

1 **Traits related to efficient acquisition and use of phosphorus promote**
2 **diversification in Proteaceae in phosphorus-impooverished landscapes**

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21 **Abstract**

22 *Background and aims* Plant species richness increases with declining soil phosphorus (P) availability,
23 especially for Proteaceae in old infertile landscapes. This difference in richness might be attributed
24 to faster diversification in lineages adapted to P-impooverished soils, *i.e.* species that possess
25 specialised P-acquisition strategies, and have lower leaf P concentration ([P]) and higher seed [P].
26 Alternatively, a longer time for species accumulation might contribute to high species richness in
27 low-P environments due to the geological stability of the landscapes in which they evolved.

28 *Methods* We assessed differences in diversification of Proteaceae in P-impooverished vs. nutrient-rich
29 environments and whether these were linked to adaptations to P-impooverished soils. We explored
30 mature leaf and seed [P] and investigated how these traits changed over the evolutionary history of
31 the family, and within two species-rich genera (*Banksia*, *Hakea*).

32 *Results* Faster diversification was correlated with lower leaf and higher seed [P] for species-rich
33 genera across the Proteaceae. For *Banksia* and *Hakea*, diversification rates peaked at relatively low
34 leaf [P], but not at the lowest leaf [P]. Ancestral state reconstructions indicated that low leaf [P] is a
35 trait that was likely present in the early evolution of the Proteaceae, with recent transitions to
36 higher leaf [P] across several species-poor rainforest genera.

37 *Conclusions* Diversification of Proteaceae correlated strongly with P-related traits. In an evolutionary
38 context, functional cluster roots, low leaf [P] and high seed [P] were likely key innovations allowing
39 diversification. Selection for low leaf [P] early in the evolutionary history of Proteaceae pre-adapted
40 ancestors of this family to diversify into oligotrophic environments. We discuss how our findings are
41 likely relevant for understanding diversification dynamics of other plant families that occur in P-
42 impooverished environments.

43

44 **Keywords** Carboxylates; Cluster roots; Diversification; Evolution; Extinction; Phosphorus;
45 Phosphorus-remobilisation proficiency; Phosphorus-use efficiency; Proteaceae; Seed phosphorus
46 concentration; Speciation

47

48 **Introduction**

49 Global vascular plant diversity is greatest in the tropics and declines towards the poles, with centres
50 of diversity in Costa Rica-Chocó, Atlantic Brazil, Tropical Eastern Andes, Northern Borneo, and New
51 Guinea (Barthlott et al. 2005; Cámara-Leret et al. 2020). However, this striking biogeographical
52 pattern is interrupted by conspicuous centres of exceptional diversity in regions outside the world's
53 tropical forests in both hemispheres (Kier et al. 2009). Plant diversity in these hotspots is strongly
54 underestimated by global models, which are based predominantly on patterns of climate and
55 environmental heterogeneity (Kreft and Jetz 2007). Regions supporting megadiverse vegetation
56 comprise 8% of all vascular plant species on only 0.3% of the vegetated land area (Wang et al. 2006).
57 These regions all occur in old climatically-buffered infertile landscapes (OCBILs, *sensu* Hopper 2009)
58 that are exceptionally poor in nutrients, particularly soil phosphorus (P) (Hopper et al. 2021). In
59 south-western Australia, half of all soils contain less than 100 mg P kg⁻¹ soil (0-300 mm), compared

60 with only 13% for eastern Australia and 25% for the entire continent (Kooyman et al. 2017), which is
61 a low-P continent (Viscarra Rossel and Bui 2016). Such broad comparisons are not available for Brazil
62 and South Africa, but data for campos rupestres (Zemunik et al. 2018; Abrahão et al. 2019) and the
63 Cape Floristic Region (Bond 2010; Mitchell et al. 1984) show similarly low values as for south-
64 western Australia. We have no information on how quickly soil fertility changed across Australia
65 during the lifespan of the continent, but based on information on a range of chronosequences
66 around the globe, it is clear that after 120,000 to 500,000 years, soils have reached very low total P
67 concentrations (Richardson et al. 2004; Turner et al. 2018; Walker and Syers 1976; Chen et al. 2015).
68 Therefore, the time scale of changes in soil fertility is relatively short, compared with the time scale
69 of evolution explored in our study, provided there is no rejuvenation, as is typical for OCBILs. In
70 contrast, the volcanic soils of southern South America experience regular rejuvenation and so never
71 reach OCBIL status and contain high P concentrations (Borie and Rubio 2003).

72 In the southern hemisphere, prominent plant diversity hotspots outside tropical forests are the
73 campos rupestres in eastern Brazil (Silveira *et al.*, 2016), the Greater Cape Floristic Region in South
74 Africa (Cowling et al. 2009), and the Southwest Australian Floristic Region (Hopper and Gioia 2004).
75 South-western Australia has remained unglaciated since the Permian and comprises extremely
76 infertile soils that are especially poor in P (Lambers et al. 2010; Kooyman et al. 2017; Viscarra Rossel
77 and Bui 2016). The Greater Cape Floristic Region shares many characteristics with the Southwest
78 Australian Floristic Region, including a Mediterranean climate, severely P-impoverished soils
79 (Cowling et al. 2015; Verboom et al. 2017), and numerous Proteaceae species (Pate et al. 2001). The
80 Brazilian campos rupestres, on the other hand, has a subtropical climate with a prolonged dry
81 winter, yet also contains severely P-impoverished soils like the other two regions (Oliveira et al.
82 2015; Silveira et al. 2016), but harbours very few Proteaceae (Lambers et al. 2015a; Zemunik et al.
83 2018). Within low-P biodiverse regions, plant species diversity increases with decreasing soil P
84 concentration ([P]) (Huston 1994; Le Brocq and Buckney 2003; Lambers et al. 2010; Adam 2012;
85 Zemunik et al. 2016). Here, we explore whether low P availability, a factor not considered in global
86 models, which instead focus on potential evapotranspiration, the number of wet days per year, and
87 measurements of topographical and habitat heterogeneity (Kreft and Jetz 2007), contributes to the
88 failure of these models to accurately predict plant diversity of these anomalous regions. Specifically,
89 we explore the contribution of P-acquisition and P-utilisation strategies to the origin and
90 maintenance of this exceptional species richness, focusing on the Gondwanan family Proteaceae. In
91 doing so, we acknowledge the importance of the global models (Kreft and Jetz 2007), but focus on P
92 as a major driver for the evolution of Proteaceae, rather than aridity, which has been the focus of
93 previous studies (Carpenter et al. 2014; Hill 1998; Onstein et al. 2016). We acknowledge that the
94 availability of other nutrients, especially nitrogen (N) is also low in OCBILs, but adhere to the classic
95 Sprengel-von Liebig concept that a single nutrient is the key limiting one (Sprengel 1828; von Liebig
96 1855), and therefore that P availability limits N availability (through constraints on biological N
97 fixation) on old, infertile soils. Moreover, species in OCBILs have evolved traits that enhance the
98 efficiency of acquisition and utilisation of P, and exhibit traits that restrict the acquisition of N
99 (Prodhan et al. 2016; 2019).

100 Proteaceae comprise 83 genera and c. 1700 species (Christenhusz and Byng 2016), with numerous
101 species in OCBILs as well as in younger landscapes where the total soil [P] is high, but the availability
102 of P is low, *e.g.*, Chile and Brazil (Hayes et al. 2018; Ávila-Valdés et al. 2019; Pate et al. 2001; de
103 Britto Costa et al. 2016). The five most species-rich genera include *Grevillea* (362), *Banksia* (c. 170),

104 *Hakea* (150), *Protea* (112), and *Persoonia* (c. 100) (Sauquet et al. 2009), all of which primarily occur
105 in OCBILs. Proteaceae are an ideal case study, because the family has representatives across both
106 low-P and nutrient-rich environments, with stark disparities in species-richness and diversification
107 rate between the two environments (Sauquet et al. 2009; Onstein et al. 2016). In addition, it is one
108 of the few species-rich families with a similar diversification history in both south-western and
109 south-eastern temperate regions of Australia during the Eocene–Oligocene extinction event (Nge et
110 al., 2020a). They show a decline in diversification rate across both regions, in contrast to other
111 families showing a decline in south-eastern, but not south-western Australia during that period.
112 Accounting for different diversification histories because of other factors (e.g., climate, extinction) is
113 encouraged, as these factors could mask or compromise tests for diversification signals of P-related
114 traits. While Proteaceae does not exhibit significantly faster diversification rates than most other
115 angiosperm families (Magallon and Sanderson 2001), Sauquet et al. (2009) showed that within
116 Proteaceae, diversification rates were significantly faster in Mediterranean regions (Cape Floristic
117 Region, south-western Australia) than elsewhere. Open sclerophyllous biomes associated with
118 Mediterranean regions have also been linked to increases in diversification rates for Proteaceae
119 (Onstein et al. 2016; Reyes et al. 2015). However, an explicit investigation of potential links between
120 diversification rates in Proteaceae and how these may be driven by adaptations (i.e. biotic factors) to
121 low-P environments is lacking.

122 Proteaceae also offer a good case study from the perspective of traits related to P acquisition and P
123 use, which have been studied in numerous genera (Lambers et al. 2015a; Lambers et al. 2015b).
124 Most Proteaceae produce functional cluster roots, with very few exceptions, meaning most meet the
125 criteria of having a specialised P acquisition strategy (Zhong et al. 2021; Lambers et al. 2021).
126 Proteaceae from severely P-impooverished habitats function at low leaf [P] ($<0.3 \text{ mg P g}^{-1}$ dry weight,
127 compared with a global average value of 1.1 mg g^{-1} (Wright et al. 2004), unlike those from habitats
128 where the total [P] is greater, but the P availability is low (Lambers et al. 2012a). Despite low leaf [P],
129 Proteaceae from low-P habitats exhibit relatively fast rates of photosynthesis. This is accounted for
130 by replacement of phospholipids in leaves by lipids that do not contain P during leaf development
131 (Lambers et al. 2012b), functioning at very low abundance of rRNA (Sulpice et al., 2014), and
132 preferentially allocating P to mesophyll cells (Hayes et al. 2019a; Hayes et al. 2018). The association
133 of proteaceous sclerophyllous heath communities with oligotrophic soils dates back to the
134 Cretaceous (Carpenter et al. 2015). Indeed, scleromorphy is commonly considered an adaptation to
135 oligotrophic soils (Beadle 1966), and is considered to be the ancestral trait for a number of species-
136 rich Proteaceae genera (e.g., Carpenter et al. 2017). These studies suggest adaptations to nutrient-
137 poor environments might have been present early in the evolutionary history of the family, or that
138 there was earlier selection for these traits compared to that in other lineages that inhabit nutrient-
139 rich environments.

140 We propose two main hypotheses. First, we hypothesise that lineages with specific adaptations to P-
141 impoverished environments (i.e. specialised P-acquisition strategies, low leaf [P], and high seed [P])
142 exhibit faster diversification rates (i.e. the net result of speciation minus extinction) in OCBILs. This
143 hypothesis pertains both across the Proteaceae family, and within highly diverse genera (e.g., *Hakea*
144 and *Banksia*). The efficient P-acquisition strategies we consider are carboxylate-releasing cluster
145 roots (Shane and Lambers 2005) or their functional equivalent. Almost all Proteaceae possess
146 specialised P-acquisition strategies and so we cannot compare groups directly, but we do compare
147 the few exceptions. Because both mycorrhizal and non-mycorrhizal plant species that lack a

148 carboxylate-releasing strategy are less effective at acquiring P from soils with very low P availability
149 (Parfitt 1979; Raven et al. 2018), we envisage that without an efficient P-acquisition strategy, rapid
150 diversification would be less likely in OCBLs. Since Proteaceae on severely P-impooverished soils
151 exhibit relatively fast rates of photosynthesis at very low leaf [P] (*i.e.* they have an exceptionally high
152 photosynthetic P-use efficiency; PPUE) (Denton *et al.*, 2007; Sulpice *et al.*, 2014), we used mature
153 leaf [P] as an indicator for efficient P utilisation in leaves. As a second measure for efficient P use in
154 leaves, we used P-remobilisation proficiency, which is the leaf [P] in fully senesced leaves (Killingbeck
155 1996). Leaf longevity, which is associated with nutrient residence time (Tsuji *et al.* 2020; Aerts
156 1990), would also be a good measure for P-use efficiency, but there are insufficient data on leaf
157 longevity in the literature. For seeds, we considered both seed [P] and seed P content. Second, we
158 hypothesised that the aforementioned P-related traits (P-acquisition, leaf [P], seed [P] and seed P
159 content) are conserved in Proteaceae and were present early in its evolutionary history, allowing it
160 to diversify into P-impooverished landscapes, with lower P-use efficiency traits found in extant
161 rainforest lineages having originated later in their evolution.

162 To test these hypotheses, we collated a dataset of P-related traits in Proteaceae. This data set was
163 used to investigate the relationships between traits and genus size, diversification rates, and stem
164 ages across the Proteaceae. The second hypothesis was tested by considering changes and
165 estimated ancestral states for some of the above P-related traits during the course of evolution
166 across Proteaceae. We extended this analysis by exploring two species-rich Proteaceae genera that
167 dominate low-P environments (*Banksia*, *Hakea*) as further case studies of within-genera
168 diversification dynamics.

169 **Materials and Methods**

170 *Leaf and seed P data collection*

171 We collated data for mature leaf [P], senesced leaf [P], seed [P], and seed P content across 329
172 species of Proteaceae, covering 44 genera – over half of all extant genera within the family (53%,
173 44/83) (Christenhusz and Byng 2016) (Tables S1-S3). These data were sourced from 61 studies
174 (published and unpublished), and included 148 sites across Australia, South Africa, Brazil, Chile,
175 China, and Japan. The full data set contained 970 mean trait values for individual species at
176 individual sites. We included only plants growing in natural habitats, as has been done for similar
177 studies dealing with global comparisons of plant traits (Wright *et al.* 2004; Reich *et al.* 1997).

178 *Leaf and seed sampling and P analyses*

179 In addition to published data (Tables S1-S3), we also collected data from unpublished sources and
180 complemented this with our own collection of leaf and seed samples from plants growing in natural
181 habitats. For unpublished material collected in south-western Australia and China, five mature and
182 healthy plants of each species were randomly selected at each site. Undamaged, mature, fully-
183 expanded leaves from four aspects of the canopy of each individual plant were sampled. Leaf
184 samples were rinsed with deionised water to remove dust and dried with paper towels. Petioles
185 were removed, if present. Seed episperms were removed. Leaf and seed samples were oven-dried at
186 70°C for two days, followed by ball-mill grinding (Geno/Grinder 2010, Spex SamplePrep, Metuchen,
187 NJ, USA) using zirconium beads. Ground leaf and seed samples from south-western Australia and

188 China were digested in a 3:1 mixture of concentrated nitric and perchloric acid (Zasoski & Burau,
189 1977). The leaf [P] in samples from south-western Australia was determined by inductively coupled-
190 plasma optical-emission spectrometry (ICP-OES, Optima 5300DV, Perkin Elmer, Shelton, CT, USA)
191 and those from China by inductively coupled-plasma atomic-emission spectrometry (ICP-AES, IRIS
192 Advantage-ER, Thermo Jarrell Ash Corporation, Waltham, MA, USA) at the Biogeochemical
193 Laboratory, Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences.

194 *Genus diversity, diversification and stem age in Proteaceae and P-related traits*

195 We explored possible relationships between the size of a genus (*i.e.* number of extant species) in
196 Proteaceae, their diversification and speciation rates, and stem age with leaf [P] and seed [P]. We
197 sourced a dated genus-level phylogeny for Proteaceae (Onstein et al. 2016) and densely-sampled
198 species-level phylogenies for two species-rich genera (*Hakea*, *Banksia*) from previous studies
199 (Cardillo et al. 2017; Cardillo and Pratt 2013), for details of gene regions used, see Table S4. The two
200 species-rich genera were selected due to our targeted collection of P trait data for these groups,
201 given that the majority of their extant species diversity are found in south-western Australia, but are
202 also present in other nutrient-richer regions (*e.g.*, south-eastern Australia). These phylogenies were
203 pruned to only include taxa with available P trait data. The coverage after applying this filter was
204 50% (41/83) of Proteaceae genera, 51% (77/150) of *Hakea* species, and 20% (34/170) of *Banksia*
205 species for leaf [P]. For seed [P], the pruned datasets covered 22% (18/83) of Proteaceae genera,
206 29% (43/150) of *Hakea*, and 16% (27/170) of *Banksia* species. Due to the paucity of available seed
207 [P] data for both *Banksia* and *Hakea*, they were not included in our formal diversification analyses
208 for these genera.

209 Diversification rates and stem ages of genera were obtained from Onstein *et al.* (2016), as we
210 sourced the dated genus-level phylogeny from their study. We tested for associations of P traits with
211 lineages occupying low-P vs. higher-P environments. Low-P OCBIL environments were classified as
212 'hotspots' (south-western Australia, Cape Floristic Region) following Onstein *et al.* (2016), with
213 differences in P traits compared between them and 'other' regions with higher soil fertility (Table
214 S2). We also tested for associations of P traits with lineages inhabiting open vs. closed habitats, as
215 sclerophyllous nutrient-poor environments are usually classified as open vegetation compared with
216 rainforests (closed). Vegetation types were scored based on Onstein *et al.* (2016) (Table S2). For the
217 *Hakea* dataset, diversification rates of each tip were derived from Bayesian analysis of macro-
218 evolutionary mixtures (BAMM) (Rabosky 2020), and stem ages obtained from Cardillo *et al.* (2017).
219 Stem ages for *Banksia* species were obtained from Cardillo and Pratt (2013); however, obtaining
220 accurate diversification rates from our *Banksia* dataset was hampered by a paucity of data for P-
221 related traits as the majority of lineages were pruned, affecting the stem ages (and hence
222 diversification rates) of the remaining taxa; hence the diversification analyses for the *Banksia* subset
223 should be treated with caution. We compared these variables against the available data on leaf [P], a
224 proxy for photosynthetic P-use efficiency (Guilherme Pereira et al. 2019; Sulpice et al. 2014; Denton
225 et al. 2007).

226 *Statistical analyses*

227 The species-level mean for each trait was determined by taking the average of each species across
228 all sites. This species-level mean was then used to determine the genus-level mean, by averaging all
229 species within each genus (Table S2). The genus-level mean was used in all analyses, comparing it

230 against the genus size, diversification rate, and stem age. The genus *Xylomelum* was removed from
231 all main analyses, because two of the species within this small genus that are endemic to south-
232 western Australia do not produce functional cluster roots (Zhong et al. 2021), and hence lack a trait
233 we consider vital to support rapid diversification. In addition, we excluded the genus *Persoonia* from
234 seed [P] regression analysis, because, although it has seeds with very low seed [P], the seeds are
235 large (Leishman and Westoby 1998), resulting in a relatively high seed P content, unlike other genera
236 where low seed [P] is indicative of low seed P content (due to their smaller size).

237 The relationships between genus diversity, stem age, speciation and diversification rates, and P-
238 related traits were assessed through regression analysis using generalised least squares models
239 (Pinheiro and Bates 2000). Data were transformed when they were not normally distributed, and
240 models were selected based on Akaike Information Criterion (AIC). The residuals of each model were
241 visually inspected for heteroscedasticity, and appropriate variance structures were specified if they
242 significantly improved the model, based on AIC and Bayesian Information Criterion (BIC) values
243 (Pinheiro and Bates 2000). Relationships between P traits vs. hotspot (*i.e.* low-P OCBIL areas) and
244 open or closed vegetation types were also assessed using ANOVA tests and subsequent *post-hoc*
245 Tukey tests. Kruskal-Wallis tests were used where data were not normally distributed even after log-
246 transformation. Statistical analyses were performed using the R software platform
247 (R_Development_Core_Team 2019) and the *nlme* package (Pinheiro et al. 2020).

248 Phylogenetic signal for the P traits and genus size was assessed across all three phylogenies using
249 Pagel's λ (Pagel 1999) and Blomberg's K (Blomberg et al. 2003), with values between 0–1; 0
250 indicating no phylogenetic signal and 1 indicating a strong phylogenetic signal (*i.e.* traits are
251 conserved). These tests were implemented using *caper* (Orme et al. 2018) and *ape* (Paradis et al.
252 2004).

253 *Trait-dependent diversification*

254 To test for trait-dependent diversification (leaf and seed [P]), we used the Maximum Likelihood-
255 based quantitative-state speciation and extinction model (FitzJohn 2010), implemented using the
256 *diversitree* package (FitzJohn 2012). The QuaSSE model compares a null model of constant-rate
257 diversification with sigmoidal, linear, or modal models. Inflated type-1 error rates have been
258 attributed to state-dependent diversification (SSE) models (Rabosky and Goldberg 2015). We
259 addressed these concerns by assessing whether QuaSSE would return a significant result based on
260 diversification-neutral traits (see Supplementary Material for details).

261 We also utilised a non-model trait-dependent approach, BAMM-based STructured Rate
262 Permutations on Phylogenies (STRAPP; Rabosky and Huang 2015), to formally assess the relationship
263 between speciation rates, and leaf and seed [P] of Proteaceae. In addition, we used semi-parametric
264 methods including phylogenetic generalised least squares tests (PGLS) in an attempt to address
265 these issues of type-1 error and phylogenetic pseudoreplication associated with SSE-type and
266 STRAPP models (see Supplementary Material for details).

267 For *Hakea*, we applied the methods-of-moments approach (Magallon and Sanderson 2001) to assess
268 whether lineages with lower leaf [P] and higher seed [P] had faster diversification rates than the
269 background rate of the genus. A similar approach has been applied in a Proteaceae-wide analysis in
270 Sauquet et al. (2009). Diversification rates were calculated using equation 6 from Magallon and

271 Sanderson (2001), assuming either no extinction ($\kappa = 0$) or high relative extinction ($\kappa = 0.9$) rates.
272 The diversification rate of each clade was calculated using both the stem and crown ages of the
273 clade separately and number of extant species within the clade. The background diversification rate
274 of *Hakea* was calculated using its stem age and total number of extant genera. The 95% confidence
275 intervals (CI) on clade size based on stem age were calculated using equation 10 (Magallon and
276 Sanderson 2001) implemented through the *geiger* package (Harmon et al. 2007; Pennell et al. 2014).

277 *Ancestral-state reconstructions for P traits in Proteaceae*

278 We estimated ancestral states of leaf [P] as continuous characters using the 'contMap' function in
279 *phytools* package (Revell 2012) in R. Missing Seed [P] tip values were reconstructed using the
280 maximum likelihood function 'anc.ML' in *phytools*, overlaid onto the pruned leaf [P] subset
281 phylogeny. We assessed and visualised our dated trees against the defined trait space using the
282 'fancyTree' function in *phytools*.

283 **Results**

284 *Genus size and mature and senesced leaf P concentrations*

285 The size of a genus in Proteaceae was negatively correlated with the mean mature leaf [P] of extant
286 species within that genus ($P = 0.007$; Fig. 1a). This negative correlation indicates that the most
287 species-rich genera tended to have a low leaf [P], a strong indicator of high P-use efficiency.
288 Similarly, there was a general negative trend between genus size and [P] of fully senesced leaves,
289 another indicator of high P-use efficiency. However, in this case the result was not statistically robust
290 ($P = 0.099$; Fig. 1b). Far fewer data were available for senesced leaf [P] ($n = 15$) and these covered
291 only a small range in [P]; additional data would be required to arrive at a firm conclusion. It is noted
292 that the three most species-rich genera included in this analysis (*Banksia*, *Grevillea*, *Hakea*) all
293 showed very low senesced leaf [P], whilst the genus with the highest senesced leaf [P] is a
294 monospecific genus (*Embothrium*). Leaf [P] of genera in hotspots and open vegetation were lower
295 than those in non-hotspot regions and closed vegetation areas ($P < 0.001$, $P = 0.004$, respectively;
296 Table S5).

297 *Seed P concentration and content*

298 The size of a genus was positively correlated with seed [P] ($P = 0.005$; Fig. 2). Too few data were
299 available for seed P content; using available data from 13 genera, we found no significant correlation
300 between genus size and seed P content (Fig. S1); more data would be required to confidently test
301 this relationship. The average seed [P] across genera in hotspots and open vegetation tended to be
302 higher than that in non-hotspots and closed vegetation areas, but these differences were not
303 significant ($P = 0.172$, $P = 0.06$, respectively; Table S5).

304 *Diversification rate, stem age and leaf and seed P concentrations*

305 Since the number of species in a genus depends on taxonomic process (Strand and Panova 2015),
306 diversification rate and stem age, we also compared these against the same P-related traits that
307 were correlated with genus size and for which we had sufficient data: mature leaf [P] and seed [P].
308 As with genus size, genus diversification rate was negatively correlated with mature leaf [P] ($P =$

309 0.015) and positively correlated with seed [P] ($P = 0.013$; Fig. 3a, b). Genus stem age was not
310 significantly correlated with mature leaf [P] ($P = 0.312$; Fig. 3c). In contrast, seed [P] was positively
311 correlated with stem age ($P = 0.015$; Fig. 3d), suggesting that older genera tended to have higher
312 seed [P] than younger genera. The average stem age (Ma) of Proteaceae genera in hotspots and
313 closed vegetation tended to be older than that in non-hotspot regions and open vegetation areas;
314 however, these differences were not significant ($P = 0.779$, $P = 0.503$, respectively; Table S5).

315 *Phylogenetic patterns in P-related traits and genus size*

316 Leaf [P] and genus size showed significant phylogenetic signals ($P < 0.05$) across the family-wide
317 phylogeny, whereas seed [P] did not (Tables S6). In contrast, both leaf and seed [P] traits showed no
318 or very little phylogenetic signal for *Hakea*. For *Banksia*, only seed [P] showed a significant
319 phylogenetic signal.

320 *Trait-dependent diversification*

321 A significant modal relationship was inferred from QuaSSE for the Proteaceae-wide dataset for both
322 leaf and seed [P] traits ($P < 0.001$; Table S7). However, 89% of the simulation tests were significant
323 for diversification-neutral traits (Table S8a). Therefore, we only relied on the STRAPP and semi-
324 parametric tests for this dataset. The QuaSSE analyses for *Hakea* indicated a significant modal
325 relationship between each of the two P-related traits (leaf [P] and seed [P]) and diversification rate
326 of the genus ($P < 0.05$; Table S7), peaking at relatively low leaf [P] and high seed [P] values. However,
327 similar to the Proteaceae-wide dataset, more than 5% of the simulations returned a significant result
328 based on diversification-neutral traits; hence, this finding should be interpreted with caution (37% of
329 simulations were significant for diversification-neutral traits; Table S8b). For *Banksia*, no significant
330 relationship was detected between the P-related traits and diversification rate of the genus (Table
331 S7) (5% of simulations were significant for diversification-neutral traits).

332 Unlike QuaSSE, STRAPP analyses did not detect a significant effect of state changes in P-related traits
333 on speciation rates across Proteaceae or within *Hakea* (Table S9). PGLS tests for both leaf and seed
334 [P] against genus size and seed [P] with diversification rates were significant for the Proteaceae-wide
335 dataset (Table S10), whereas other semi-parametric tests and associations were non-significant for
336 our three datasets (Proteaceae-wide, within *Hakea*, and within *Banksia*) (Tables S10, S11).

337 In a method-of-moments analysis, none of the lineages in *Hakea* that displayed high P-use efficiency
338 were significantly species-rich or species-poor (excluding clades with only one taxon, as they have a
339 net diversification rate of 0) (Fig. S2).

340 *Ancestral-state reconstructions*

341 The ancestral leaf [P] for Proteaceae was estimated to be low (0.4 mg g^{-1} , 95% confidence interval
342 (CI): $0.2\text{--}0.6 \text{ mg g}^{-1}$), compared with a modern value of 0.3 (Table S1) and a global average value of
343 1.1 mg g^{-1} (Wright et al. 2004), with the majority of genera (88.6%, 39/44) falling within the 95% CI of
344 the Most Common Recent Ancestor (MRCA) and clustering at the lower half of the range ($0.2\text{--}0.5 \text{ mg g}^{-1}$)
345 (Fig. 4a). Only the genus *Isopogon* fell below the 95% CI, compared with five genera falling above
346 the 95% CI (all of which are associated with rainforest or closed vegetation). Importantly, all five
347 genera with high leaf [P] were derived from lineages that had low leaf [P].

348 For *Hakea*, the ancestral leaf [P] was also estimated to be low (0.2 mg g⁻¹, 95% CI: 0.1–0.4 mg g⁻¹),
349 with the majority of species clustering at the lower half of the range (0.1–0.3 mg g⁻¹) (Figs 5 & S3).
350 The few outlying species that had a leaf [P] above the 95% CI range (>0.4 mg g⁻¹) were all late-
351 branching from low leaf [P] lineages (Fig. 5). *Hakea* species exhibiting the lowest leaf [P] were also
352 late-branching, arising independently at least five times over the course of their evolution (Figs 5,
353 S3). Clades with the lowest leaf [P] were of recent origin, diversifying in the last 5 Ma to 10 Ma,
354 during the late Miocene (e.g., *H. eneabba*–*H. acuminata*, *H. obliqua*–*H. brownii* clades) (Fig. 5).

355 In *Banksia*, the ancestral leaf [P] was estimated to be intermediate (0.25 mg g⁻¹, 95% CI: 0.10–0.35
356 mg g⁻¹) (Fig. 6). The two outlying species *B. prionotes* and *B. integrifolia* fell above the 95% CI and
357 were derived from lineages that had lower leaf [P] (Figs 6 and S4). Lineages with the highest and
358 lowest values in both leaf and seed [P] were derived, compared with older lineages with low leaf [P]
359 and moderate seed [P] (Figs 6 and S4).

360 Interestingly, the ancestral seed [P] was estimated to be low in Proteaceae and increased in lineages
361 that occupy environments with low nutrient availability (Fig. 4). In contrast, the ancestral seed [P]
362 was intermediate for both *Hakea* and *Banksia*, with low and high seed [P] lineages being late-
363 branching lineages, though it is worth noting that intermediate values for *Hakea* and *Banksia*
364 lineages are high compared with other Proteaceae genera. However, greater sampling is required to
365 confirm these trends due to limited seed [P] sampling in this study (Fig. S5). Hence, we express
366 caution when interpreting this section of our results.

367 Discussion

368 *Proteaceae-wide diversification dynamics and P-related traits*

369 Our study provides compelling evidence that soil P availability, which affects leaf and seed [P], is
370 associated with the genus size of Proteaceae. A strength of our study is that it compares species
371 traits collected in natural habitats, similar to analyses of global-scale relationships between
372 photosynthesis and leaf nutrient concentrations (Reich et al. 1997; 1999) used to develop the leaf
373 economic spectrum. Our results support our first hypothesis that diversification (*i.e.* the net result
374 of speciation minus extinction) on P-impoverished OCBILs is likely greater in lineages with low leaf
375 [P], high seed [P], and functional cluster roots or their equivalent. A low mature leaf [P] in
376 Proteaceae and co-occurring species in south-western Australia is associated with a high PPUE
377 (Denton *et al.*, 2007; Sulpice *et al.*, 2014; Guilherme Pereira *et al.*, 2019). This study demonstrates a
378 clear link between traits and environment in Proteaceae, which agrees with studies of other traits
379 across Proteaceae, *i.e.* stomatal size (Jordan et al. 2015) and sclerophylly (Onstein et al. 2016).

380 The negative correlation between both genus size (Fig. 1a) and diversification rate (Fig. 3a) with
381 mature leaf [P] strongly supports our first hypothesis, with the majority of species-rich genera that
382 have higher diversification rates, found in low-P environments (Specht and Moll 1983; Kooyman et
383 al. 2017; Bond 2010), exhibiting low leaf [P]. Photosynthetic P-use efficiency is a complex trait; thus,
384 the individual traits underpinning high PPUE must be the target of selection during the course of
385 evolution. In Proteaceae and other eudicots, preferential allocation of P to mesophyll cells, which
386 underpins a high PPUE, is exhibited only by those species that evolved in P-impoverished landscapes,
387 and not in species that evolved in habitats with higher total soil [P], such as volcanic landscapes in

388 southern South America and cerrado in Brazil (Guilherme Pereira et al. 2018; Hayes et al. 2018). In
389 the present study, genera with the majority of extant species confined to Mediterranean hotspots
390 with severely P-impooverished soils showed faster diversification rates (Sauquet *et al.*, 2009) and
391 lower leaf [P]. In contrast, rainforest genera such as *Darlingia*, *Embothrium* and *Stenocarpus* had
392 higher leaf [P] and slower diversification rates (Fig. 4). *Helicia* species, which inhabit low-P
393 rainforests of southeast Asia, New Guinea and eastern Australia, is a genus with one of the fastest
394 diversification rates within the family (Sauquet et al. 2009). It also had a lower leaf [P], similar to
395 other genera found in OCBILs; this supports our first hypothesis, although the variation among
396 species in the genus was large, likely reflecting variation in soil [P]. For the ForestGEO plot at Fushan,
397 Taiwan, where *Helicia formosana* is the second-most common species on the plot (Su et al. 2007),
398 mean Bray [P] (0-100 mm) was relatively low, 4.1 mg P kg⁻¹ dry soil (B.L. Turner and I.-Fang Sun,
399 unpubl. data).

400 Ideally, we would compare seed P content, which is determined by both seed mass and seed [P].
401 However, there are few data on seed P content in the literature, and hence we used seed [P] in our
402 analysis. Positive correlations between both genus size (Fig. 2), diversification rate (Fig. 3b) and seed
403 [P] further support our first hypothesis. Mineral nutrient reserves in seeds are important for early
404 plant growth, especially for plants growing on impoverished soils (Zhu & Smith, 2001). In P-
405 impoverished and seasonally-dry habitats, seed P is vital for early growth and seedling establishment
406 of perennials (Allsopp and Stock 1995). Seeds that contain more P germinate earlier, giving rise to
407 plants that produce shoot and root mass faster than plants that produce seeds with less P (Bolland
408 and Paynter 1990; Thomson and Bolger 1993; Kolawole and Kang 1997; Ros et al. 1997; Zhu and
409 Smith 2001). Early vigour is particularly important in a seasonally-dry environment to ensure that
410 seedlings reach the water table before the onset of the dry season. Species with smaller seed P
411 reserves typically show greater mortality towards the end of the dry season (Shi et al. 2020).

412 In fire-prone Mediterranean vegetation, two contrasting life histories with respect to fire exist:
413 obligate seeders and resprouters (Lamont and Markey 1995; Pate et al. 1990; Bell 2001). Seeders
414 tend to produce smaller seeds, grow faster, and reach reproductive maturity before the next
415 catastrophic event, whereas resprouters have fewer larger seeds, grow more slowly and allocate a
416 greater proportion of their resources below ground, where reserves are stored to sustain regrowth
417 (Hansen et al. 1991; Bowen and Pate 1993; Knox and Morrison 2005; Keeley et al. 2011; Power et al.
418 2011). These contrasting requirements may account for some of the variation in seed [P] in the
419 present study.

420 Interestingly, while we demonstrate significant correlations between both genus size and
421 diversification rate and leaf [P] in this study, the correlations of leaf [P] with speciation rates
422 (STRAPP) and diversification (QuaSSE) are less clear from the formal diversification analyses. Even
423 though clear differences in species richness and diversification rates exist across different regions,
424 diversification tests from STRAPP still returned non-significant results, as also found in other studies,
425 due to the method requiring replication, rather than clade-specific radiations in explaining
426 diversification shifts (Rabosky, 2020). Faster diversification rates in a particular region might be due
427 to only one (or a few) specific radiations dominating the diversification signal, as has been shown for
428 fish in freshwater vs. marine environments (Rabosky 2020), rather than small multiple shifts in
429 diversification across numerous clades. Hence, in Rabosky (2020), transitions from marine to
430 freshwater environments did not result in elevated speciation rates in general, even though

431 freshwater fish have substantially faster speciation rates in general – driven by one species-rich
432 clade. In our case, non-significant shifts in speciation rates from nutrient-rich to nutrient-poor OCBIL
433 environments, despite faster diversification rates associated with the latter, is due to the presence
434 of not only a few dominant species-rich genera (*e.g.*, *Hakea* and *Banksia*), but also many species-
435 poor lineages (including monospecific genera) in OCBILs. Indeed, south-western Australia and Cape
436 Floristic Region hotspots harbour not only more species-rich genera, but also more species-poor
437 genera than other landscapes with nutrient-richer soils. Therefore, OCBILs contain higher species
438 richness and higher diversification rates of selected species-rich genera, but still have lower average
439 diversification rates due to these species-poor lineages (Nge et al. 2020a). While selection of high P-
440 use efficiency traits was evident for the faster diversification rates of species-rich genera in OCBILs,
441 other factors might explain the lower diversification rates of a few species-poor lineages that exhibit
442 high P-use efficiency in OCBILs (*e.g.*, clade-specific competition with other Proteaceae) (Crisp *et al.*,
443 2011; Carpenter *et al.*, 2015). It is also worth noting that diversification rates in this study were
444 derived from stem ages of genera across Proteaceae only. This may explain why non-significant
445 results were obtained from the PGLS analyses for stem-derived diversification rates, but not for
446 genus size (a proxy for crown diversification) in relation to P traits. In addition, no significant
447 correlation was detected for stem age and leaf [P] from PGLS tests, suggesting that higher
448 diversification rates associated with P traits in oligotrophic environments probably occurred at or
449 after the crown radiations, rather than the stems. The finding of significant correlation between
450 stem age and seed [P] is tentative and warrants further investigation due to limited sampling at
451 present. It would be worth expanding hypotheses in future studies with greater sampling, as this
452 would allow for more accurate estimates of relationships between P-related traits and diversification
453 rates, by using rates derived from crown radiations instead of stem ages which reflect more on the
454 origin of a lineage, rather than its diversification. Comparisons of paleo-records with our findings
455 based on molecular phylogenies of extant taxa are also encouraged. These would allow for a more
456 holistic understanding of diversification across Proteaceae in relation to these traits, since estimating
457 extinction rates from extant taxa is currently fraught with difficulties (Louca and Pennell 2020;
458 Rabosky 2010).

459 We were not able to use correlation analyses to test for the role of P-acquisition traits in increasing
460 diversification, because most Proteaceae exhibit functional cluster roots or their equivalent, and
461 lacking them is the exception. However, the correlations of diversification with P-related traits were
462 greatly strengthened when the genus without functional cluster roots (*Xylomelum*) was removed.
463 Note that we did not remove this genus because it was an outlier, but because it lacks functional
464 cluster roots. This supports our hypothesis that functional cluster roots or their equivalent are a vital
465 trait in P-impooverished habitats. We surmise that the absence of functional cluster roots (Zhong et
466 al. 2021) restricted diversification in *Xylomelum*. Carboxylate-releasing roots allow plants to acquire
467 P that is not available for most mycorrhizas (Parfitt 1979; Lambers et al. 2008), albeit at much
468 greater costs than those incurred by mycorrhizas (Lambers et al. 2006; Raven et al. 2018). Functional
469 cluster roots are also absent in mature plants of *Stirlingia latifolia* (Lambers et al. 2021), a member
470 of another small genus of seven species, but we do not know how widespread the absence of cluster
471 roots is in this genus. *Persoonia*, a large genus of 101 species, lacks cluster roots, but the measured
472 species all show high leaf [Mn] indicating high levels of carboxylate release (Lambers et al. 2021) and
473 thus their roots are likely the functional equivalent of cluster roots. Therefore, these three genera

474 highlight the importance of efficient carboxylate-releasing P-acquisition strategies to support
475 diversification in severely P-impoverished landscapes.

476 *Diversification dynamics within genera of Proteaceae and P-related traits*

477 *Hakea* lineages with relatively low leaf [P] exhibited faster speciation rates based on our STRAPP
478 analyses. In *Hakea*, and more broadly in the closely-related *Grevillea*, P is preferentially allocated to
479 the mesophyll, the site of photosynthesis, thus increasing their PPUE (Hayes *et al.*, 2018; P.L. Clode &
480 K. Ranathunge, unpubl.). Low leaf [P] in *Banksia* and *Hakea* is associated with a low abundance of
481 ribosomal RNA (Sulpice *et al.*, 2014) and phospholipids (Lambers *et al.* 2012b). Extant species of
482 *Hakea* with the lowest leaf [P] arose independently at least five times within the genus, indicating a
483 selection for low leaf [P], and thus high PPUE, in low-P environments compared with species
484 exhibiting high leaf [P], which arose only twice from our sampled taxa (Fig. 5). Interestingly, lineages
485 with either low or high leaf [P] within *Hakea* were of recent origin, but lineages with the lowest leaf
486 [P] and high seed [P] diversified faster. In contrast, lineages with high leaf [P] did not diversify or
487 diversified at a slower rate (*H. erinacea*, *H. lissocarpha*), and were limited to one or two species. The
488 present dataset for *Grevillea*, the most species-rich genus within the family, is limited (unpublished
489 data), and more data on [P] are warranted to study clades in this species-rich genus more closely, as
490 we did for *Hakea*. This is of particular interest as several clades of *Grevillea* are sister to *Hakea* (with
491 *Hakea* being nested within *Grevillea*) and *Grevillea* functions at 60% higher leaf [P] than *Hakea*
492 (Table S1).

493 For the species-rich genus *Banksia*, we detected no significant difference in diversification rates
494 across lineages with contrasting leaf [P]. The diversification of lineages in *Banksia* with low leaf [P]
495 was more complicated than that in *Hakea*. Some of these lineages with low leaf [P] were relatively
496 old (*e.g.*, *B. chaemaephyton*, *B. coccinea*), whereas the majority show a similar pattern noted for
497 *Hakea*, being of more recent origin, *e.g.*, *B. burdettii* (a close relative of *B. prionotes*, which exhibited
498 higher than average leaf [P] for *Banksia*), *B. robur*, *B. candolleana*, *B. armata*, *B. cirsioides*, and *B.*
499 *lehmanniana* (Fig. 6). Independent origins of lower leaf [P] within *Banksia* throughout its
500 diversification history, as shown by relatively older and younger clades that exhibited this trait,
501 suggest that it might be under repeated selection throughout the evolution of the genus in P-
502 impoverished landscapes. A stabilising mode of selection might explain these patterns, with trade-
503 offs against lower leaf [P] as the average leaf [P] of this sclerophyllous genus is relatively low
504 compared with that of other Proteaceae genera. If we consider *B. prionotes* as the only endemic
505 south-western Australian taxon with relatively high leaf [P], compared with other taxa with relatively
506 low leaf [P], then the diversification trend of *Banksia* is similar to *Hakea* in supporting our first
507 hypothesis that lineages with low leaf [P] in low-P environments have greater diversification rates
508 than lineages with higher leaf [P]. Within P-impoverished landscapes of south-western Australia, *B.*
509 *prionotes* is also able to grow on younger dunes along the Jurien Bay chronosequence (Zemunik *et al.*
510 *et al.* 2016; Hayes *et al.* 2019b) that have higher soil [P] (Turner *et al.* 2018). It is killed by fire, but on
511 locations with lower soil [P] it germinates abundantly and does particularly well after a fire, when
512 soil [P] increases, followed by self-thinning in post-fire vegetation (Pate and Bell 1999).

513 Interestingly, sister species pairs of *Banksia* tended to show divergent selection for leaf [P] (Figs 6,
514 S4). This phenomenon could be a driver for the co-occurrence of multiple species of this genus
515 across south-western Australia, with different species occupying different nutrient niches, as we

516 discussed for *Grevillea* vs. *Hakea*. Similarly, while a correlation between low leaf [P] and high seed
517 [P] was evident in *Banksia*, several lineages did not show this trend. For example, *B. chaemaephyton*
518 and *B. spinulosa* had low leaf [P], but also low seed [P], and *B. sphaerocarpa* had moderate leaf [P],
519 but the highest seed [P] among all sampled *Banksia* species (Fig. 6). Not many species of *Banksia*
520 have both low leaf [P] and high seed [P], unlike in *Hakea*. Therefore, an alternative hypothesis for
521 *Banksia* is that there are trade-offs for each of the P-related strategies. Some species have low leaf
522 [P] and others have high seed [P] allowing them to co-exist on different soils in low-nutrient
523 landscapes – high beta diversity due to niche-partitioning (Paoli et al. 2006). However, more
524 sampling is required to confirm this pattern in *Banksia* (Fig. S5e-f).

525 Why are P-efficiency traits associated with a high species diversity, rather than P-efficient species
526 becoming dominant in low-P environments? When resources are abundant, successful colonisation
527 can, indeed, lead to large populations and few fast-growing species at one extreme of the plant
528 economic spectrum. That is typically what happens in nutrient-rich habitats where fast-growing
529 species outcompete those that have a lower growth potential, but plant species diversity is low
530 (Huston 1979; Lambers and Poorter 1992). In habitats where resources are scarce, this is not a likely
531 option, because there are no ‘Darwinian demons’ that are good at everything (Carlson and Taffs
532 2010; Law 1979; Morris 2009). The reason we do not see these ‘demons’, even though life history
533 traits evolve, is that different life history traits are not independent; there are trade-offs. Proteaceae
534 are good at acquiring P, but this is traded off against susceptibility to oomycete pathogens (Lambers
535 et al. 2018; Albornoz et al. 2017). Efficiency of resource use is what matters in P-impooverished
536 habitats, rather than rapid growth. Here we deal with the opposite end of the plant economic
537 spectrum, and facilitation, rather than only competition is the norm (Lambers et al. 2018; Wright et
538 al. 2017). However, within the Southwest Australian Biodiversity Hotspot, the giant karri forests
539 (*Eucalyptus diversicolor*) are distinctly ‘cool spots’ (Wardell-Johnson et al. 2007), although their soils
540 contain little P (Adams 1992) and strongly sorb P (Barrow and Shaw 1975). It would be interesting to
541 explore to what extent ‘Darwinian demons’ account for this anomaly.

542 *Proteaceae* pre-adapted to oligotrophic soils

543 From our ancestral-trait reconstructions, the most common recent ancestor (MCRA) of Proteaceae
544 likely exhibited relatively low leaf [P], thus a high PPUE, which enabled it to diversify in low-P
545 habitats. Indeed, low leaf [P] is not limited to OCBIL genera in Proteaceae, but is pervasive across the
546 family (Figs 3–5), although OCBILs contain a greater proportion and number of genera exhibiting low
547 leaf [P] compared with other regions. Extant species-poor rainforest genera with high leaf [P], and
548 thus low P-use efficiency, all descended from ancestors that exhibit lower leaf [P] within the family.
549 The clustering of genera towards the lower end of the leaf [P] scale with only a few outlying younger
550 rainforest genera exhibiting high leaf [P], suggests a general selection for traits representing
551 adaptation to low-P environments across the family (Fig. 3a). The megadiversity of lineages with
552 efficient P-use strategies in old landscapes could be linked with a greater fraction of these
553 landscapes containing P-impooverished soils, with less area of P-richer soils compared with younger
554 regions (Hopper 2009; Hopper et al. 2016).

555 From the present phylogenies, it appears that several groups within Proteaceae are pre-adapted
556 (exapted *sensu* Gould and Lewontin 1979) to oligotrophic soils, spurring their subsequent
557 diversification in P-impooverished landscapes. This is especially apparent for south-western Australia

558 and the Cape Floristic Region. These include both species-rich genera such as *Hakea* and *Banksia*,
559 included in our study where their MCRA have relatively low leaf [P], with species showing high leaf
560 [P] being derived within the respective genera (Figs 5, 6). However, these results based on ancestral
561 reconstructions should be regarded with caution, due to potential biases from directional evolution
562 (Oakley and Cunningham 2000; Holland et al. 2020), and ancestral traits perhaps having no modern
563 analogue (Finarelli and Flynn 2006; Hinojosa et al. 2016). Also, early-branching lineages of the family-
564 wide phylogeny (*e.g.*, *Bellendena*, *Symphionema*, *Beaupreopsis*) were pruned from the phylogeny
565 due to missing data, and may affect these ancestral reconstructions, though all three genera
566 mentioned are from open habitats and oligotrophic environments (Carpenter et al. 2014; Onstein et
567 al. 2016) and hence should not affect our results significantly. Despite these limitations, our results
568 are supported by other sources of evidence. Paleobotanical records from Australia show the
569 presence and dominance of Proteaceae in open sclerophyllous communities that inhabited
570 oligotrophic wetland soils fringing rainforest through the continent's history since the Cretaceous
571 (Crisp and Cook 2013; Hill 1998; Carpenter 2012). Indeed, this pattern might apply more broadly to
572 the family as a whole, with genera inhabiting environments with non-Mediterranean climates also
573 exhibiting relatively low mature leaf [P] compared with rainforest congeners. Thus, it would not be
574 surprising that ancestors of extant Proteaceae lineages would exhibit low leaf [P] as an adaptation
575 allowing them to occupy these oligotrophic environments in addition to other traits that are
576 associated with these environments, such as sclerophylly (Onstein et al. 2016; Carpenter et al. 2014).
577 Further studies are required to test this hypothesis and to assess whether other P-utilisation traits
578 and adaptations to oligotrophic environments are found in the MRCA of Proteaceae.

579 **Concluding remarks**

580 Plant species diversification in Proteaceae in OCBILs is strongly associated with functional traits
581 related to P nutrition (*i.e.* adaptations to low-P oligotrophic environments). For Proteaceae to be
582 successful in such landscapes in an evolutionary context required functional cluster roots or their
583 equivalent. In addition, a high P-use efficiency, based on a low–moderate mature leaf [P], is linked to
584 large genus size and likely increased diversification within the family. Our study indicates that this
585 trait was already present early in the evolutionary history of Proteaceae, spurring its diversification
586 in oligotrophic environments. Finally, high seed [P] likely also contributed to diversification of
587 species-rich genera across the family, but additional sampling is required to confirm this. It is likely
588 that our findings are also relevant for other plant families or genera inhabiting low-P environments
589 in OCBILs, *e.g.*, eucalypts (Hopper 2021), some of which release carboxylates (Lambers et al. 2021; X.
590 Zhou et al., unpubl.). An interesting family to further consider is Fabaceae, because at least some
591 species in the large *Daviesia* group (Mirbelioids) in this family in Australia produce cluster roots (Nge
592 et al. 2020b) and at least one of these species functions at mature leaf [P] similar to *Banksia* and
593 *Hakea* (Lambers et al. 2019). Likewise, in fynbos, *Aspalathus* and *Cyclopia* species (Fabaceae)
594 produce cluster roots (Maseko and Dakora 2013; Allsop and Stock 1993; Power et al. 2010). In the
595 same family, *Lupinus* species in the Mediterranean and the Americas are non-mycorrhizal, and some
596 produce cluster roots or release carboxylates without these specialised structures (Lambers et al.
597 2013). Along the Jurien Bay chronosequence, P-related leaf traits converge in several families
598 (Guilherme Pereira et al. 2019; Hayes et al. 2014), lending further support for the contention that at
599 least some of the traits we studied can be expected outside the Proteaceae. Further investigations
600 should therefore explore whether our findings are specific to Proteaceae or if some can be expanded

601 to include other lineages in P-impooverished landscapes. This research is critical in understanding
602 how P-impooverished landscapes on different continents formed such exceptional megadiversity.

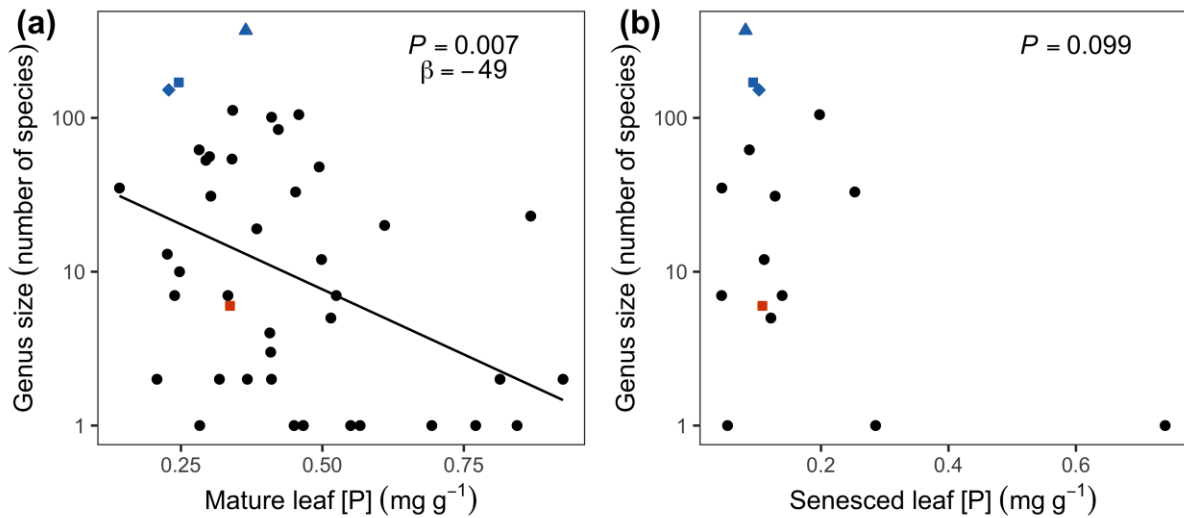
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604 **Acknowledgements**

605 Funding was provided by Australian Research Council Discovery Grants, DP130100005 to HL, and
606 DP200101013 to HL and PMF. HL acknowledges support from the School of Plant Biology and the
607 Deputy Vice Chancellor Research at the University of Western Australia (DVCR-UWA) towards a
608 writing workshop that laid the foundation for this study. FJN was supported through an Australian
609 Government Research Training Program (RTP) Scholarship. Further support from DVCR-WA was used
610 towards field trips to collect some of the data included in this paper. We acknowledge the
611 Department of Biodiversity, Conservation and Attractions (WA; permit no. SW019140 and no.
612 FT61000353) for their permission to examine plants on land under their administration. PMF thanks
613 the Chinese Academy of Sciences President's International Fellowship Initiative (No.2018VBB0008)
614 for funding. PMF and PF thank Zhou Bo for assistance, and the Biogeochemistry Laboratory,
615 Xishuangbanna Tropical Botanical Garden for analysing the leaf P concentration of *Helicia* spp. in
616 southern China. SDH acknowledges funding from an Australian Research Council Discovery
617 Outstanding Researcher Award associated with a Discovery Grant DP140103357, as well as grants
618 from the Great Southern Development Commission and the Jack Family Trust. We would like to
619 thank the following people for their help in sample collection Roberta Dayrell, Qi Shen, Christiana
620 Staudinger, and Jun Wasaki. We also thank Renske Onstein for discussions and help on theory
621 relevant to the simulations of QuasSE analyses.

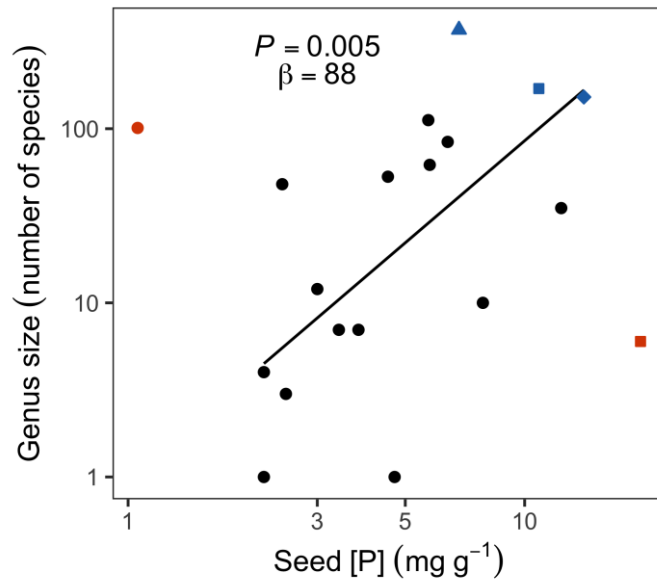
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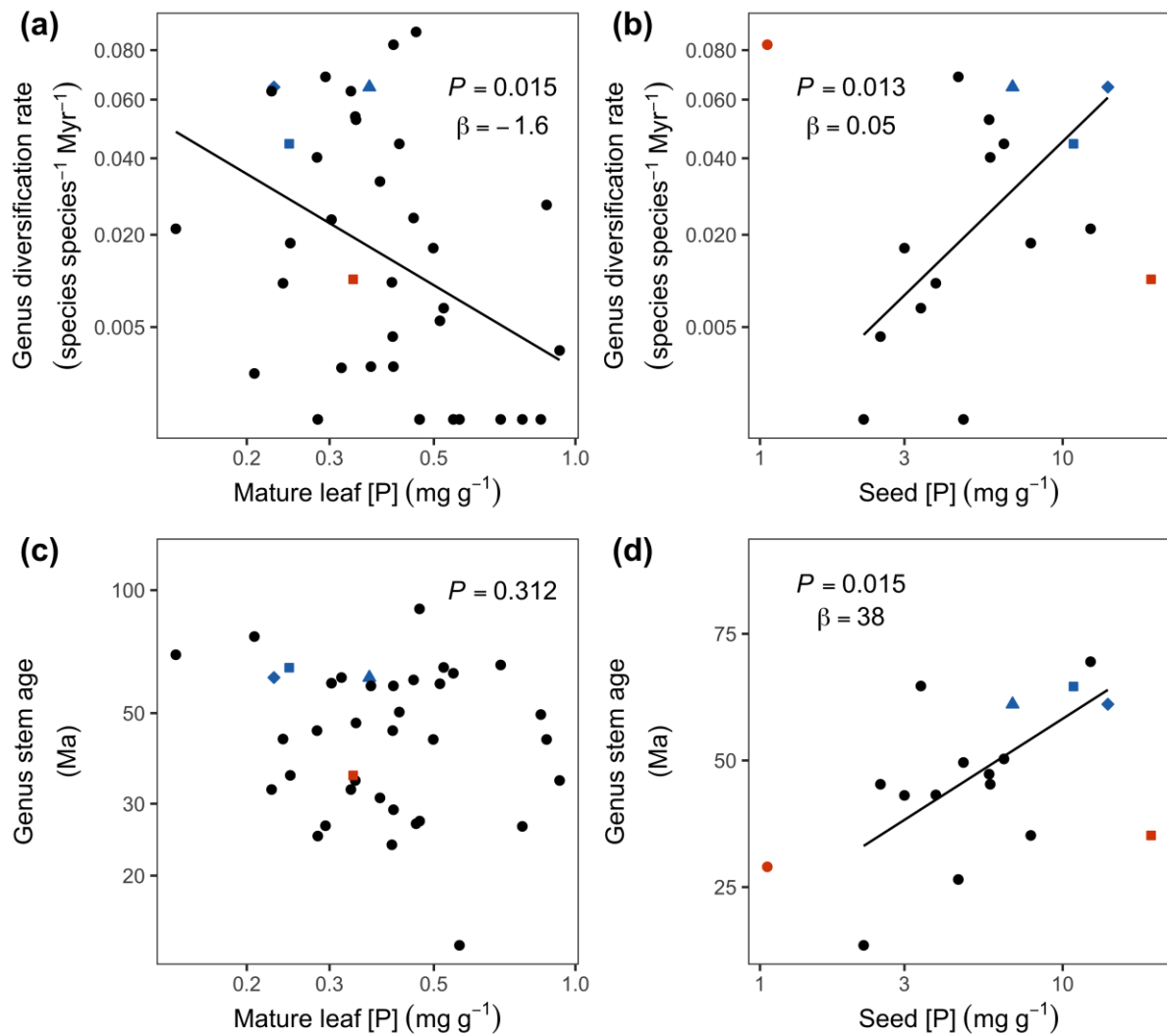
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Fig. 1 Number of species in a genus plotted against (a) mature and (b) senesced leaf phosphorus (P) concentration ([P]). Each point represents the mean leaf [P] of a single genus. Genus size is on a logarithmic scale. The black line represents the line of best fit derived from a generalised least square model, using log-transformed genus size. The genus *Xylomelum* (orange square) was excluded, because they are believed to not produce functional cluster roots (Zhong *et al.*, 2020), a trait we considered pivotal to facilitate speciation. The three largest genera, *Hakea* (blue diamond), *Banksia* (blue square), and *Grevillea* (blue triangle) are indicated by unique symbols. β = regression coefficient.

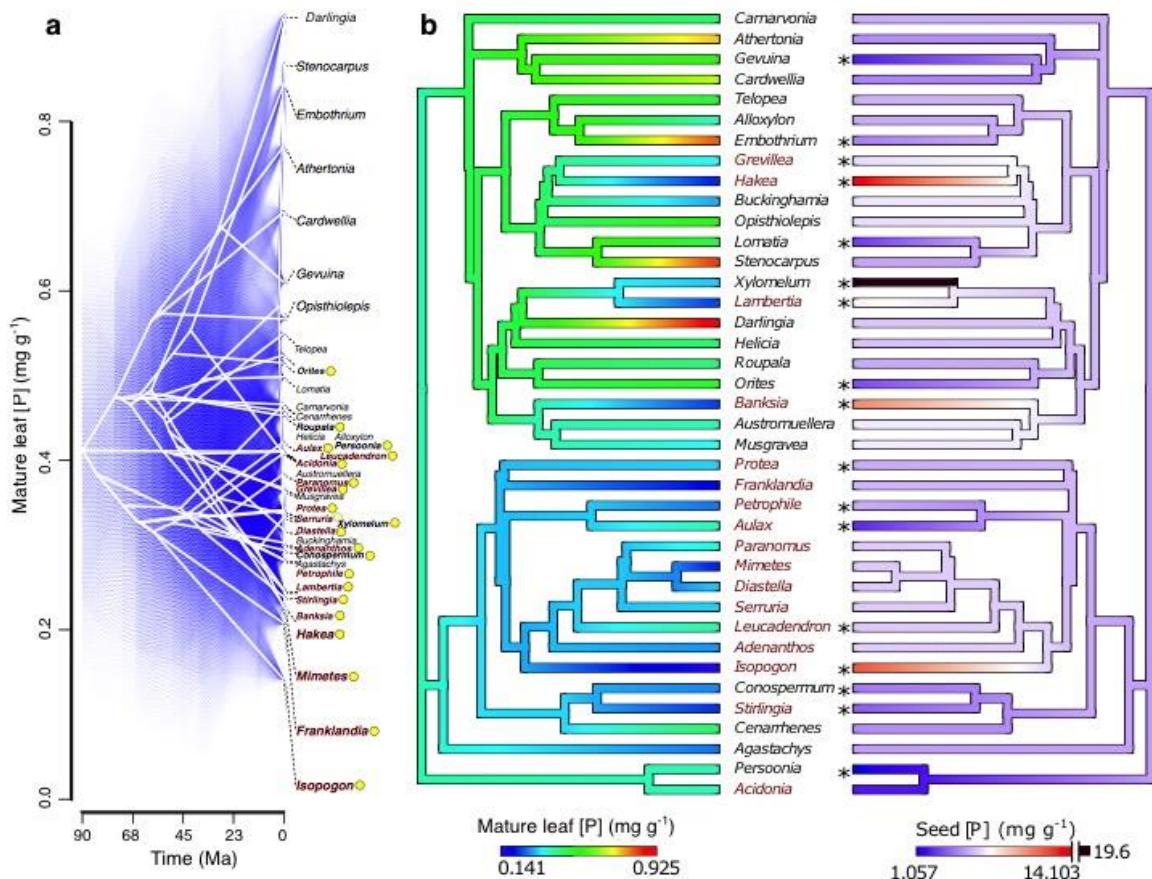


634

635 **Fig. 2** Number of species in a genus plotted against seed phosphorus (P) concentration ([P]). Each
 636 point represents the mean seed [P] of a single genus. Genus size and seed [P] are on a logarithmic
 637 scale. The black line represents the line of best fit derived from a generalised least square model,
 638 using log-transformed data. The genus *Xylomelum* (orange square) was excluded, because they are
 639 believed to not produce functional cluster roots (Zhong *et al.*, 2020); the genus *Persoonia* (orange
 640 circle) was excluded, because it has exceptionally large seeds with very low seed [P] (Leishman and
 641 Westoby 1998), resulting in a reasonable seed P content. The three largest genera, *Hakea* (blue
 642 diamond), *Banksia* (blue square), and *Grevillea* (blue triangle) are indicated by unique symbols. β =
 643 regression coefficient.



644
 645 **Fig. 3** (a,b) Genus diversification rate and (c,d) genus stem age plotted against (a,c) mature leaf and
 646 (b,d) seed phosphorus (P) concentration ([P]). Each point represents the mean of a single genus.
 647 (a,b) Genus diversification rate is on a square-root scale, genus stem age is on a (c) logarithmic or (d)
 648 linear scale, all other traits are on a logarithmic scale. The black line represents the line of best fit
 649 derived from a generalised least square model, using transformed data where shown. The genera
 650 *Xylomelum* (orange square) and *Persoonia* (orange circle) were excluded where shown in orange
 651 (see Methods). The genera *Banksia* (blue square), *Grevillea* (blue triangle), and *Hakea* (blue
 652 diamond) are indicated by unique symbols. β = regression coefficient.



654

655 **Fig. 4.** Mature leaf phosphorus (P) concentration ([P]) and seed [P] traits in Proteaceae. (a) traitgram
 656 projecting the Proteaceae tree into morphospace defined by mature leaf [P] and time (Ma). Blue
 657 shading indicates the 95% confidence interval of ancestral states. (b) Maximum likelihood ancestral
 658 reconstructions of mature leaf [P] and seed [P] performed using the contMap function in phytools
 659 (Revell 2012). Branch lengths are proportional to time based on divergence-dating in Onstein et al.
 660 (2016). Genera highlighted in red occur in low-P environments (hotspots/OCBILs). Yellow circles and
 661 genera in **bold** occur in open vegetation, in the traitgram. * indicate genera with available seed [P]
 662 trait, others are inferred through maximum likelihood using the ‘anc.ML’ function in phytools.

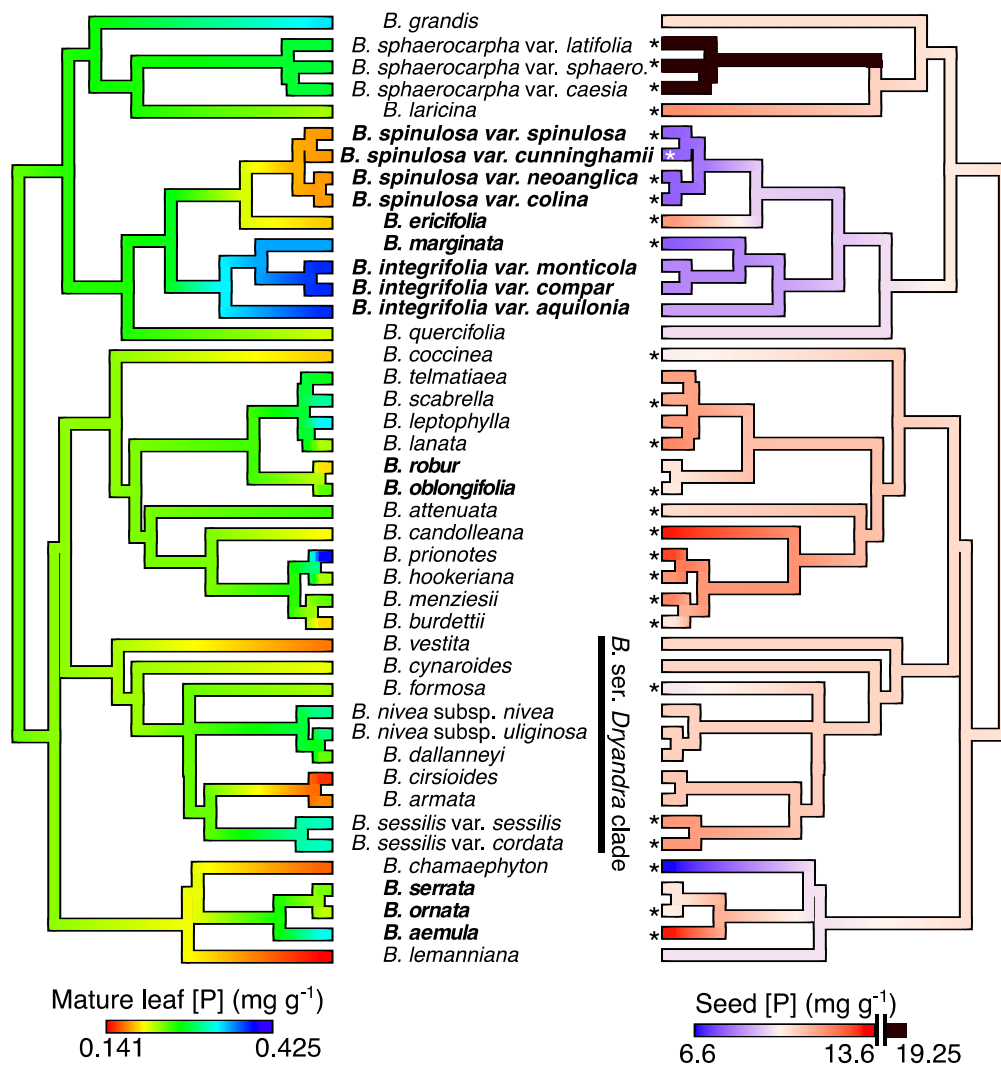


663

664 **Fig. 5.** Maximum likelihood ancestral reconstructions of mature leaf phosphorus concentration ([P])
 665 and seed [P] for *Hakea* performed using the contMap function in phytools (Revell 2012). Branch
 666 lengths are proportional to time based on divergence-dating in Cardillo et al. (2017). Species in bold
 667 occur in non-hotspot regions. * indicates species with available seed [P] trait, others are inferred
 668 through maximum likelihood using the 'anc.ML' function in phytools.

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672 **Fig. 6.** Maximum likelihood ancestral reconstructions of mature leaf phosphorus concentration ([P])
 673 and seed [P] for *Banksia* performed using the contMap function in phytools (Revell, 2012). Branch
 674 lengths are proportional to time based on divergence-dating in Cardillo & Pratt (2013). Species in
 675 bold occur in non-hotspot regions. * indicates species with available seed [P] trait, others are
 676 inferred through maximum likelihood using the 'anc.ML' function in phytools.

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