among years using boxplots. Note  
616 that the values plotted do not exactly reflect the number of lightnings and the number of fires as  
617 the data are constrained by the spatial resolution of the sensor and the temporal resolution of the  
618 satellite, however, they are a good indicator of the fire and lightning activity (Pausas and Ribeiro  
619 2013).

620

## 621 **Additional references**

622

623 Giglio L (2013) MODIS Collection 5 Active Fire Product User's Guide. Version 2.5:

624 Department of Geographical Sciences, University of Maryland.

625 Pagel M (1994) Detecting correlated evolution on phylogenies: a general method for the

626 comparative analysis of discrete characters. *Proceedings of the Royal Society B* 255: 37–  
627 45.

628 Pagel M, Meade A (2006) Bayesian analysis of correlated evolution of discrete characters by  
629 reversible - jump markov chain monte carlo. *The American Naturalist* 167: 808–825.

630 Pausas JG, Ribeiro E (2013) The global fire–productivity relationship. *Global Ecology and*  
631 *Biogeography* 22: 728–736.

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633 **Notes S2: Why are subshrub geoxylic proteas so short?**



634

635 There are three possible explanations. 1. Species have a fixed ontogeny for an inherently dwarf  
636 growth form. This can only apply to SGs with a creeping (procumbent) habit and some species  
637 with ephemeral erect stems or those that only produce rhizomes and therefore remain low  
638 whatever the growing conditions (Table S1). Among grassland species, 25% have a fixed SG  
639 morphology for the 32 taxa with available records, and 89% of shrubland species are fixed  
640 because of the preponderance of rhizomatous species. This fixed growth form has the advantage  
641 of a) ensuring mutual protection from the 'elements' among the grass sward and b) guaranteeing  
642 flowering among species with pyrogenic flowering. 2. Species have a flexible ontogeny but  
643 growing conditions are so poor that only a dwarf form can be supported. This might apply to the  
644 sandy, waterlogged sites originally proposed by White (1977) as typical of SGs in the Zambebian  
645 region but which we show to be atypical overall. A websearch using the terms arid, desert,  
646 sandstone and alpine yielded *P. welwitschii* on quartzitic sandstone but not the SG form (Hyde *et*  
647 *al.* 2016), the alpine *P. dracomontana* that is burnt at 2–3-year intervals and appears to show  
648 fire-stimulated flowering ([https://www.ispotnature.org/node/658456?nav=parent\\_ob](https://www.ispotnature.org/node/658456?nav=parent_ob),  
649 6/10/2016), and the Mt Kilimanjaro form of *P. caffra* that may reach a height of 4 m (Rebelo  
650 2001). Thus, growing conditions have a negligible role in stunting proteas.

651 3. Species stature is reduced by damage due to herbivory/trampling, fire and/or frost but  
652 they have a resprouting ontogeny that enables tolerance. Given that proteas are ignored by  
653 mammal herbivores (Lamont *et al.* 2013) and frost is either rare or fitful and historically recent  
654 as shown here, fire is the most likely cause of stem mortality. In addition, trampling by large  
655 mammals and dieback from frost increases the dead fuel load and exacerbates the pruning effect  
656 of fire (Holdo 2005). Among grassland species, the morphology of 75% examined here appears  
657 to be the outcome of the interaction between genetic predisposition and environmental pruning  
658 such that frequent fire can be held responsible for transferring many of them from the shrub  
659 geoxyle to the subshrub class. This is true for only 10% (*P. speciosa* and *P. nitida*) in the  
660 shrublands because of their fixed rhizomatous habit.

661 Thus, strongly fire-exposed proteas may be short as they are continually burnt back to the  
662 lignotuber and/or leafless rhizome. They respond by resprouting from numerous accessory buds  
663 on the lignotuber and/or axillary/terminal buds on the rhizomes to give an increasingly  
664 interwoven and spreading structure (Witkowski and Lamont 1997). Such plants are more likely  
665 to survive subsequent fires and to reach reproductive maturity quicker (Hoffmann and Solbrig  
666 2003; Gignoux *et al.* 2009). Proteas, either different (Maurin *et al.* 2014) or the same (Chisumpa  
667 and Brummitt 1987) species, in fire-protected rock outcrops or rarely-burnt woodland/forest  
668 pockets grow tall, unconstrained by early fire and promoted by shade and competition (Table  
669 S2). They tend to develop a single trunk without a lignotuber but with thick bark and highly

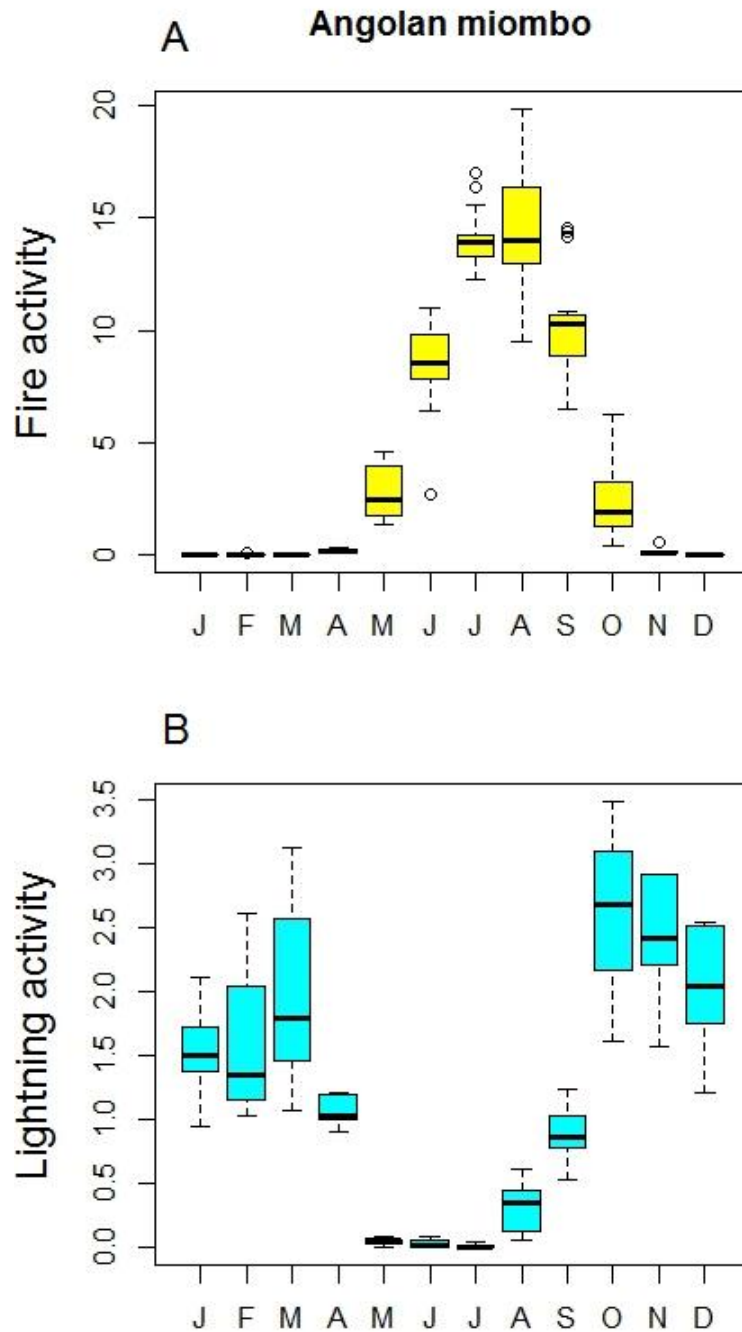
670 divided upper branches, and to resprout epicormically, as in *P. rubropilosa*, or from scale-  
671 protected terminal buds, as in *P. roupelliae* subsp. *roupelliae* ('fire-escapers', Clarke *et al.*  
672 2013). Plants at intermediate or low fire frequencies become shrubs or trees respectively, making  
673 it difficult to define a taxonomic limit to SGs. Thus, we recognize subshrub, shrub and mallee  
674 geoxyles, and fire-escaping aeroxyles, here (Tables 3, S2). For example, *P. wentzeliana* is a  
675 geoxyle to 0.4 m tall with short undivided stems in Angola but a 5-m 'aeroxyle' with highly  
676 divided branches in Tanzania (Chisumpa and Brummitt 1987, Table 3). Despite the absence of  
677 translocation studies to confirm their genetic basis, subspecific ranks are often recognized among  
678 proteas (Table S2) that may eventually prove to have a merely proximate explanation.

679 A few species have ephemeral stems or leaves that abscise at the start of the dry season. It  
680 is difficult to interpret this as an ultimate response to either fire or frost but it is more in keeping  
681 with a drought response akin to that of geophytes (since they die back to a dormant lignotuber).  
682 *P. simplex* does display fire-stimulated flowering (Rebelo 2001) and the dead material around the  
683 plant might ensure that the heat-derived cue is adequate to stimulate flowering (Lamont and  
684 Downes 2011).

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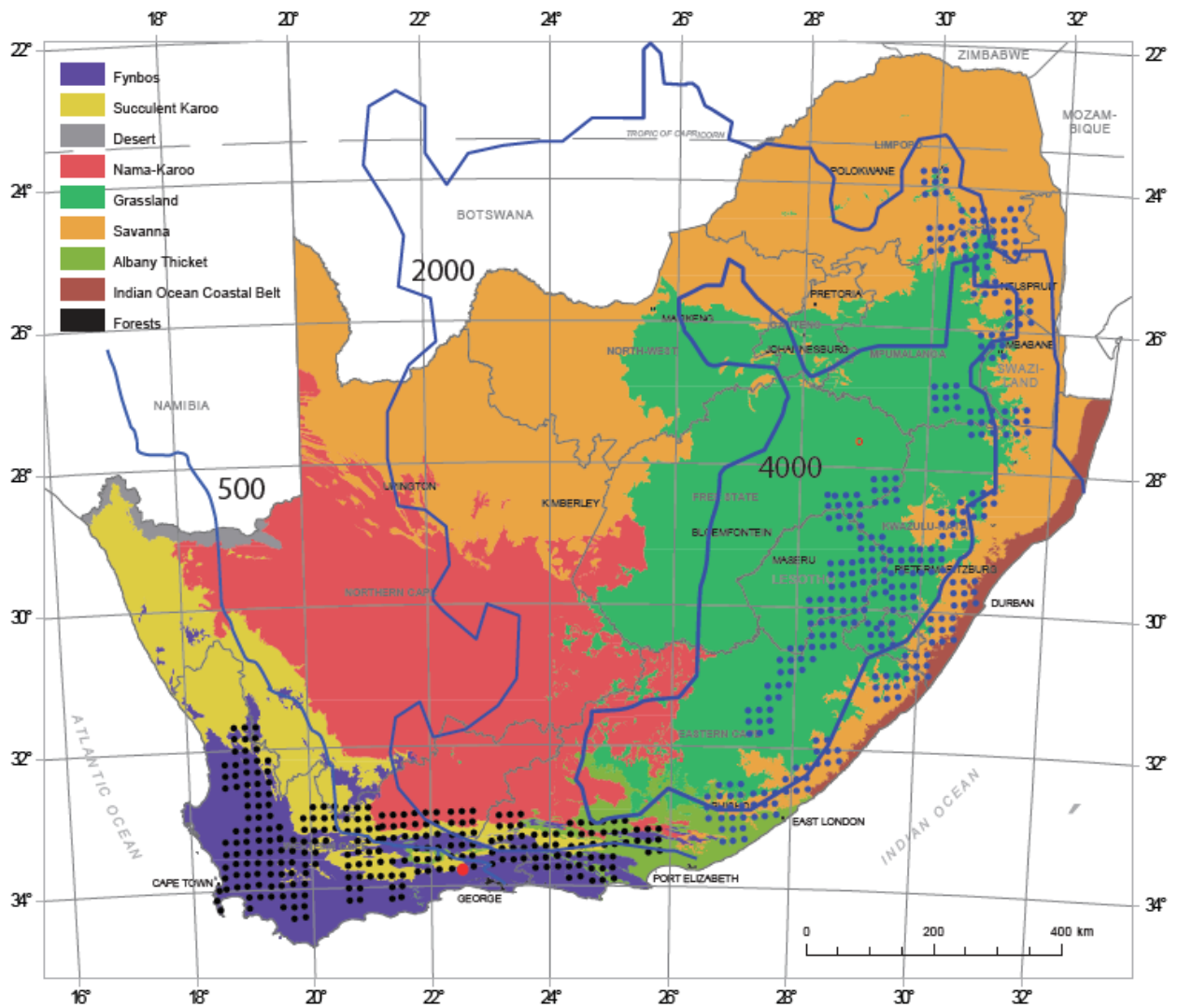
## 686 **References**

687 All listed in the main text



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**Fig. S1.** Monthly fire activity (A) and lightning activity (B) for the Angolan miombo. The Y-axis indicates relative activity and therefore has no units. See details in Notes S1, Supplementary Material.



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 696 **Fig. S2** Biome map of South Africa (from Mucina and Rutherford 2006) to which has been  
 697 added the distribution of subshrub geoxylis proteas in shrubland (black dots) and grassland (blue  
 698 dots) (from Rebelo 2001). Also added are three isolines for the total number of lightning strikes  
 699 for the period Jan 1998 to May 2009 (drawn from  
 700 [http://en.wikipedia.org/wiki/File:Global\\_lightning\\_strikes.png](http://en.wikipedia.org/wiki/File:Global_lightning_strikes.png), downloaded Nov 2010, available  
 701 from us as no longer online in this form).

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**Table S1** Habitat and growth form traits of *Protea* species categorized as suffrutescent (subshrub) geoxyles: 25 under summer rainfall climate and 17 under winter (sometimes becoming uniform) rainfall. **Habitat:** G = grassland, W = woodland, S = shrubland

Climate	Habitat	<i>Protea</i> species/ subspecies/variety	Habit	Max. Stem height (m)	Stem branching	Fire- stimulated flowering	Reference for subshrub geoxyle status	Reference for traits
Summer rainfall	G	<i>angolensis</i> var. <i>angolensis</i>	dwarf	1.0	simple	no	Maurin <i>et al.</i> 2014	Chisumpa and Brummitt 1987
	G	<i>angolensis</i> var. <i>roseola</i>	multistemmed	1.0	simple	no	Maurin <i>et al.</i> 2014	Chisumpa and Brummitt 1987
	G, savanna	<i>argyrea</i> subsp. <i>zambiana</i> (subsp. <i>argyrea</i> intended?)	tree (subshrub)	3.0 (0.6)	highly branched (simple)		Maurin <i>et al.</i> 2014	Chisumpa and Brummitt 1987
	W, seeps, dambos	<i>baumii</i> subsp. <i>robusta</i>	creeping stems to 1.6 m wide	0.15?	simple		Maurin <i>et al.</i> 2014	Chisumpa and Brummitt 1987
	S*	<i>enervis</i>	creeping stems	0.15?	simple		Maurin <i>et al.</i> 2014	villege.ch/musinfo/bd/cjb/af rica/details.php?langue=ana ndid=82805
	G	<i>heckmanniana</i> subsp. <i>heckmanniana</i>	subshrub	0.35 (0.5)	simple (rarely 2)		Maurin <i>et al.</i> 2014	Brummitt and Marner 1993
	G	<i>humifusa</i>	decumbent/suberect	0.35	simple		Maurin <i>et al.</i> 2014	Brummitt and Marner 1993
	G	<i>inyanganiensis</i> = <i>dracomontana</i>	erect	1.0	rarely branched	yes? (for <i>dracomontana</i> )	Maurin <i>et al.</i> 2014	<a href="http://www.zimbabweflora.co.zw/speciesdata/species.php?species_id=120760">http://www.zimbabweflora.co.zw/speciesdata/species.php?species_id=120760</a> , Rebello 2001
	G	<i>kibarensis</i> subsp. <i>cuspidata</i>	subshrub, erect	0.35 (0.5)	simple		Maurin <i>et al.</i> 2014	Chisumpa and Brummitt 1987
G	<i>lemairei</i>	erect	0.35	simple		Maurin <i>et al.</i>	Chisumpa and Brummitt	

G	<i>linearifolia</i>	erect	0.7	simple, sparsely branched		<i>al.</i> 2014 1987 Maurin <i>et al.</i> 2014
G	<i>matonchiana</i>	rhizomatous with erect terminal stems <sup>#</sup>	0.3	simple		Maurin <i>et al.</i> 2014 1987
W or dambos	<i>micans</i> subsp. <i>micans</i>	erect	0.6 (0.9)	simple		Maurin <i>et al.</i> 2014 1987
G	<i>micans</i> subsp. <i>makutuensis</i>	erect	0.9	sparsely branched		Maurin <i>et al.</i> 2014 1987
W	<i>micans</i> subsp. <i>trichophylla</i>	erect	1.0	simple, shortly branched		Maurin <i>et al.</i> 2014 1987
W, edge dambos	<i>minima</i>	rhizomatous, erect	0.15	simple, ephemeral and renewed annually		Maurin <i>et al.</i> 2014 <a href="http://villege.ch/musinfo/bd/cjb/af-rica/details.php?langue=anandid=82811">villege.ch/musinfo/bd/cjb/af-rica/details.php?langue=anandid=82811</a>
G, with shrubs	<i>ongotium</i>	prostrate	0.10?	simple		Maurin <i>et al.</i> 2014 <a href="http://villege.ch/musinfo/bd/cjb/af-rica/details.php?langue=anandid=82812">villege.ch/musinfo/bd/cjb/af-rica/details.php?langue=anandid=82812</a>
G	<i>paludosa</i> subsp. <i>secundifolia</i>	decumbent	0.5	simple, deciduous and renewed annually		Maurin <i>et al.</i> 2014
G	<i>parvula</i>	rhizomatous, prostate branches to 1 m	0.16	sparsely branched	no	Maurin <i>et al.</i> 2014 Beard 1958, Rebelo 2001
W	<i>poggei</i> subsp. <i>mwinilungensis</i>	dwarf, suberect	?	simple, slender		Maurin <i>et al.</i> 2014 1987

	G	<i>praticola</i>	decumbent	0.35	simple		Maurin <i>et al.</i> 2014
	G	<i>rouPELLIAE</i> subsp. <i>hamiltonii</i>	many decumbent to erect stems	0.3	simple		Brummitt and Marner 1993
	G	<i>suffruticosa</i> = <i>micans</i> subsp. <i>suffruticosa</i>	suberect	0.9 (1.2)	rarely branched		Rebello 2001
	G	<i>simplex</i>	dwarf, erect	1.0	simple <sup>®</sup>	yes	Chisumpa and Brummitt 1987
	G	<i>nubigena</i>	erect, many stems	0.7	much branched	no?	Lamont <i>et al.</i> this paper
			<b>Mean (range) (m)</b>		<b>0.66</b> <b>(0.15–1.0</b> <b>0)</b>		
Winter rainfall (extending to uniform)	S	<i>acaulos</i>	low shrub to 1 m across, rhizomatous	0.25	simple from rhizome	no	Lamont <i>et al.</i> this paper
	S	<i>angustata</i>	Shrublet, mat to 1.5 m across, erect stems, rhizomatous	0.35	simple	no	Lamont <i>et al.</i> this paper
	S	<i>aspera</i>	shrublet to 0.5 m across, rhizomatous	0.2	simple	yes	Lamont <i>et al.</i> this paper
	S	<i>cordata</i>	shrublet, erect stems from woody base to 0.3 m diamater	0.5	simple, ephemeral, renewed at intervals	no	Lamont <i>et al.</i> this paper
	S	<i>decurrens</i>	shrublet, erect stems from woody base	0.6	Simple, ephemeral, renewed at intervals	no	Lamont <i>et al.</i> this paper

S	<i>intonsa</i>	dense, dwarf shrub, 0.3 rhizomatous		simple from rhizome	no	Lamont <i>et</i> Rebelo 2001 <i>al.</i> this paper
S	<i>lorea</i>	shrublet, leaves 0.4 from ground to 1 m across, rhizomatous		simple from rhizome	yes	Lamont <i>et</i> Rebelo 2001 <i>al.</i> this paper
S	<i>piscina</i>	shrublet to 1 m 0.3 across, rhizomatous		simple from rhizome	yes	Lamont <i>et</i> Rebelo 2001 <i>al.</i> this paper
S	<i>restionifolia</i>	shrublet to 1 m 0.3 across, rhizomatous		simple from rhizome	yes	Lamont <i>et</i> Rebelo 2001 <i>al.</i> this paper
S	<i>revoluta</i>	prostrate shrublet to 0.2 2 m across, rhizomatous		simple from rhizome	no	Lamont <i>et</i> Rebelo 2001 <i>al.</i> this paper
S	<i>scabra</i>	shrublet to 0.5 m 0.3 across, rhizomatous		simple from rhizome	yes	Lamont <i>et</i> Rebelo 2001 <i>al.</i> this paper
S	<i>scolopendrifolia</i>	shrublet to 1.0 m 0.6 across, rhizomatous		simple from rhizome	no	Lamont <i>et</i> Rebelo 2001 <i>al.</i> this paper
S	<i>scorzonifolia</i>	shrublet to 1.0 m 0.4 across, rhizomatous (also a dwarf form)		simple from rhizome	yes	Lamont <i>et</i> Rebelo 2001 <i>al.</i> this paper
S	<i>speciosa</i>	low shrub, stems 0.5-1.2 short, erect		seldom branched	no	Lamont <i>et</i> Rebelo 2001 <i>al.</i> this paper
S	<i>subulifolia</i>	shrublet, erect 0.7 stems from woody base		many branchlets, ephemeral, renewed at intervals	no	Lamont <i>et</i> Rebelo 2001 <i>al.</i> this paper
S	<i>tenax</i>	low trailing to 4 m 0.2		Sparsely	no	Lamont <i>et</i> Rebelo 2001



		across,		branched		<i>al.</i> this
S		dwarf shrublet,	0.25	simple	no	paper
	<i>vogtsiae</i>	rhizome atous to				Lamont <i>et</i> Rebelo 2001
		0.5 m across				<i>al.</i> this
		<b>Mean (range) (m)</b>		<b>0.41 (0.2-</b>		paper
				<b>1.20)</b>		

705 \*ericaceous scrub or fynbos

706 @dying 2-5 years after fire (and renewed?)

707 #illustration in Chisumpa and Brummitt (1987) shows three resprouts at the apex of the sobole from the base of three blackened stumps

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709 References

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711 Beard, JS 1958. The *Protea* species of the summer rainfall area of South Africa. *Bothalia* 7: 41-63.

712 Brummitt RK, Marner SK (1993) Flora of tropical East Africa: Proteaceae. Balkema, Rotterdam 30 pp.

713 Chisumpa SM, Brummitt RK (1987) Taxonomic notes on tropical African species of *Protea*. *Kew Bulletin* 42: 815-853.

714 Maurin O, Davies TJ, Burrows JE, Daru BH, Yessoufou K, Muasya AM et al. (2014) Savanna fire and the origins of the ‘underground

715 forests’ of Africa. *New Phytologist* 204: 201-214.

716 Rebelo AG 2001. A field guide to the proteas of southern Africa. Fernwood Press, Cape Town, South Africa.

