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8 **Pollen and plant diversity relationships in a Mediterranean montane area**

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20

21 **Abstract**

22

23 Understanding the significance of pollen diversity is key to reconstructing plant diversity over long
24 timescales. Here we present quantitative pollen–plant diversity comparisons for a mountainous area
25 of the Western Mediterranean region. Samples were collected between 430–1865 m elevation and
26 pollen–plant diversity assessed through richness and turnover (beta-diversity) metrics. We found
27 statistically significant relationships between pollen diversity metrics and the diversity of pollen-
28 equivalent plant taxa in the surrounding vegetation. The strongest richness relationships emerged
29 from the exclusion of trees and with rarefaction applied to both plant and pollen datasets. Three
30 different metrics for turnover produced similar results, but emphasise different components of beta
31 diversity (replacement vs richness differences). These results pave the way for reconstructing
32 biodiversity trends from pollen sequences, with a number of caveats. Fossil pollen is a potentially a
33 rich source of information on past biodiversity in the Mediterranean region.

34

35 **Keywords**

36 plant diversity, Mediterranean, palynological richness, turnover, vegetation

37

38 **Declarations**

39 *Funding:* This work was funded by the Laboratory for Palaeobotany and Palynology, State University
40 of Utrecht, Utrecht, The Netherlands.

41 *Conflicts of interest/Competing interests:* not applicable

42 *Availability of data and material:* Data are available through the Neotoma Palaeoecology Database
43 (www.neotomadb.org)

44 *Code availability:* R code is provided in Electronic Supplementary Material

45 *Authors' contributions:* JFNvL and WOK collected the field data; JFNvL analysed the pollen samples;
46 WOK and SEC analysed the data; SEC drafted the manuscript with all co-authors (JFNvL, WOK, RBA,
47 MAA, MM).

48

49

50 **Introduction**

51

52 Biodiversity is key to the stability, functioning and productivity of ecosystems (Cardinale et al. 2012).

53 On a global scale, biodiversity is unevenly distributed, with the greatest diversity in equatorial

54 regions and the least at high latitudes (Kreft & Jetz 2007; Kraft et al. 2011; Jenkins et al. 2013). This

55 global biodiversity gradient is linked to resource availability, competition and the history of past

56 climatic changes (Mittelbach et al. 2007; Rabosky and Hurlbert 2015; Grace et al. 2016). Climate

57 warming in the coming decades is expected to force species to migrate poleward, altering the

58 diversity gradient (Parmesan and Yohe 2003). However, the last major climatic warming 12–10

59 thousand years ago did not alter the biodiversity gradient through poleward migration, at least in

60 Europe (Giesecke et al. 2019). Instead, it seems that past human choices have had a major bearing

61 on today's biodiversity patterns (Ellis et al. 2012; Giesecke et al. 2019; Di Marco et al. 2019). In

62 floristically and culturally rich regions, such as the Mediterranean, mitigation of biodiversity loss can

63 only be achieved when we understand the drivers and long-term trajectories of biodiversity change

64 (Willis et al. 2010).

65

66 Fossil pollen is the most abundant and reliable indicator of past vegetation communities, land cover

67 and floristic diversity (Birks et al. 2016a). At hemispheric and continental scales, pollen richness

68 tracks floristic richness gradients closely (Flenley 2005; Giesecke et al. 2019; Reitalu et al. 2019).

69 Pollen and plant richness relationships are also evident at smaller spatial scales (Felde et al. 2016),

70 though some studies have failed to find a relationship (e.g. Gosling et al. 2018). Pollen data have a

71 number of inherent biases that complicate their comparison to plant diversity, particularly issues of

72 taxonomic precision and pollen representation (Odgaard 1999; Birks et al. 2016a). Adjustments,

73 both in terms of taxonomy and study design, are required to allow fair comparisons between pollen

74 and plant diversity. Plant diversity knowledge itself is biased by sampling approaches that overlook

75 the influence of sampling effort on richness estimation, necessitating adjustments such as

76 rarefaction (Gotelli and Colwell 2001; Meyer et al. 2016).

77

78 This study concerns the Mediterranean region, the most biodiverse region of Europe and one of the
79 world's richest floristic hotspots. Previous studies have analysed the relationships between pollen,
80 vegetation and environmental gradients in several parts of the Mediterranean basin (e.g. Davies and
81 Fall 2001; Ejarque et al. 2011; see Davis et al. 2013 for others). Quantitative relationships between
82 plant diversity and pollen diversity are yet to be explored, despite several palaeoecological studies of
83 past diversity in the Mediterranean region (Colombaroli and Tinner 2013, Connor et al. 2019;
84 Lestienne et al. 2020). Pollen–plant diversity relationships open the way for past diversity changes
85 to be translated into ecologically meaningful trends (Nieto-Lugilde et al. 2015).

86

87 The aim of this paper is to analyse pollen–plant diversity relationships to enable past biodiversity
88 trends to be reconstructed. We present pollen and floristic data from the Western Mediterranean
89 to test the following null hypotheses: 1) there is no relationship between plant richness and pollen
90 richness in the study area and 2) there is no relationship between plant community turnover and
91 pollen turnover in the study area. We then apply the relationships to assist interpretation of past
92 diversity changes at a site in the same area.

93

94

95 **Materials and methods**

96

97 The study area is continental Portugal's highest mountain range, the Serra da Estrela (maximum
98 elevation 1993 m). The range is granitic with relatively poor soils and vegetation arranged
99 altitudinally, from lowland forests to open vegetation above 1600 m (Fig. 1; Silva and Teles 1999).

100 The Serra da Estrela, like much of the Mediterranean region, is an ancient cultural landscape with
101 arable fields, tree plantations and grazed areas.

102

103 Sampling occurred at 42 locations along a 31-km elevational gradient between 430 and 1865 m (Fig.
104 1). Sampling sites ranged from meso-Mediterranean cultural landscapes with nearby *Pinus*
105 plantations, up into supra-Mediterranean heathlands of *Juniperus communis*, *Calluna vulgaris*, *Erica*
106 and *Cytisus* spp., and finally high-elevation grasslands and sparse herbfields of the oro-
107 Mediterranean zone (Fig. 1). Samples consisted of surface moss cushions which trap and aggregate
108 pollen from the surrounding vegetation (Pardoe et al. 2010).

109 Species abundance of all vascular plants was surveyed in 1 x 1 m quadrats at the time of sampling
110 (06/1987). Species occurring outside the quadrat and up to 200 m away were recorded using a
111 'nearest individual' method of plotless sampling, which uses measured distance to the nearest
112 population to calculate cover abundance (Bryant et al. 2005). This calculation relies on the empirical
113 and theoretical demonstration that the distance from the sampling point to the nearest individual is
114 equal to 50% of the square root of its area (Cottam and Curtis, 1956). $Area = 1 / (distance / 0.5)^2$.
115 Plant taxonomy follows the *Nova Flora de Portugal* and *Flora Europaea* (Franco, 1971, 1984; Tutin et
116 al. 1964 ff.), updated using Flora-On (flora-on.pt).

117

118 Pollen extraction involved soaking in 10% KOH, sieving to remove coarse debris, and acetolysis
119 (Faegri et al. 1989). Pollen grains and pteridophyte spores were counted to a minimum of 600 per
120 sample. The second author (JFNvL) identified these at the lowest taxonomic level possible using the
121 *Northwest European Pollen Flora* (references in van der Knaap and van Leeuwen 1995) and
122 reference material collected from plants in the Serra da Estrela.

123

124 Since pollen identifications are typically less taxonomically resolved than botanical ones, fair
125 comparisons can only be achieved by transforming plant taxa to pollen-taxon equivalents (Felde et

126 al. 2016). Taxonomic richness of pollen and plants (number of species and pollen-equivalents) was
127 estimated by rarefaction (Birks and Line 1992). Rarefaction removes bias from different sampling
128 sizes by producing richness estimates relative to the smallest of the samples. Here we apply
129 rarefaction to the vegetation survey data as well as the pollen assemblages to correct for reduced
130 sampling effort (*sensu* Gotelli and Cowell 2001), and thus reduced plant richness, in quadrats with
131 sparse vegetation. Rarefaction was implemented through repeated random resampling (1000
132 replicates; Felde et al. 2016) to the minimum pollen count (608 grains) and minimum plant cover
133 value (0.3 m² vegetated area). Species beyond the quadrat were rounded up and added to the
134 quadrat data prior to rarefaction.

135 We also tested whether removing entire structural groups (Felde et al., 2016; Reitalu et al. 2019;
136 Blaus et al. 2020) improved pollen–plant richness relationships. We did this to understand which
137 structural groups and individual taxa introduce the most bias into those relationships. Data were
138 rarefied to a new minimum count each time taxa were excluded. Relationships between plant
139 richness, elevation, location and pollen richness were sought using linear models. We checked the
140 fidelity of the linear models using split-sampling (Birks et al. 2010). This involved randomly splitting
141 the dataset in half 10000 times, each time using one half to develop linear models and applying
142 them the other half to assess model fit.

143 Community-level turnover (beta-diversity) assessments employed three metrics appropriate to
144 palaeoecological assemblages: Hellinger standardisation with Euclidean distance (Hellinger-
145 Euclidean; Plumpton et al. 2019), square-chord distance (SCD; Overpeck et al. 1985; Seddon et al.
146 2015) and Detrended Correspondence Analysis on square-root transformed abundances (DCA;
147 Jacobson and Grimm 1986; Giesecke et al. 2019). Spatial turnover between samples from different
148 vegetation types can be used to define ‘critical values’ for temporal turnover in fossil records
149 (Overpeck et al. 1985). Critical values indicate whether a change in pollen assemblages is likely to
150 represent variation within similar vegetation or a transition from one type of vegetation to another

151 (Simpson 2012). Rates of change are produced when temporal turnover is expressed relative to
152 standardised time steps (Grimm and Jacobson 1992; Finsinger et al. 2017).

153 We assessed turnover metrics by using cluster analysis (Ward's method) to group the vegetation
154 quadrats into 'similar' and 'dissimilar' groups as measured using the Hellinger-Euclidean, SCD and
155 DCA metrics. These groups contained approximately half the samples in the dataset. The same
156 groupings were applied to the pollen metrics and inter-sample dissimilarities within these groups
157 presented as boxplots (see explanatory figure in Supplementary Material S1). The approach is similar
158 to analogue matching (Simpson 2012), although in our case the vegetation groups are not
159 predefined. Mantel tests were used to assess whether the dissimilarities between pollen samples
160 relate to dissimilarities between vegetation assemblages (i.e. turnover). Partial Mantel tests were
161 performed with spatial information as a third variable, in an attempt to control for elevation
162 differences and geographic differences (Cartesian distance) between sites. Beta diversity was
163 decomposed into replacement and richness difference components using the Jaccard coefficient
164 (Legendre 2014; Abraham et al. 2020) to understand how the three dissimilarity coefficients
165 represent different aspects of turnover. Input data were randomly resampled to the minimum sum.

166

167 To interpret temporal richness on the basis of spatial richness relationships, a palynological richness
168 trend was produced for an existing pollen record from the study area. Charco da Candieira
169 (40°20'37"N, 7°34'41"W, 1400 m elevation, see #20 in Fig. 1) is an infilled glacial lake above the
170 anthropogenic treeline, surrounded by shrub and herb vegetation communities with significant
171 conservation and biodiversity values (Jansen 2011). The site yielded a high-resolution
172 palaeoecological record spanning the last 14,300 years (van der Knaap and van Leeuwen 1995,
173 1997). The record comprises 301 samples and its Bayesian chronological model is based on 30 ¹⁴C
174 dates (Connor et al. 2012).

175 For community-level turnover reconstruction, samples from the Charco da Candieira record were
176 binned into time steps of 100, 200, 500, 1000 and 2000 years. This pollen record has an average
177 temporal resolution of 48 years, but we opted for longer time bins to examine centennial- and
178 millennial-scale turnover and to reduce temporal autocorrelation. Inter-sample dissimilarities were
179 obtained using a randomisation procedure to avoid statistical artefacts introduced by uneven
180 temporal sampling (Birks 2012). Once the record was binned, one sample was selected at random
181 from each bin. Dissimilarity was calculated between samples from adjacent bins, from oldest to
182 youngest. This process was repeated 100 times with different random samples drawn from each bin
183 to obtain a mean dissimilarity (Connor et al. 2019). The resulting trend was compared to the
184 ‘similar’ and ‘dissimilar’ vegetation distributions to qualitatively assess turnover through time.

185 Numerical analyses were all performed with the R packages ‘vegan’, ‘adespatial’ and ‘analogue’
186 (Dray et al. 2020; Oksanen et al. 2019; Simpson and Oksanen 2020; R Core Team 2020).

187

188

189 **Results**

190

191 A summary of the pollen and vegetation data is given in Fig. 2. The most frequent plant species in
192 the sampling quadrats were two grasses (*Micropyrum tenella*, *Agrostis delicatula*) and the herb
193 *Rumex angiocarpus*, while the grass *Nardus stricta* was the most abundant plant species. The most
194 abundant pollen taxa were grasses (Poaceae) and the tree *Pinus pinaster*, which were present in all
195 samples.

196

197 Pollen–plant richness relationships are compared in Fig. 3 using four different metrics for plant
198 richness and two different vegetation datasets. Where plant richness in the quadrats was based on
199 data without corrections for pollen taxonomy or sampling effort, the pollen–plant richness

200 relationship was weak (r^2 : 0.04). Rarefaction led to a slight improvement (r^2 : 0.07). Plant taxa
201 converted to pollen-equivalents showed stronger relationships (r^2 : 0.10). Rarefaction of pollen-
202 equivalents yielded the strongest correlation (r^2 : 0.18). Richness relationships based on pollen-
203 equivalents in the quadrats were significant at $p < 0.05$ (Fig. 3). Inclusion of plotless sampling data
204 yielded weaker and non-significant relationships, except for pollen equivalents in the first metre and
205 rarefied pollen equivalents in the first 20 metres of the central quadrat (Fig. 4). Rarefied pollen-
206 equivalents from the quadrat-only data also produced the strongest results in split-sampling
207 experiments (Supplementary Material S4). Neither site elevation nor site location
208 (latitude/longitude) were significant predictors or co-predictors of pollen richness estimates (r^2 : 0.01
209 and 0.00 respectively).

210

211 Fig. 5 shows how exclusion of structural groups affects pollen–plant relationships in the study area.
212 Exclusion was most effective when tree taxa were excluded (r^2 : 0.49), followed by exclusion of herbs
213 (r^2 : 0.46), shrubs (r^2 : 0.38), and least effective for exclusion of trees and shrubs (r^2 : 0.35; Fig. 5).

214

215 Pollen and plant turnover relationships were statistically significant for all three dissimilarity
216 measures considering turnover in shrub and herb communities (Table 1).. Partial Mantel tests,
217 controlling for site coordinates and elevation, yielded weaker significant relationships and, for some
218 DCA combinations, non-significant relationships (Table 1). Beta diversity decomposition is compared
219 to the three dissimilarity measures in Table 2. Total beta diversity was most strongly reflected in
220 these measures. Plant replacement was also reflected, but richness differences were not (Table 2).

221 Pollen turnover between samples with ‘similar’ vegetation was lower than between ‘dissimilar’
222 vegetation (Fig. 6; see Supplementary Material S1-3 for cluster dendrograms, further explanation of
223 the grouping method and results based on a fewer similar samples). Sites with ‘similar’ vegetation
224 also tended to be closer to each other in terms of elevation and geographic distance (Fig. 6). Mantel

225 correlograms exhibit significant positive autocorrelation to a distance of approx. 4 km in the
226 vegetation data and 7 km in the pollen data (Supplementary Material S5). Significant autocorrelation
227 was evident for elevation differences up to 300 m for Hellinger-Euclidean and SCD metrics, and 450
228 m for DCA. Autocorrelation was greater for DCA than the other metrics (Supplementary Material S5).

229
230 The Charco da Candieira richness trend for shrub and herb communities is presented in Fig. 7. The
231 results indicate a steep rise in shrub and herb richness at the terminal Pleistocene, followed by an
232 Early Holocene decline. Richness estimates peaked in the mid-Holocene, followed by low richness
233 during the last 3000 years, particularly in the last millennium. Pollen turnover trends for Charco da
234 Candieira are shown in Fig. 8a in relation to the pollen boxplots from Fig. 6. The amount of
235 compositional change and rate of change are dependent on the time steps between observations.
236 The 100-year bins exhibit the highest turnover in the early-mid Holocene, whereas longer intervals
237 place the highest turnover earlier, around the Pleistocene–Holocene transition (ca 11700 cal. yr BP;
238 Fig. 8). DCA produced different trends to the other metrics, exhibiting a stronger late-Holocene
239 increase. Beta diversity decomposition shows Hellinger-Euclidean and SCD turnover to be similar to
240 replacement, whereas the DCA trend mimics richness difference (Fig. 9).

241

242

243 **Discussion**

244

245 *Richness relationships*

246 Pollen and plant richness in the study area are positively related. The strength of this relationship
247 depends greatly on the metrics used to quantify richness in the vegetation. Only those metrics that
248 compared pollen richness to pollen-equivalent plant richness in the quadrats allowed us to reject the
249 null hypothesis in relation to richness. In other words, significant relationships were found between
250 pollen and pollen-equivalent plant richness, but not between pollen and plant taxa richness. There

251 are at least two key biases that distort the pollen–plant relationship presented here: pollen
252 representation and sampling bias, which we discuss below (see Giesecke et al. 2014 for synthesis).

253

254 The most palynologically diverse assemblages in Europe are found in open vegetation dominated by
255 herbaceous plants (Meltsov et al. 2013; Matthias et al. 2015; Giesecke et al. 2019). In our data,
256 shrubs and herbaceous plants in the direct surroundings of the moss sample control palynological
257 richness to a great degree, indicated by exclusion experiments (Fig. 4). Many of these plants are
258 insect-pollinated and have poorly dispersed pollen. In contrast, tree species in this region are often
259 wind-pollinating and have good pollen dispersal (e.g. *Pinus*, *Quercus*). This means that many trees
260 contribute to every pollen sample’s richness, regardless of whether they are present in the
261 surrounding vegetation. Pollen–plant relationships improved substantially with the exclusion of
262 trees (Fig. 4) because they constitute a source of palynological noise while being virtually absent
263 from the vegetation data. In northern Europe, correction for differential pollen productivity has
264 improved pollen–plant richness correlations (Felde et al. 2016; Reitalu et al. 2019; Blaus et al. 2020).
265 As yet, the Mediterranean region lacks taxon-specific corrections such as Andersen’s (1970)
266 correction factors or pollen productivity estimates (PPEs; e.g. Broström et al. 2008).

267

268 Our data show that pollen–plant richness relationships can be teased out by addressing vegetation
269 sampling biases that have not been considered in previous studies (e.g. Felde et al. 2016; Gosling et
270 al. 2018, and articles cited therein). The strongest pollen–plant relationships were produced when
271 rarefied pollen was compared to rarefied vegetation data converted to pollen-equivalents (Fig. 3).
272 Rarefaction standardises vegetation data for sampling effort and is therefore recommended for
273 meaningful richness comparisons (Gotelli and Colwell 2001). In our case, rarefaction makes sense
274 because the sampling effort at sites dominated by bare rock is very different to sites with multi-
275 layered vegetation (i.e. a herb layer growing beneath a shrub layer). While it is possible to remove

276 sites with sparse vegetation from analysis (e.g. Felde et al. 2016), we suggest that there are good
277 theoretical and practical reasons for favouring rarefaction. It must be noted, however, that not all
278 vegetation data will be amenable to rarefaction, particularly presence/absence and semi-
279 quantitative abundance data typically collected for pollen–plant richness comparisons.

280

281 An unexpected result was the poor pollen–plant richness relationships that emerged from combined
282 vegetation data (plotless and quadrat samples) compared to the quadrat data alone (Fig. 3). One
283 might imagine that a greater sampling radius would improve richness relationships, at least until the
284 relevant source-area of pollen was reached. However, this source area for richness appears to be
285 quite small, as indicated by recent studies that likewise found pollen–plant richness relationships
286 became weaker with increasing vegetation survey distance (Blaus et al. 2020; Abraham et al. 2020).
287 In species-rich grasslands, this distance can be as low as 1.5 m and tends increase in forested
288 landscapes (Abraham et al. 2020). Plotless sampling also increases the risk of overlooking rare taxa.
289 A key limitation of the richness index is that it considers the number of species irrespective of their
290 spatial location in relation to the pollen sampling point. The index does not recognise that plants
291 situated further away from the pollen sampling point are less likely to contribute to the pollen
292 sample’s richness. Just as pollen abundance decreases with distance from the source plant
293 (Theuerkauf et al. 2012), the likelihood of encountering a pollen taxon decreases with distance from
294 the source plant. This effect is slight for wind-pollinating plants such as *Rumex* and *Plantago* spp.,
295 but insect-pollinated species with poor pollen dispersal are likely to go unrecorded unless found in
296 close proximity to the pollen sampling location (Shaw and Whyte 2020). As far as pollen
297 representation is concerned, there is little equivalence between a plant growing next to the pollen
298 sampling point and a plant growing 200 m away, yet for richness metrics the two plants are treated
299 the same.

300

301 Sampling design therefore becomes critical for detecting richness differences. Had we extended our
302 vegetation survey to the surrounding 50 km of each pollen sampling point, plant species richness
303 would be the same for all samples, while the pollen richness would still differ. We suspect that
304 these problems of scale may explain some of the variation in pollen–plant relationships reported in
305 the literature. For instance, in Norway, richness relationships between pollen assemblages from
306 medium-sized lakes (median 28 ha) and plants in the surrounding 500m were not significant unless
307 samples from sparse vegetation were excluded or taxon-specific corrections applied (Felde et al.
308 2016). In the tropics, comparisons of pollen trap assemblages with the surrounding hectare of
309 vegetation found no relationship (Gosling et al. 2018). Strong relationships emerged in a comparison
310 of pollen from lakes in northern Europe (median area 7 ha) and plant richness in the surrounding
311 7500 km² (Reitalu et al. 2019). In this case the richness gradient extended over a latitudinal range of
312 30° and a longitudinal range of 60° and hence factors apart from plant richness, such as climate and
313 elevation, were co-predictors of pollen richness (Reitalu et al. 2019).

314 Measured geographic factors had negligible influence on richness estimates in our dataset, but we
315 were unable to test the full range of climatic and landscape factors that influence pollen and plant
316 richness (Birks et al. 2016; Reitalu et al. 2019) due to a lack of suitably spatially-resolved data
317 relevant to the time of sampling. Even so, the results here show that plant richness relationships are
318 reflected in pollen assemblages, paving the way for pollen-based reconstructions (with appropriate
319 caveats). Further improvements to pollen–plant calibration could be achieved through spatially-
320 explicit indices of association (Shaw and Whyte 2020). These taxon-specific indices could be
321 employed to overcome the significant shortcomings of comparing pollen assemblages to vegetation
322 data from a single quadrat or region.

323

324

325

326 *Turnover relationships*

327 Community-level turnover in pollen assemblages is strongly related to turnover in pollen-equivalent
328 plants and plant species in the study area (Table 1). These relationships allow us to reject the null
329 hypothesis of no relationship between plant community turnover and pollen turnover in the study
330 area. That said, spatial autocorrelation appears to be a significant factor in the turnover results,
331 given that both site coordinates and elevation also correlate with pollen turnover metrics. Partial
332 Mantel tests were performed to gauge how much variance in pollen turnover is explained by
333 vegetation turnover versus geographic factors (Table 1). The Hellinger-Euclidean and SCD results
334 were similar, given similarities in these metrics when applied to proportional data (Legendre and
335 Gallagher 2001). These results indicate that the null hypothesis (i.e. of no relationship between plant
336 community turnover and pollen turnover in the study area) can still be confidently rejected. The
337 relationship between DCA-derived turnover in pollen and vegetation data was insignificant once
338 elevation was taken into account. Hellinger-Euclidean distance consistently produced stronger
339 relationships than the other two metrics when assessed using Mantel tests (Table 1). However, some
340 authors suggest that Mantel tests are less than optimal for dealing with spatial data and have
341 proposed alternative analyses that could prove more powerful and appropriate (Legendre et al.
342 2015).

343

344 Pollen turnover metrics are capable of differentiating similar from dissimilar vegetation groups (Fig.
345 6), in agreement with previous studies (e.g. Overpeck et al. 1985; Nieto-Lugilde et al. 2015; Abraham
346 et al. 2020). In geographic terms, pollen turnover between dissimilar samples is equivalent to
347 moving 250–1000 m in elevation or 5–15 km in distance, regardless of metric (Fig. 6). Pollen
348 turnover values < 0.6 Hellinger-Euclidean distance, < 30 square-chord distance and < 0.3 DCA
349 distance could be expected within a single vegetation type. Greater values indicate turnover from
350 one vegetation type to another (Fig. 6).

351 Differentiations such as these have been used to define ‘critical values’, i.e. thresholds for when
352 pollen turnover represents vegetation change (Overpeck et al. 1985; Seddon et al. 2015; Connor et
353 al. 2019). Hellinger-Euclidean distances in our dataset are relatively low compared to a previous
354 study where much greater distances and a larger range of vegetation types were involved (Plumpton
355 et al. 2019). Square-chord distances for ‘similar’ vegetation around SCD 30 (Fig. 6) are slightly higher
356 than previously adopted thresholds (e.g. SCD 15, Overpeck et al. 1985), perhaps due to the inclusion
357 of all pollen types in the turnover metric and the higher diversity of Mediterranean pollen
358 assemblages compared to temperate forests. Our thresholds are derived from the most similar 50%
359 of the samples in the dataset and from different branches of the cluster dendrogram. Selecting only
360 the most similar 25% did not change the thresholds appreciably (Supplementary Material S3).
361 Various numerical techniques exist for determining dissimilarity thresholds for data with *a priori*
362 groupings (Simpson 2012), which is not the case for our data.

363

364 Despite the attractiveness of thresholds, their application is complicated by the substantial
365 differences in pollen source-areas of moss samples and lake sediments. Lake basins gather pollen
366 from many kilometres around the site, while moss samples tend to gather pollen from a much
367 smaller area (Sugita 1994). In patchy vegetation, turnover between small pollen-collecting basins is
368 therefore expected to be much higher than between larger basins (see Fig. 5 in Sugita 1994).

369

370 The precise limits of pollen source areas are difficult to define, as factors like taxon-specific pollen
371 productivity, atmospheric conditions and basin characteristics play a role (Jacobson & Bradshaw
372 1981; Theuerkauf et al. 2013; Shaw and Whyte 2020). Mechanistic models such as REVEALS (Sugita
373 2007), combined with detailed information on the spatial distribution of plant species (Shaw and
374 Whyte 2020), could be used to develop spatially-explicit corrections for pollen representation
375 differences between moss samples and lake basins of different sizes. Applied to turnover

376 reconstructions, such corrections could help identify sites where recent turnover has exceeded past
377 limits, signalling ecosystems perhaps at risk of critical transitions.

378

379

380 *Interpreting diversity trends*

381

382 Strong linear relationships between pollen and pollen-equivalent plant richness in the Serra da
383 Estrela's shrub and herb communities (Fig. 4) offer the possibility of assessing long-term richness
384 dynamics in these communities via fossil pollen records. The Charco da Candieira palynological
385 richness trend (Fig. 7) clearly reflects the vegetation history at Charco da Candieira. Landscapes
386 dominated by pines and grasses 14,000 years ago gave way to richer vegetation as habitats
387 diversified at the Pleistocene–Holocene transition. Oak forest expansion led to slight
388 impoverishment during the Early Holocene, but mid-Holocene heathland expansion equated to
389 increasing richness. Proliferation of fires, ericaceous shrubs and grasses through the last 3000 years
390 led to richness decline (van der Knaap and van Leeuwen 1995, 1997; Connor et al. 2019). A
391 contemporaneous richness decline is observed at many sites across the Mediterranean region
392 (Giesecke et al. 2019).

393 The strength of richness relationships in this work and other recent papers (e.g. Felde et al. 2016,
394 Reitalu et al. 2019, Blaus et al. 2020) suggests that quantitative reconstruction of past richness
395 should be achievable with appropriate modelling of pollen source-area. Summed pollen samples and
396 vegetation data could be used to produce taxa accumulation curves (Giesecke et al. 2014), which
397 could form the basis of modelling that seeks to extend richness relationships from the sample scale
398 to a whole lake surface (following Sugita 1994, 2007).

399

400 Turnover cannot easily be translated from change through space to change through time. Apart from
401 geographic distance, competition, environmental dissimilarity and other factors influence turnover
402 through space (Qian and Ricklefs 2012). These factors also come into play in temporal turnover, but
403 usually cannot be directly measured, only inferred from proxy data. Spatial and temporal
404 autocorrelation are also inherent properties of our pollen and vegetation datasets (see
405 Supplementary Material S5). Pollen-based turnover rates through time are influenced by pollen
406 source-area issues and the choice of time bins, as is apparent in Fig. 8.

407 Turnover metrics also exhibit different properties that may make them more or less appropriate for
408 palaeoecological data (Overpeck et al. 1985). DCA was the least effective metric for spatial turnover
409 in the study area. This is shown by weak relationships between pollen and plants/equivalents once
410 the influence of elevation was removed (Table 1). The DCA trends in Fig. 8 are probably less reliably
411 indicative of turnover than those produced using Hellinger-Euclidean and SCD metrics. This may
412 relate to the dominance of replacement (average 0.80 BD) over richness difference (average 0.46
413 BD) in contributing to the total beta diversity of our vegetation data, which DCA was less successful
414 in capturing (Table 2). This does not indicate any deficiency in DCA as a turnover metric.

415 Over millennial timescales, the reorganisation of ecosystems at the Pleistocene–Holocene transition
416 is clearly evident at Charco da Candieira (Fig. 8). Compositional change was high enough during this
417 period to represent a shift from one type of vegetation to another (Fig. 8a), consistent with the
418 pollen-inferred transition from glacial-era steppic vegetation to early-Holocene woodland vegetation
419 (van der Knaap and van Leeuwen 1995, 1997). Subsequent vegetation change was less dramatic,
420 attaining greater stability through the mid-late Holocene and increasing again over the last 3000
421 years.

422 A different pattern is seen at the centennial-scale, with turnover peaking through the early-mid
423 Holocene as replacement came to dominate (Fig. 9). This was followed by a period of stability as
424 heathland-forest mosaics established themselves through the mid-late Holocene (van der Knaap and

425 van Leeuwen 1995). Stability was punctuated by phases of higher turnover, often lasting centuries to
426 millennia. Differences in turnover patterns on millennial and centennial timescales could affect
427 other measures of vegetation change through time, such as the emergence of novelty (Finsinger et
428 al. 2017).

429

430 The Late Holocene brought a decline in species richness (Fig. 7) that is also reflected in increasing
431 compositional change, mostly from richness differences (Figs 8, 9). Fire has always been a key
432 disturbance in the Serra da Estrela, but in the last 3500 years the amount of burning has increased
433 (Connor et al. 2012). The last century appears to have been a time of particularly rapid turnover
434 compared to the Holocene as a whole, attaining centennial rates-of-change that have only been
435 exceeded twice in the last 7000 years (Fig. 8, left and middle panels). These trends are unexpected
436 in nutrient-poor landscapes, in which relative stability in richness and turnover is the norm (Birks et
437 al. 2016b). Impoverishment and instability in the Serra da Estrela are almost certainly linked to over-
438 exploitation, burning and climate change in recent centuries (Jansen et al. 1997; Connor et al. 2012).
439 Whether these recent biodiversity trends can be reversed is a major question for management of
440 the Serra da Estrela Natural Park and for Mediterranean montane ecosystems generally.

441

442

443 **Conclusions**

444

445 Based on coupled modern pollen-vegetation analyses in the western Mediterranean, this work
446 shows that a significant correlations exist between plant richness (pollen-equivalents) and pollen
447 richness, and between plant turnover and pollen turnover. These relationships between pollen and
448 plant diversity metrics indicate that pollen is capable of reconstructing long-term biodiversity

449 changes, but further research is needed to better control for spatio-temporal autocorrelation in
450 calibration and reconstruction datasets. Improved knowledge of biodiversity change in the past is
451 essential for contextualising and responding to current threats to biodiversity in floristically rich
452 regions such as the Mediterranean.

453

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455

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637

638

639 **Figure Captions**

640

641 **Fig. 1** Map of Portugal (left) with the location of the Serra da Estrela. Detailed map (right) of the
642 study area showing the sampling locations along the 31-km transect. Bioclimatic zones are shaded:
643 from meso-Mediterranean (lightest shade) through supra-Mediterranean to oro-Mediterranean
644 (darkest shade). The palaeoecological site of Charco da Candieira is found at location 20.

645

646 **Fig. 2** Composition of a) pollen samples and b) vegetation quadrat surveys at 42 locations in the
647 Serra da Estrela, Portugal, expressed as percentages of major functional groups. *Pinus pinaster* (in
648 black) is present only in the pollen samples. Complete pollen and plant datasets are available from
649 Neotoma (www.neotomadb.org).

650

651 **Fig. 3** Pollen–plant richness relationships in the Serra da Estrela. Left panels show relationships
652 between plant species richness and rarefied pollen richness. Right panels show relationships
653 between pollen-equivalent richness and rarefied pollen richness. The right-hand panel in each pair
654 (green) shows rarefied plant and pollen-equivalent data to the minimum area (0.03 m²). Minimum
655 pollen sum for rarefaction is 608. Each panel compares richness estimates for quadrat-only data,
656 along with r-squared values for each of the least-squares regressions (* $p < 0.05$, ** $p < 0.01$, *** $p <$
657 0.001).

658

659 **Fig. 4** a) Effect of including plotless sampling results from increasing distances on the strength of
660 pollen–plant richness relationships (r^2). Black dots are significant at $p < 0.05$. b) An example of
661 ‘nearest individual’ plotless sampling. Plant A is 200 m from the quadrat and Plant B is 100 m away.
662 Relative to the 1 m² quadrat, the area of Plant A is 0.0006 and Plant B is 0.003 m².

663

664

665 **Fig. 5** The effect of excluding structural groups on richness relationships between pollen and pollen-
666 equivalent plant taxa (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

667

668 **Fig. 6** Pollen turnover as a reflection of vegetation similarity in the Serra da Estrela. The ‘similar’
669 group in each boxplot is a comparison between the half of the sites with the most similar vegetation;
670 the ‘dissimilar’ group is the rest. The lower boxplots show how the ‘similar/dissimilar’ vegetation
671 groups correspond in terms of Cartesian distance and elevation above sea level.

672

673 **Fig. 7** a) Palynological richness through time at a site in the Serra da Estrela (Charco da Candieira
674 record) compared to the pollen record (trees excluded). Dots are sample-based rarefied richness.
675 The line is a lowess smoother (span 0.25) with standard errors shaded.

676

677

678 **Fig. 8** a) Pollen turnover trends through time at a site in the Serra da Estrela (Charco da Candieira
679 pollen record), shown for different temporal binning intervals. Turnover represents change in shrub

680 and herb communities since tree taxa were excluded (see Supplementary Material S6 for trends with
681 tree pollen included). Left panel: Hellinger-Euclidean distance. Middle panel: square-chord distance.
682 Right panel: Detrended Correspondence Analysis. The lines are lowess smoothers (span 1). Similar
683 and dissimilar groups (Fig. 5) shown to the right of top panels for comparison. b) the same results
684 presented as rates-of-change per century. N.B. DCA did not produce significant pollen–plant
685 relationships with elevation partialled out (Table 1), so DCA trends must be interpreted with caution.

686 **Fig. 9** Beta diversity decomposition applied to the Charco da Candieira record, presented as rates-of-
687 change for comparison with Fig. 8b. Left panel: total beta diversity; middle: replacement diversity;
688 right: richness difference.

689

690

691