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9 **Variability and trends of black truffle production in Spain (1970-**
10 **2017): linkages to climate, host growth, and human factors**

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29 **Abstract**

30 Black truffle (*Tuber melanosporum*) is a highly-appreciated fungus that grows below
31 ground during several months, undergoing a series of morphogenetic stages before it is
32 harvested in late autumn or winter. Black truffle production in Spain has been subject to
33 important temporal variation in recent decades. The records of the Spanish Truffle
34 Growers Association from 1970 to 2017 were analysed using additive models to
35 investigate the relative roles of climate, host tree growth and other environmental and
36 human factors on the variability and trend of fruiting body production and phenology.
37 Climatic factors largely explained the variability in annual truffle production, but not
38 the major time trend observed in the studied period. Temperature and precipitation
39 during fruiting body development showed the highest relationship with truffle
40 production. Atmospheric evaporative demand during fruiting induction and temperature
41 during maturation showed a significant relationship to how truffle production was
42 distributed throughout the fruiting season. The relationship between truffle production
43 and host growth was mostly explained by summer rainfall and by temperatures in
44 several periods spanning from host tree bud burst to fruiting body ripening. The
45 temporal trend of Spanish truffle production in the last decade reflected the recent
46 transition from a wild harvest to an agricultural production, with an abrupt increase in
47 annual production and a decrease in year-to-year variability. In the context of the
48 expected evolution of regional climate according to current models, our results point to
49 drier and warmer summer conditions as major threats to truffle production in Spain.
50 Spring and autumn warming could induce an advance in the mean day of truffle
51 fruiting.

52

53 **Key words:** *Tuber melanosporum*; fungal fruiting; climate; *Quercus ilex*; phenology;
54 dendroecology.

55 **1. Introduction**

56 The highly-appreciated black truffle (*Tuber melanosporum* Vittad.) is an
57 ectomycorrhizal fungus that lives in obligate symbiosis with the roots of several tree
58 species. It is endemic to southern Europe, where it preferentially associates with
59 Mediterranean oaks such as the evergreen *Quercus ilex* L or the deciduous *Quercus*
60 *faginea* Lam. (Garcia-Barreda et al., 2012; Ricard et al., 2003). The fruiting body grows
61 below ground for several months, with fruiting induction typically happening in late
62 spring (Montant et al., 1983). During summer and early autumn, the fruiting body
63 undergoes a series of sequential morphogenetic stages to develop its complex structure
64 as its weight rapidly increases (Montant et al., 1983; Zarivi et al., 2015). At the end of
65 this development and swelling stage, the fruiting body has practically achieved its final
66 size. Then, during the maturation stage, typically in late autumn, the spores acquire their
67 characteristic pigmentation and the gleba darkens (Garcia-Barreda et al., unpublished;
68 Zarivi et al., 2015). Throughout late autumn and all winter, the fruiting bodies are
69 progressively harvested as they develop the aroma that allows dogs to locate them (the
70 so-called ripening).

71 Climatic factors have a major influence on the outcome of the fruiting body formation
72 process. The close relationship between summer rainfall and annual truffle production
73 has for long been known to truffle harvesters and experts (Rebière, 1981; Ricard et al.,
74 2003), with researchers confirming its importance (Büntgen et al., 2012a; Le Tacon et
75 al., 1982, 2014). However, there is much less information and more controversy on the
76 role of other climatic factors throughout the rest of the fruiting body lifespan (Bardet
77 and Fresquet, 1995; Büntgen et al., 2019; Le Tacon et al., 2014; Montant and Kulifaj,
78 1990; Ricard et al., 2003). The physiological status of the host tree is also likely to
79 influence annual truffle production, since the fruiting body depends on carbon allocated

80 by the host tree to its fine roots (Le Tacon et al., 2013). Büntgen et al. (2012a) found a
81 close relationship between tree-ring width of host trees and truffle production,
82 pioneering the use of tree radial growth data to investigate the influence of climate on
83 truffle production. A better knowledge of climate-growth-truffle relationships would
84 help improve our knowledge on truffle ecology and adapt the cultivation practices to
85 specific environmental conditions and to the different stages of the fruiting body
86 formation.

87 Socio-economic and technological factors have also been claimed to affect trends in
88 truffle production (Baragatti et al., 2019). Harvesters and experts agree that wild
89 Spanish production of black truffle suffered a sharp decline during the 1970s and the
90 1980s (Reyna, 2012). Overexploitation due to high market price, and natural habitat
91 deterioration due to forest encroachment and the increase in tree cover (linked to rural
92 depopulation and land use change) are generally considered as major causes, although
93 climate warming has also been claimed as an important factor (Büntgen et al., 2012a;
94 Garcia-Barreda et al., 2018; Garcia-Barreda and Reyna, 2013). The high market price of
95 black truffle and the decline of wild harvests were the spur for its cultivation in Spain, a
96 key technological advance which began in 1968, although in the late 1990s only around
97 10% of the national production was harvested in plantations (Garcia-Barreda et al.,
98 2018). Nowadays the share of black truffles produced in plantations is estimated to be
99 80-90%, with Spain accounting for 43% of the European production from 2013 to 2017
100 according to the records of the European Group for Truffle and Trufficulture.

101 The relationship of truffle fruiting with climatic conditions, host tree growth and other
102 environmental or human factors should be contemplated in the context of the current
103 regional climatic trends and their expected evolution based on climate change models
104 (Vicente-Serrano et al., 2017a). Climatic trends associated with global warming have

105 been linked to alterations in the production and phenology of epigeous fungi (Büntgen
106 et al., 2012b; Kauserud et al., 2012). Assessing the impact of climate warming on truffle
107 production and examining which biological mechanisms are involved is essential to
108 foresee risks and plan for future needs of the truffle sector. A particularly intriguing
109 challenge is to link climatic conditions and tree growth to truffle fruiting phenology. For
110 epigeous fungi, Kauserud et al. (2012) found that the climatic trends associated with
111 global warming were related to changes in the length and dates of the fruiting period,
112 although the response was highly species-specific. In the case of black truffle, with a
113 much longer fruiting season (typically spanning from November to March), no data on
114 the first and last day of fruiting is available, although some hints exist that climatic or
115 soil environmental conditions influence how truffle production is distributed throughout
116 the fruiting season (Garcia-Barreda et al., unpublished; Montant and Kulifaj, 1990).
117 The main objectives of this study are: (i) to investigate which climatic variables are
118 important for explaining the variability in the annual black truffle production of Spain,
119 (ii) to analyse whether climatic conditions and/or host plant growth are sufficient to
120 explain the temporal variation in Spanish black truffle production since 1970, and (iii)
121 to evaluate the effect of climatic conditions and host plant growth on how black truffle
122 production is distributed throughout the fruiting season. We hypothesised that: (i)
123 climatic conditions throughout the lifespan of the fruiting body, and not only in
124 summer, influence black truffle production, (ii) socio-economic and technological
125 factors need to be accounted for explaining the temporal evolution of black truffle
126 production, and (iii) warm conditions promote earlier fruiting of black truffle. Finally,
127 we discuss the likely effects of expected regional climate trends on Spanish black truffle
128 production, based on trends for the 1970-2017 period and those pointed out by climate

129 change models, as well as the implications of our results on the management of truffle
130 plantations.

131

132 **2. Materials and methods**

133 *2.1. Statistical sources for truffle harvests*

134 Data on the annual black truffle production in Spain from the fruiting season November
135 1970 – March 1971 (hereafter called season 1970) to the season November 2017 –
136 March 2018 (i.e. season 2017) was obtained from the Spanish Federation of
137 Associations of Truffle Growers (FETT) (Fig. S1, Supplementary Material). This record
138 proved to be the most consistent among the Spanish statistical sources, being useful for
139 climate change studies (Büntgen et al., 2012a; Garcia-Barreda et al., 2018).

140 Black truffle production data from the FETT record is not disaggregated by month,
141 making it impossible to analyse the patterns of fruiting phenology within a season.

142 However, the International Trade Statistics Yearbook (ITSY) from the Spanish Ministry
143 of Finance, which has specific categories for truffles, incorporated monthly
144 disaggregated data since 1995. Garcia-Barreda et al. (2018) showed that ITSY record
145 for fresh truffles exported worldwide from December to February was a good predictor
146 of the FETT record. Here, we use the February/December ratio of Spanish fresh truffle
147 exports, according to Dirección General de Aduanas (1969-2018), as a late/early ratio of
148 truffle production (L/E ratio), in order to explore how black truffle production is
149 distributed throughout the fruiting season.

150

151 *2.2. Climatic records*

152 The climatic variables selected as predictors were mean minimum and maximum
153 temperature, atmospheric evaporative demand (AED) and precipitation for bimonthly

154 periods with a biological relevance. Relative humidity, wind speed and sunshine
155 duration (as a surrogate of the global solar radiation) were also obtained, but only to
156 calculate the AED by means of the FAO-56 Penman-Monteith reference
157 evapotranspiration equation (Allen et al., 1998). Temperature, AED and precipitation
158 are generally considered key to explain the variation in truffle production from year to
159 year (Bardet and Fresquet, 1995; Le Tacon et al., 2014). We decided not to test the
160 climatic variables on a monthly basis, but on periods selected on the basis of truffle life
161 cycle and fruiting body morphogenesis (Table 1), similarly to Le Tacon et al. (2014).
162 *Tuber melanosporum* does not show a particularly high degree of reproductive
163 synchrony, neither in fruiting induction nor in fruiting body development or ripening,
164 with these processes spreading over more than one month (Pacioni et al., 2014; Sourzat
165 et al., 1993; Zarivi et al., 2015).

166 Climatic data for the 1970-2017 period was retrieved from the dataset described in
167 Vicente-Serrano et al. (2017b). This dataset was generated using the entire climate
168 records of the Spanish Meteorological Agency, which were quality controlled and
169 homogenised. The data was interpolated to a regular grid of 1.21 km² and a weekly
170 temporal resolution by means of a universal kriging method. Details of the climate
171 dataset creation and validation can be found in Vicente-Serrano et al. (2017b).

172 When it comes to analyse regional data on truffle production, it is key to select climatic
173 data with an appropriate spatial and temporal representativeness. Truffle production
174 data from Spanish statistical sources is not spatially disaggregated. Black truffle is
175 harvested from wild forests over large parts of eastern Spain, mostly concentrated in the
176 Iberian System and the Pre-Pyrenees (Garcia-Barreda et al., 2019). In recent decades the
177 wild truffle production is being progressively substituted by cultivated production
178 (Garcia-Barreda et al., 2018), but almost all of the currently productive plantations are

179 located in areas with wild production (Fig. 1, Table S1). The widespread spatial
180 distribution of Spanish truffle production makes it difficult to obtain climatic and tree
181 growth data that are sufficiently representative to allow for the assessment of the
182 climate effect on truffle production at the national level. Here, we use the dataset of
183 natural truffle-producing forests in Garcia-Barreda et al. (2019) as a meaningful
184 representation of the spatial distribution of the black truffle production in Spain for the
185 1970-2017 period (Fig. 1). For each year of the 1970-2017 period, the climatic data was
186 calculated as the average of the climatic values retrieved for each forest in the dataset.

187

188 2.3. Dendrochronological data

189 The two most common host tree species of black truffle in Spanish wild stands and
190 plantations, *Q. ilex* and *Q. faginea* (Garcia-Barreda et al., 2012), were selected for the
191 dendrochronological analyses. Host trees supply carbon to truffle fruiting bodies over
192 all their formation period through mycorrhizae (Le Tacon et al., 2013), which has led to
193 propose *Q. ilex* ring-width as a proxy for truffle production (Büntgen et al., 2012a).

194 For each tree species, two chronologies were selected to be representative of the two
195 main truffle-producing regions of Spain, the Iberian System (Teruel province) and the
196 Pre-Pyrenees (Huesca province). For both tree species, the series from the two regions
197 were significantly related ($P = 0.03$ and $P = 0.001$), but with low R^2 (0.11 and 0.21).

198 Thus, the indexed ring-widths of the two regions were averaged to obtain a chronology
199 that could better reflect the conditions for host tree growth throughout all the truffle-
200 producing region of Spain.

201 We used residual ring-width indices instead of raw ring-width data because each
202 chronology showed a specific long-term time trend, due to differences in tree age, size,
203 density, site quality and historical factors. As a result, ring-width indices are exclusively

204 used to assess the effect of tree growth on the interannual variability of truffle
205 production, not to assess relationships between long-term trends of growth and truffle
206 production.
207 Data on annual tree growth for the period 1970-2017 was obtained from previously
208 studied forest stands, updated with the collection of new trees in recent years (Alla and
209 Camarero, 2012; Camarero et al., 2016, 2012). Each ring-width chronology was based
210 on at least 15 randomly sampled dominant trees. Wood samples were sanded until tree
211 rings were clearly visible and then visually cross-dated. Once dated, tree ring-widths
212 were measured to the nearest 0.01 mm from the pith to the bark along two radii per tree
213 using a binocular scope and a LINTAB measuring device (Rinntech, Heidelberg,
214 Germany). The accuracy of visual cross-dating was checked with the program
215 COFECHA (Holmes, 1983). Tree-ring widths were standardised and detrended by
216 applying a 15-year long cubic-smoothing spline curve and autoregressive modelling to
217 remove the first-order autocorrelation from the individual tree-ring width series. This
218 resulted in pre-whitened or residual ring-width indices that were averaged for each site
219 using biweight robust means. Chronology building procedure was carried out using the
220 software ARSTAN v 4.4 (Cook, 1985).

221

222 *2.4. Statistical analyses*

223 A generalised additive model (GAM) with a Gaussian (normal) error distribution was
224 used to analyse the effect of the climatic factors on the annual production of black
225 truffle, and its temporal trend ($n = 48$). All the variables were treated as smoothed
226 predictor variables, specified using shrinkage smoothers (cubic regression spline). After
227 adding the effect of time to the model, the climatic variables were sequentially added.
228 The final model was selected through several rounds of model fitting, iteratively

229 comparing models with and without particular terms based on the Akaike information
230 criterion (AIC). At each step, the variables with the highest partial correlation
231 coefficients with the response were tested. The basis dimension of each smooth term
232 was checked and increased when necessary. The assumptions of normal distribution,
233 constant variance, temporal independence and concurvity (the analogue of multi-
234 collinearity in GAMs) were tested in the final model. The analysis was conducted with
235 the package *mgcv* in R (Wood, 2011), using a 0.05 threshold for statistical significance.
236 Deviance partitioning was used to discriminate the proportion of variation in the
237 response variable that is explained by each single predictor, calculated as the reduction
238 in deviance after dropping the corresponding term while maintaining the same
239 smoothing parameters throughout. The difference between the deviance explained by
240 the final model and the sum of deviances explained by the individual predictors was
241 attributed to the shared effect of predictors (i.e. affected by concurvity).
242 The final model including the time trend and the climatic variables was used to test the
243 hypothesis of whether the growth of the host tree (ring-width index) is important to
244 explain truffle production beyond the general climatic parameters previously analysed.
245 Ring-width indices were added to that final model and both AIC and the deviance
246 explained by the model were used to compare the model with and without a ring-width
247 index.
248 We selected GAMs to analyse the data because they allow to bring together: (i) the
249 possibility of non-linear relationships between response and predictor variables, and (ii)
250 the possibility of simultaneously analysing and modelling the time trend and the effect
251 of climatic variables. In order to complement the GAM analysis of the time trend, we
252 also explored the segmentation approach by Bai and Perron (2003), conducted with the
253 *strucchange* R package (Zeileis et al., 2002). Segmentation approaches allow to detect

254 breaking points (level shifts) and periods significantly different. Simple linear
255 regression was used to estimate the time trends within each segment.
256 The L/E ratio was also analysed through a GAM with a Gaussian error distribution ($n =$
257 22). After testing for its temporal trend, the climatic variables were sequentially added,
258 as explained above. In addition to the selected climatic variables, the climatic conditions
259 for the April–May period were also tested. Montant and Kulifaj (1990) increased the
260 proportion of fruiting bodies harvested in early season by experimentally modifying soil
261 temperature and water content, attributing a key role in truffle induction and early
262 development to April–May soil temperature.

263

264 **3. Results**

265 *3.1. Annual truffle production*

266 After controlling for the time trend ($F = 9.5$, $P < 0.001$), the following climatic variables
267 were included as significant predictors in the GAM analysing the annual production of
268 black truffle: July–August precipitation ($F = 9.1$, $P < 0.001$), mean maximum
269 temperature of March–April ($F = 3.1$, $P = 0.003$), mean maximum temperature of July–
270 August ($F = 4.7$, $P < 0.001$), mean maximum temperature of September–October ($F =$
271 1.7, $P = 0.025$), and mean maximum temperature of January–March ($F = 3.5$, $P =$
272 0.004).

273 The GAM met the assumptions of constant residual variance (Fig. S2a), normal
274 distribution (Shapiro-Wilks test: $W = 0.98$, $P = 0.41$, Fig. S2b-c) and temporal
275 independence (Fig. S2d). The predictors showed worst-case concurrencies between 0.55
276 and 0.72, with all the pairwise values of worst-case concurrency being lower than 0.5
277 (Table S2).

278 According to the GAM, the time trend was negative from 1970 until the early 2000s,
279 significantly increasing in the last six years (Fig. 2a). July–August precipitation showed
280 a positive relationship with annual truffle production, tending to plateau above 120 mm
281 (Fig. 2b). March–April temperature showed a non-significant relationship with
282 production for most of the range, and a positive relationship with temperatures above
283 16°C (Fig. 2c). July–August temperature showed an almost linear and negative
284 relationship with truffle production (Fig. 2d). September–October maximum
285 temperature showed a quadratic relationship with truffle production, with a maximum
286 occurring at around 20–21 °C (Fig. 2e), whereas January–February temperature showed
287 a positive relationship with truffle production (Fig. 2f).

288 The total deviance explained by the GAM was 90.8%, from which the time trend
289 accounted for 15.7%, July–August precipitation accounted for 9.1%, July–August
290 temperature accounted for 4.1%, March–April temperature accounted for 3.2%,
291 January–February temperature accounted for 3.2%, and September–October
292 temperature accounted for 2.2%. An additional 24.9% deviance was shared among the
293 combined effects of climatic variables, and another 28.4% deviance was attributable to
294 the shared effect of the time trend and climatic variables.

295 When the series of *Q. ilex* ring-width indices was added to the GAM including time
296 trend and climatic variables, it showed a significant relationship with annual truffle
297 production, which was positive only for years with high growth rates (ring indices > 1,
298 $F = 4.1$, $P < 0.001$, Fig. 3a). The addition of *Q. ilex* ring-width index decreased the AIC
299 from 343.2 to 332.6, and increased the deviance explained by the model from 90.8% to
300 92.8%. All the other predictors in the GAM remained significant, except for the March–
301 April temperature which became non-significant ($F < 0.1$, $P = 0.61$).

302 The addition of the series of *Q. faginea* ring-width indices to the GAM including time
303 trend and climatic variables had a more limited effect on the model results. The ring-
304 width index showed a non-significant relationship with annual truffle production ($F =$
305 0.9 , $P = 0.060$, Fig. 3b). The AIC decreased from 343.2 to 340.6 and the deviance
306 explained by the model increased from 90.8% to 91.7%. All the other predictors in the
307 GAM remained significant despite the addition.

308 Finally, the segmentation approaches used to detect level shifts in annual truffle
309 production from 1970 to 2017 indicated years 1977 and 2012 as breaking points (Fig.
310 S3). The first segment (1970-1977) corresponded to a period without a significant linear
311 time trend ($F = 0.3$, $P = 0.60$) and 56 t of mean annual truffle production (coefficient of
312 variation $CV = 21\%$). The second segment (1978-2012) corresponded to a period
313 without a significant linear trend ($F = 1.5$, $P = 0.23$) and 22 t of mean annual truffle
314 production ($CV = 69\%$). The last segment (2013-2017) corresponded to a period
315 without a significant linear trend ($F = 0.3$, $P = 0.63$) and 47 t of mean annual truffle
316 production ($CV = 5\%$).

317

318 3.2. Late/early ratio for black truffle production

319 Two climatic variables were included as significant predictors in the GAM analysing
320 the L/E ratio: mean minimum temperature of November–December ($F = 10.4$, $P <$
321 0.001) and the AED of April–May ($F = 1.7$, $F = 0.02$). Time did not show a significant
322 relationship with the L/E ratio ($F < 0.1$, $P = 0.49$). *Quercus ilex* and *Q. faginea* ring-
323 width indices also presented non-significant relationships with the L/E ratio ($F < 0.01$, P
324 $= 0.43$ and $F < 0.1$, $P = 0.63$, respectively).

325 The GAM met the assumptions of constant residual variance (Fig. S4a), normal
326 distribution (Shapiro-Wilks test: $W = 0.97$, $P = 0.82$, Fig. S4b-c), and temporal
327 independence (Fig. S4d). The predictors showed a worst-case concavity of 0.44.
328 According to the GAM, the mean minimum temperature of November–December
329 showed a negative relationship with the L/E ratio, but only below 0-1°C (Fig. 4a),
330 whereas April–May AED showed an almost linear and negative relationship with the
331 L/E ratio (Fig. 4b). The total deviance explained by the GAM was 76.9%, from which
332 November–December temperatures accounted for 58.1% and the AED of April–May
333 accounted for 10.3%. An additional 8.5% deviance was attributable to the shared effect
334 of both predictors.

335

336 **4. Discussion**

337 *4.1. Climatic conditions favouring truffle production*

338 The GAM showed that the variability in annual black truffle production in Spain is
339 largely related to variability in the climatic conditions. The climatic variables explaining
340 the highest share of model deviance were those related to the development and swelling
341 stage. July–August precipitation was the climatic variable with the closest relationship
342 to truffle production, in agreement with Büntgen et al. (2012a) and Le Tacon et al.
343 (2014). Wet summers were associated to high truffle harvests. During July and August
344 the truffle fruiting body typically shows an intense weight increase (Montant et al.,
345 1983), coinciding with the hottest and driest period in inland Mediterranean Spain
346 (Lionello et al., 2006). Soil water content during summer is critical for the fruiting body
347 survival and the productivity of black truffle plantations (Le Tacon et al., 1982; Pacioni
348 et al., 2014), although it also affects the physiological status of *Q. ilex* by constraining
349 carbon uptake and arresting growth (Lempereur et al., 2015; Martin-StPaul et al., 2013).

350 Interestingly, our results point that in wet summers rainfall has a decreasing
351 effectiveness on truffle production.

352 July–August temperatures showed a negative relationship to truffle production. Warmer
353 summer conditions lead to increased AED and water deficit (Vicente-Serrano et al.,
354 2014a). Our results agree with Büntgen et al. (2012a), but apparently contradict the
355 observations by Bardet and Fresquet (1995). In this regard, the relationship of truffle
356 production with temperatures in the July–October period suggests that the optimum
357 mean maximum temperature during the development and swelling stage is around 20°C
358 (Fig. 2d-e). It would be interesting to quantify the likely interaction between
359 precipitation and temperature, which determines the fruiting phenology of some
360 epigeous fungi (Diez et al., 2013). In the case of black truffle, summer temperature and
361 its interaction with rainfall could be playing a negative role on fruiting body survival or
362 growth rate.

363 Beyond the already well-established influence of summer climatic conditions on annual
364 black truffle production, our results showed the relationship of truffle fruiting with
365 climatic conditions in other times of the year. The positive relationship between March–
366 April temperature and annual black truffle production is seldom taken into account by
367 harvesters and experts, although Le Tacon et al. (2014) found that AED in February–
368 March positively related to the truffle production in the following fruiting season. In
369 March–April, the induction of truffle fruiting has not begun yet, but bud burst and high
370 fine root production of the main host trees typically happens between late March and
371 early May (Coll et al., 2012; Montserrat-Martí et al., 2009). Le Tacon et al. (2013)
372 concluded that the truffle fruiting body depends exclusively upon the carbon assimilated
373 by the host during the same growing season. Dates of growth onset and cessation
374 determine the length of *Q. ilex* growth period, which closely relates to its growth

375 (Lempereur et al., 2017, 2015). Thus, the positive relationship between March–April
376 temperature and annual truffle production could be due to an earlier growth onset of the
377 host tree increasing the chances of a higher annual host growth and a higher carbon
378 allocation to the fungal symbiont. Ágreda et al. (2015) attributed the positive
379 relationship between spring temperature and the productivity of epigeous fungi fruiting
380 in autumn to the increased growth of the vegetative mycelium.

381 The climatic conditions in May–June did not show any significant relationship with
382 annual truffle production, despite being the time for fruiting induction. However, the
383 negative relationship between April–May AED and L/E ratio suggests that increasing
384 AED in the time of the year just before and during which fruiting is induced brings
385 about an advance in the mean day of black truffle harvesting. Montant and Kulifaj
386 (1990) increased the proportion of fruiting bodies harvested in early season by
387 experimentally modifying soil temperature and water content, hypothesising that April–
388 May soil temperature was critical for truffle induction and early development. For
389 epigeous fungi fruiting in late summer and early autumn, Gange et al. (2007) found a
390 relationship between increasing August temperature and an earlier onset of fruiting,
391 hypothesising that it was due to increased mycelial growth before fruiting induction.

392 Pacioni et al. (2014) interpreted that the induction of *T. melanosporum* fruiting
393 depended on the existence of precipitation events effectively modifying soil
394 environmental conditions (e.g. increasing water content and decreasing temperature),
395 and on the soil conditions before and after that event, pointing to drying–rewetting
396 cycles and the resulting physiological stress for the mycelium as key factors. Thus,
397 increased AED could be playing a role in fruiting induction by promoting increased
398 mycelial growth or rapid fluctuations in soil environmental conditions due to increased
399 evapotranspiration rates in wet soils.

400 The negative relationship between L/E ratio and November–December temperature
401 suggests that a cold late autumn delays the mean date of black truffle harvesting,
402 although this result must be interpreted with caution because it is related to the few
403 years with mean minimum temperature lower than 0°C. It is widely agreed among
404 harvesters that black truffle maturation and ripening go along with soil temperature drop
405 in late autumn (Montant and Kulifaj, 1990). Previous research confirms that November–
406 December is the period with more intense spore melanisation (Garcia-Barreda et al.,
407 unpublished). On the other hand, our results suggest that late-autumn low temperatures
408 delay the senescence processes that set the moment in which dogs localise the ripe
409 fruiting body.

410 Finally, January–February temperature showed a positive relationship to annual truffle
411 production. Le Tacon et al. (2014) found a negative relationship between annual truffle
412 production and the number of severe frosts during winter, those with the higher
413 probability of damaging the fruiting body. In our case, January–February temperature
414 could be associated with the probability of severe frosts, since it is the period with
415 higher frequency of frost events in inland Spain. In January–February, the fruiting
416 bodies that remain below ground are completely developed and mature (i.e. with
417 melanised spores). Thus, the effect of frosts would be more of a reduction in marketable
418 truffle production (Le Tacon et al., 2014; Rebière, 1981).

419

420 *4.2. Host plant growth*

421 Despite improving the model fit, the addition of ring-width data to the GAM had a
422 much more modest contribution to its explanatory power than suggested by the high
423 Pearson partial correlations between truffle production and ring-width indices ($r =$
424 0.382 , $P = 0.008$ for *Q. ilex* and $r = 0.277$, $P = 0.06$ for *Q. faginea*). This does not

425 necessarily mean that the effect of the host growth is residual. The close relationship
426 between climatic conditions and annual black truffle production could be due not only
427 to a direct effect on truffle formation, but also to an indirect effect through the host
428 growth (Diez et al., 2013). The fact that adding *Q. ilex* ring-width indices to the GAM
429 makes March–April temperature become non-significant supports this hypothesis.
430 The differences in the response to climatic factors between black truffle production and
431 the growth of its hosts suggest that truffle fruiting body does not directly follow host
432 phenology throughout the entire formation process. In contrast to truffle production,
433 ring-width indices of *Q. ilex* and *Q. faginea* showed its highest correlations with
434 climatic conditions in June–July and May–June respectively (Tables S3-S4), in
435 concurrence with the main peak of stem growth typically happening in May–June
436 (Montserrat-Martí et al., 2009). Both species show some responsiveness to winter
437 precipitation, possibly related to its influence on soil water content in early spring, the
438 time for bud burst (Tables S3-S4). *Q. ilex* seems to be more responsive to variations in
439 late winter and summer temperatures, the latter possibly in relation to its ability the
440 extend the growing period after the dry summer, whereas the more drought-sensitive *Q.*
441 *faginea* seems more responsive to early spring precipitation (Granda et al., 2013). It
442 would be interesting to explore whether alternative variables reflecting *Quercus* climatic
443 requirements in May–June (e.g. soil moisture, radiation, vapour pressure deficit) and
444 early spring (soil moisture) influence annual truffle production.

445

446 4.3. Time trends for the period 1970-2017

447 Annual black truffle production in Spain over the last 48 years showed a significant
448 time trend that could not solely be explained by variations in the climatic conditions.
449 The GAM indicated that truffle production decreased by 60% from 1970 to 2000, with

450 the most important decline during the late 1970s. The segmentation approach pointed to
451 a level shift in 1977 from which mean annual truffle production decreased by 60%.
452 This decline in truffle production, which was almost exclusively harvested in natural
453 forests until the mid 1990s (Table S5), is consistent with the experience of local
454 harvesters and experts (Reyna, 2012). It has been attributed to two major factors: (i)
455 overexploitation (Garcia-Barreda et al., 2018), and (ii) the increase in tree density and
456 cover (Alcaraz-Segura et al., 2010; Lasanta-Martínez et al., 2005), which involved the
457 deterioration of natural habitats for black truffle fruiting (Garcia-Barreda and Reyna,
458 2013). In this context, a shift towards drier conditions in the 1980s (Carnicer et al.,
459 2019) could have also played a role. We have not estimated the relative contribution of
460 each factor, because neither indicators of the annual evolution of forest density for the
461 truffle-producing region of Spain nor models on the long-term effect of intensive
462 harvesting on hypogeous fungi are available.

463 The GAM also indicated that, beyond climate-driven fluctuations, annual truffle
464 production more than doubled from the early 2000s to 2017, with the most important
465 rise during the 2010s. The segmentation approaches pointed to year 2012 as a level shift
466 from which mean annual truffle production increased by 110% and year-to-year
467 variability decreased. The increase in truffle production followed, with a 10-15 year lag,
468 the sharp increase in the surface of plantations that began in the early 2000s (Table S1).

469 The weight of plantations in the sector also shows up in its increasing contribution to
470 Spanish truffle production (Table S5). It is generally accepted among harvesters and
471 experts that the level shift after 2012 went along with an increase in the volume of
472 fruiting bodies harvested in irrigated plantations. Irrigation has the potential to weaken
473 the relationship between summer precipitation and truffle production, thus reducing the
474 influence of detrimental climatic conditions on yields (Le Tacon et al., 1982). However,

475 it makes plantations vulnerable to climatic conditions reducing available water
476 resources, which are likely to deteriorate in the future (Vicente-Serrano et al., 2014b).
477 The abrupt increase in annual truffle production and the decrease in interannual
478 variability, together with the inability of climatic factors to explain this increase,
479 suggest that truffle production transitioned from a wild harvest –with climatic
480 conditions as a critical driver– to an agricultural production –with a relevant role of
481 cultural practices. However, it must be considered that most plantations (around 80% in
482 Spain) continue to be rainfed. Years with propitious climate conditions could still
483 trigger truffle production peaks in these plantations.

484

485 *4.4. Perspectives and implications for management*

486 Despite our results, it is not unlikely that the current trends in the regional climatic
487 conditions provoke an effect on truffle production, if they continue in accordance to the
488 predicted regional trends derived from global warming. Since 1960, temperature and
489 AED have significantly increased in an important portion of the year, particularly in
490 spring and summer, whereas seasonal precipitation trends since 1960 are not so clear
491 (Vicente-Serrano et al., 2017a). This is reflected in the climatic variables selected for
492 the present study (Table S6, Figs. S5-S8). Moreover, climate change models show a
493 relatively high confidence that temperatures will continue to increase in the truffle-
494 producing region of Spain during the 21st century, more intensely in summer than in
495 winter, leading to aridification trends in some areas. These models show higher
496 uncertainty about precipitation trends, pointing to no change or a moderate reduction in
497 winter precipitation and to a marked reduction in spring and summer precipitation
498 (Christensen et al., 2013). These trends and models point out that the expected climate
499 trends for summer precipitation and temperature are very likely to negatively impact

500 truffle production. On the other hand, our results point that an increase in late winter –
501 early spring temperatures could have a positive influence on truffle production, whereas
502 an increase in early spring AED and late-autumn temperature could advance the mean
503 day of black truffle fruiting. This advance could have negative consequences on truffle
504 quality (Garcia-Barreda et al., unpublished).

505 These changes are likely to make truffle cultivation in Spain more dependent on
506 irrigation. In warmer, drier areas they could increase the probability of years with
507 climatic conditions outside the range observed for the period 1970-2017. This would
508 likely require a rearrangement of cultural practices in plantations, in order not only to
509 improve soil water balance in summer, but also to moderate soil warming. It would be
510 interesting to investigate the potential of cultivation practices such as host tree pruning,
511 management of soil cover and soil tilling.

512 The absence of significance in some of the climatic variables should be judged with
513 caution. The use of climatic variables aggregated over a large geographical area limits
514 the observed range of climatic values (Table S7). These ranges do not reflect the
515 extreme climatic conditions that happen at a local scale. This is particularly pertinent in
516 the case of precipitation, which in Mediterranean Spain is highly irregular at a local
517 scale throughout the entire year (De Luis et al., 2009). The negative effect of long
518 spring or autumn droughts on black truffle production is well known to experts and
519 growers (Bardet and Fresquet, 1995; Montant and Kulifaj, 1990). It justifies the use of
520 irrigation during all the growing season, which has become standard for irrigated
521 plantations in Spain.

522 Caution should be also exercised when making future predictions of truffle production
523 with models based on past relationships between truffle production and climate
524 conditions. Our results hint at a recent technological change in the productive structure

525 of the truffle sector. In a context of an increasing share of truffle production coming
526 from irrigated plantations, the relative importance of the climatic variables will likely
527 change, reducing the predictive power of past models. Climatic conditions determining
528 the availability of water resources, particularly precipitation seasonality, could become
529 relevant controlling factors. Other factors with preferential incidence in plantations,
530 such as outbreaks of the truffle beetle (*Leiodes cinnamomea* Panzer) extensively
531 infesting fruiting bodies, could also add complexity to the relationship between climate
532 and truffle production (Garcia-Barreda et al., unpublished).

533

534 **5. Conclusions**

535 This study showed that annual production of black truffle in Spain in the 1970-2017
536 period was subject to a complex pattern of multiple factors. Its interannual variability
537 was largely explained by climatic factors. Wet-cool summers enhanced truffle
538 production, although nuanced by the non-linear relationships suggested by the GAM.
539 The relationship between climatic factors and truffle records does not support the
540 hypothesis that climatic variation explains the major time trend of black truffle in Spain
541 in the 1970-2017 period. This trend ran parallel to an increase in tree density,
542 overexploitation and the generalisation of truffle cultivation. The study showed that the
543 climatic conditions linked to the fruiting body development were critical drivers of
544 annual truffle production, although conditions during the bud burst of the host plant and
545 during the fruiting season also influenced annual truffle production. Mean maximum
546 temperature and precipitation values explained most of the relationship between
547 *Quercus* radial growth variability and truffle production. AED during fruiting induction
548 and mean minimum temperature during maturation showed a relevant role on how
549 truffle production was distributed throughout the fruiting season, thus providing insight

550 into fruiting phenology. However, inferences about truffle phenology based on these
551 results should be made with caution due to the nature of the data and the sample size.

552

553 **Declarations of interest**

554 None

555

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561

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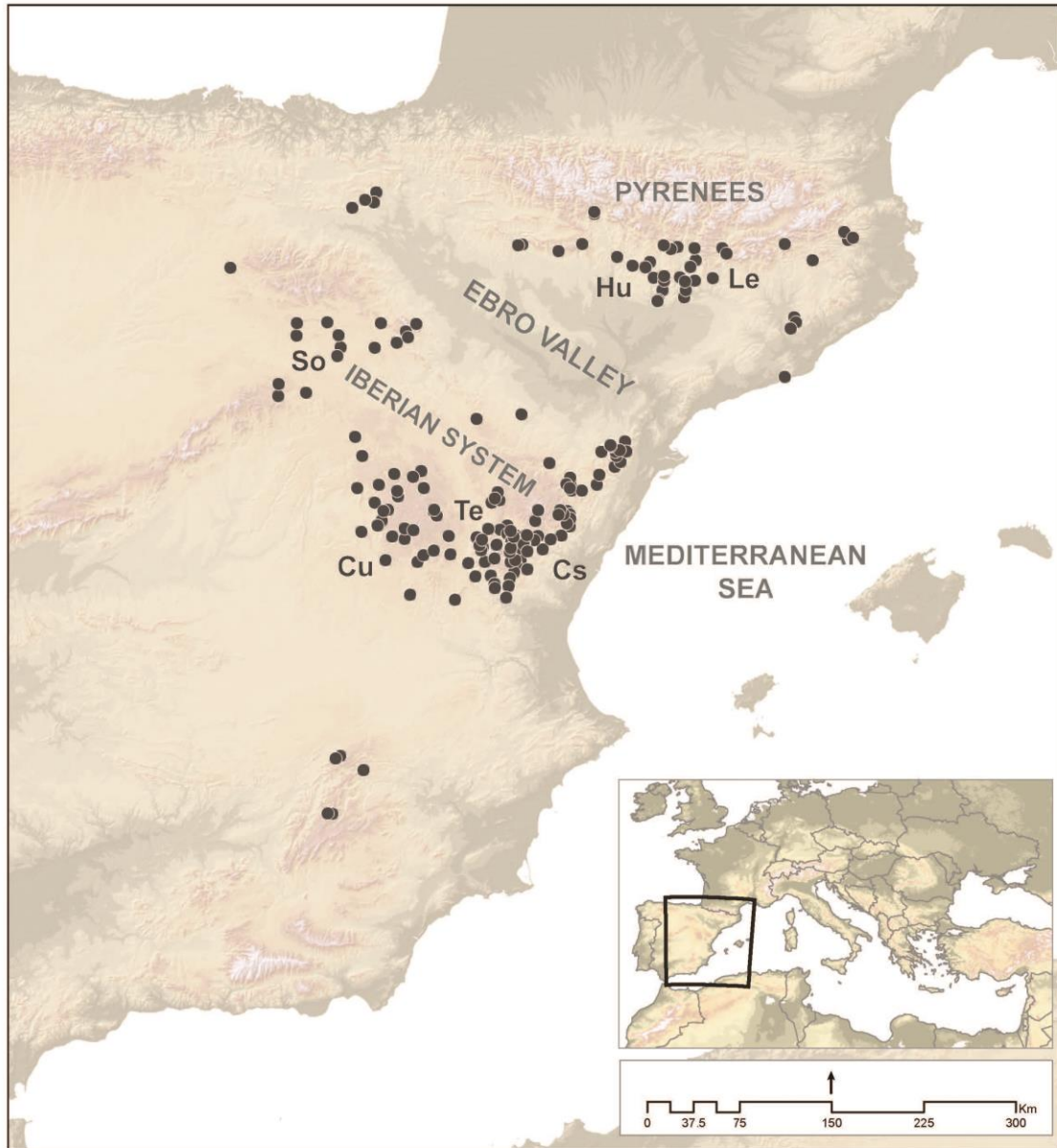
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751 **Table 1** Time basis used for investigating the effect of climatic variables on truffle
 752 production. Fruiting body (FB) morphogenetic stages that are more typically observed
 753 in each period, and typical phenology and growth periods for *Q. ilex* and *Q. faginea*
 754 (Montant et al., 1983; Montserrat-Martí et al., 2009; Pacioni et al., 2014; Zarivi et al.,
 755 2015).

Period	Stage in truffle life cycle or FB morphogenesis. Host phenology and growth
March-April	Bud burst of host oak species. Host primary and secondary growth resumes.
May-June	Fruiting induction, mating and very early stage of FB (hyphal stage). Maximum growth rates of host secondary growth and wood production.
July-August	FB development and swelling (peridial, veined and ascal stages) with maximum aridity conditions. Host growth rates decrease due to water shortage.
September-October	FB development and swelling (ascal and sporal stages) with milder climatic conditions. Maturation begins in late October. Ending of most growth processes of the host, except for acorn enlargement and maturation, which occurs in September.
November-December	FB maturation (pigmented stage). Ripening (aroma development) of part of the FBs.
January-February	Ripening of the remaining FBs. Higher risk of frost. Bud enlargement and maturation.

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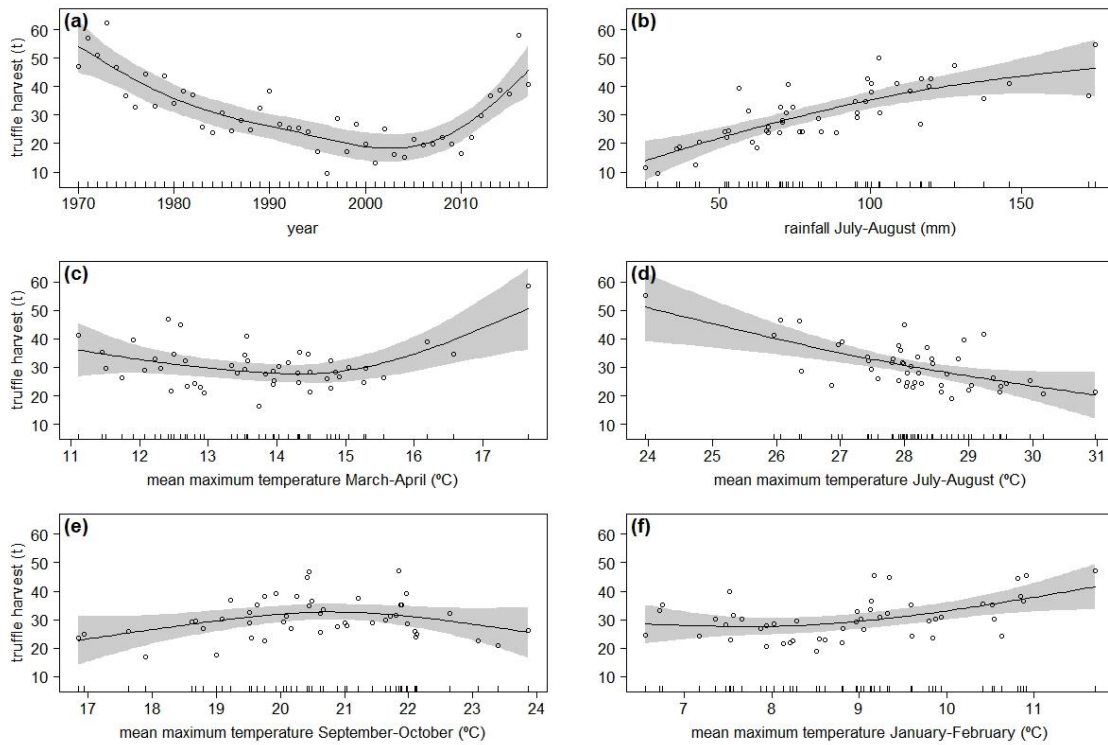


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758 **Fig. 1** Location of the selected Spanish forests naturally producing black truffle. The
 759 provinces with higher abundance of these wild truffle stands are indicated (Te: Teruel,
 760 Cs: Castelló, Le: Lleida, Cu: Cuenca, So: Soria, Hu: Huesca).

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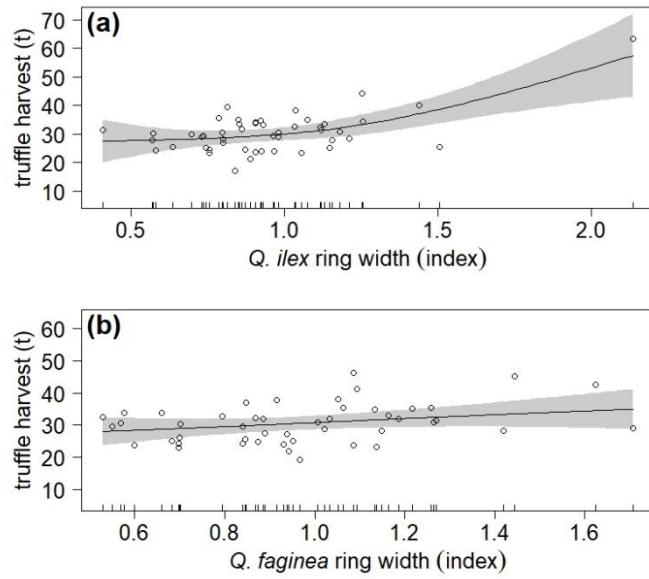


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764 **Fig. 2** Predicted relationships (mean and 95% confidence band) of annual production of
 765 black truffle in Spain with the significant predictors according to the GAM: (a) time
 766 trend, (b) July–August precipitation, (c) mean maximum temperature of March–April,
 767 (d) mean maximum temperature of July–August, (e) mean maximum temperature of
 768 September–October, and (f) mean maximum temperature of January–February. Points
 769 represent partial residuals.

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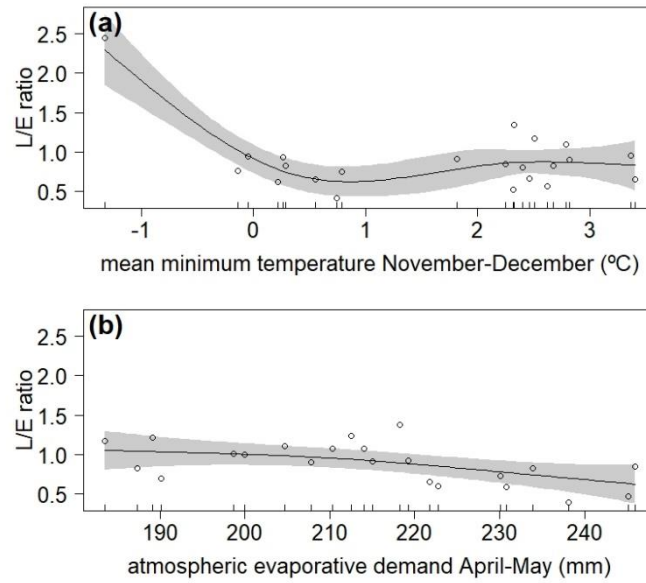
772

773 **Fig. 3** Predicted relationships (mean and 95% confidence band) of annual production of
 774 black truffle in Spain with ring-width indices when one of these variables was added to
 775 the GAM: (a) *Q. ilex*, (b) *Q. faginea*. Points represent partial residuals.

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780 **Fig. 4** Predicted relationships (mean and 95% confidence band) of the late/early ratio
781 for truffle production (L/E ratio) with the significant predictors according to the GAM:
782 (a) mean minimum temperature of November–December, (b) atmospheric evaporative
783 demand of April–May. Points represent partial residuals.

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