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Long-term irrigation effects on Spanish holm oak growth and its black truffle symbionts

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35 **Summary**

36 • The Périgord black truffle (*Tuber melanosporum*) is an exclusive culinary delicacy,
37 but harvest of this ectomycorrhizal ascomycete is declining in its Mediterranean
38 habitat. Effects of long-term irrigation, symbiotic fungi-host interaction, and microbial
39 belowground progression remain poorly understood, because experimental settings
40 miss the necessary degree of real world complexity and information from truffle
41 orchards is limited.

42 • We assess dendrochronological and wood anatomical measurements from 295
43 holm oaks (*Quercus ilex*), which have been growing under different irrigation
44 intensities in the world's largest *Tuber melanosporum* orchard in Spain. Tree-ring
45 evidence is compared with local and countrywide estimates of annual black truffle
46 yield. Growth-climate response analyses of the host chronologies disentangle direct
47 and indirect effects of climate variation on truffle fruit body production.

48 • Irrigation at medium – instead of high – intensity is most beneficial for oak growth,
49 whereas non-irrigated trees reveal lower stem increments. Warmer spring
50 temperatures (February-April) and wetter summer conditions (May-July) enhance
51 host vitality, and most likely also the interplay with its fungi symbionts via increased
52 fine root production and mycorrhizal colonization.

53 • If successful, a widespread recovery of carefully irrigated Mediterranean truffle
54 orchards might counteract some of the putative drought-induced falling yields, and
55 subsequently stabilize rural tourism, regional agriculture and global markets.

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66 **Key words:** *Dendroecology, Iberian Peninsula, irrigation systems, Mediterranean*
67 *drought, symbiotic fungi-host interaction, tree-ring anatomy, truffle orchards, Tuber*
68 *melanosporum.*

69 **Introduction**

70 The Périgord black truffle is the fruit body of *Tuber melanosporum* (Vittad.), an
71 ectomycorrhizal hypogeous fungus considered a unique delicacy by gourmets worldwide
72 (Hall *et al.*, 2003). This species is characterized by a black peridium and a dark spore-bearing
73 gleba that matures under cold conditions between November and February within its native
74 Mediterranean habitat. Naturally occurring *T. melanosporum* fruit bodies are mainly
75 harvested in Italy, France and Spain (Delmas, 1978; Ceruti *et al.*, 2003), where their range is
76 confined to calcareous soils without excesses of nitrogen and phosphorus, mild summer
77 temperatures and suitable rainfall patterns (Bonet *et al.*, 2009). A limited distribution of fruit
78 bodies across the greater Mediterranean region, together with its harvest dependency on
79 trained dogs (and historically also pigs), have weaved a component of mystery (Olivier *et al.*,
80 2012), which in turn enhanced the appeal of the Périgord black truffle to gastronomy
81 aficionados all over the globe.

82 In contrast to the increasing demand for black truffles, the bounty of its harvest has
83 decreased over the second half of the 20th century (Callot, 1999). A continuous long-term
84 decline not only resulted in global price inflation, but also triggered local cultivation attempts
85 about 40 years ago (Chevalier & Grente, 1979). Inoculation facilitated the expansion of
86 primary orchards, in which host seedlings were successfully colonized by *T. melanosporum*
87 (Chevalier *et al.*, 1973; Palenzona, 1969). Since the 1980s, plantations have begun to
88 compensate for some of the loss of wild truffle appearance (Le Tacon *et al.*, 2014), offering
89 rural landowners an economically interesting alternative to traditional crops (Samils *et al.*,
90 2003, 2008). The truffle business is currently a multi-million euro industry, not only in
91 France, Italy and Spain, but also in Australia (Reyna & Garcia-Barreda, in press). Within the
92 past decade, the prices asked for *T. melanosporum* by Mediterranean farmers lay between 100
93 and 900 euros kg⁻¹ (Reyna & Garcia-Barreda, in press). The average annual production of *T.*
94 *melanosporum* in Europe was about 68 t for the last ten seasons (2004/05 to 2013/14),
95 whereas the production in 2013/14 was 125 t with 45 t from Spain according to the European
96 Group for Truffles (GET), Federación Española de Asociaciones de Truficultores (FETT) and
97 G. Gregori, Experimental Centre for Trufficulture ASSAM Regione Marche Sant'Angelo in
98 Vado (PU) Italy (personal communication), compared to 8 t in 2013 in Australia (A. Mitchell,
99 President, Australian Truffle Growers Association, 2013, personal communication). Today,
100 more than 40'000 ha are used globally for truffle cultivation, with 14'000 ha planted in Spain
101 of which only 10-20% are in production (FETT, GET).

102 Despite a better understanding of the fungus' life cycle (Kues & Martin, 2011), and
103 advances in plantation management principles (Olivera *et al.*, 2011, 2014a, 2014b, 2014c), the
104 production of truffle sporocarps is not yet guaranteed, even when using well-inoculated
105 seedlings in a theoretically suitable orchard (Guerin- Laguette *et al.*, 2013; Molinier *et al.*,
106 2013). Despite an immense plantation effort in many regions, the total harvest of this
107 ectomycorrhizal ascomycete continued to decline. A satisfying explanation for this long-term
108 dwindling of both natural and planted truffle fruit bodies must consider desiccation
109 constraints in a warmer and dryer climate (Hall *et al.*, 2003; Büntgen *et al.*, 2012; Le Tacon *et*
110 *al.*, 2014). In this regard, it should be noted that yields of *T. melanosporum* increased after a
111 two-year summer irrigation experiment in southeastern France (Le Tacon *et al.*, 1982).
112 However, our understanding of long-term irrigation effects, symbiotic fungi-host interactions,
113 and microbial belowground processes is still limited. This profound knowledge gap is in part
114 due to erratic and proprietary information from truffle orchards, as well as the short-term
115 nature of experimental settings that are subsequently not suitable to capture the complexity of
116 long-term ecosystem functioning and productivity.

117 Disentangling biotic (host plants, fungal partners and rhizospheric bacteria), abiotic
118 (climate, pollution, land cover), and combined edaphic (soil, microbes) aspects of the
119 mutualistic relationship between the ectomycorrhizal black truffle and its tree partners
120 remains a challenging task. Given that both, host and fungi, co-evolved under the
121 Mediterranean climate where soil moisture availability is the most limiting factor, the role of
122 this symbiotic relationship in water relations and drought survival has been a focus of several
123 studies. Nardini *et al.*, (2000) demonstrated a decrease in hydraulic conductance compensated
124 by an increased root system for oak seedlings highly colonized by *T. melanosporum*
125 compared to seedlings with low colonization levels; Domínguez Nuñez *et al.*, (2006)
126 confirmed greater survival rates, nutrition levels and leaf water potential for inoculated oaks
127 versus non-inoculated plants on a site with droughty Mediterranean conditions; Martín de
128 Aragón *et al.*, (2012) also observed improved survival of *T. melanosporum* inoculated hosts
129 ten years after their plantation on a wildfire site where mean annual precipitation was very
130 low.

131 However, these fairly short-term and small-scale observations only demonstrate
132 advantages for the host trees but do not capture the influence on the symbiotic relationship or
133 on the reproductive response of the fungal partner. An integrative approach that combines
134 aspects from mycology and dendroecology might be most suitable for discovering if favorable
135 conditions for tree growth are also advantageous for truffle yield. In the specific case of the

136 drought-prone Mediterranean *T. melanosporum* habitats, consideration of the host trees' wood
137 anatomical features, such as vessel counts and transversal areas, could be further beneficial to
138 reconstruct hydroclimatic influences that may even have wide-ranging consequence for the
139 terrestrial carbon cycle (Averill *et al.*, 2014).

140 In seeking to illuminate the dark world of belowground truffle ascomycetes, we combine
141 dendrochronological and wood anatomical techniques to assess 295 oak trees, which have
142 been growing under different irrigation intensities in the world's largest truffle plantation in
143 Spain. We compare our novel tree-ring evidence with local and countrywide estimates of
144 annual black truffle harvest, and perform growth-climate response analyses of the oak host
145 chronologies to separate direct from indirect effects of climate variation on Périgord black
146 truffle fruit body production. Our results, emerging at the so far unexplored interface of
147 mycology and dendroecology, including wood anatomy, are indicative for a successful cross-
148 disciplinary approach with application-oriented implications for local farmers. The discussion
149 places abiotic drivers of the mutualistic fungi-host symbiosis within the wider context of the
150 ongoing 'global climate change' debate.

151

152 **Materials and Methods**

153 **Truffle orchard**

154 The world's largest Périgord black truffle orchard covering 600 ha is situated ~1200 m asl at
155 the southern slope of the Sierra de Cabrejas within the Sistema Ibérico mountain range (Fig.
156 1; Supplementary Information Fig. S1). This calcareous area is located on well-drained, mild
157 slopes with an average inclination of ~8% in the municipality of Villaciervos (Soria, Castilla
158 y León, northern Spain). The orchard was established on a previously open mixed-forestland
159 of Spanish juniper (*Juniperus thurifera* L.), Holm oak (*Quercus ilex* subsp. *ballota* (Desf.)
160 Samp.), Portuguese oak (*Quercus faginea* Lam.), and Scots pine (*Pinus sylvestris* L.); a region
161 that was characterized by an abundant wild truffle production.

162 Thousands of *Q. ilex* seedlings colonized with *T. melanosporum* mycorrhiza were planted
163 at a spacing of 6x6 m in the early 1970s (Fig. S1a-b). *Q. ilex* is an evergreen tree species with
164 diffuse- to semi-ring-porous wood that is widespread in the western Mediterranean (Barbero
165 *et al.*, 1992). Under continental climate conditions, such as those prevalent in inland Iberia,
166 most of the species' primary and secondary growth is restricted to spring and summer,
167 between April and July (Montserrat-Martí *et al.*, 2009). Above average spring precipitation
168 enhances the formation of wider tree rings and vessels (Corcuera *et al.*, 2004, Campelo *et al.*,
169 2010, Gutiérrez *et al.*, 2011), and also stimulates the maximum fine root production (Coll *et*

170 *al.*, 2012). The inoculated oak seedlings originated from France and Spain, and a combination
171 of agricultural and silvicultural treatments was continuously applied to reach sustainable
172 sporocarp productivity: Ploughing the soil approximately 10 cm deep every spring eliminates
173 weeds. Tree pruning in reverse cone shape during October and November increases radiation
174 levels in the understory and facilitates truffle hunting. A total of 250 ha is irrigated with doses
175 of 25 l m⁻² every two weeks between July and September. Accumulated irrigation efforts of
176 36 (50) mm month⁻¹ on medium (highly) irrigated areas correspond to more than a doubling
177 of the natural precipitation totals during this dry period of the year (Fig. S1c).

178 Climate in this part of the Central Iberian Peninsula is continental Mediterranean with a
179 mean annual rainfall and temperature of ~515 mm and ~11.0 °C, respectively (Fig. S1c).
180 Although yearly values are fairly moderate, June-August summer precipitation totals of 100
181 mm and a corresponding temperature mean of 19.2 °C are indicative for a drought-prone
182 ecosystem (Büntgen *et al.*, 2013). Early-spring (summer) temperatures averaged over
183 February-April (May-July) range from 4-8 °C (13-17 °C) and reveal a positive trend since the
184 mid-1970s (Fig. S1d). Precipitation totals during time of the year, however, stay trend-free
185 from 1976-2012 (Fig. S1e), but show slightly increased year-to-year variability since ~1997.

186

187 Tree-ring analyses

188 Core samples of 5 mm diameter were extracted from a total of 480 *Q. ilex* in six disjunct
189 sectors within the orchard and in two natural woodland sites outside the plantation (Fig. 1).
190 While the six orchard sectors represent areas with three levels of irrigation: none, medium,
191 and intense (Table 1), the adjacent natural stands were used as independent references. A core
192 microtome was utilized for surface preparation (Gärtner & Nievergelt, 2010), after which a
193 combination of black staining and white charcoal was used for contrast enhancement. Cores
194 were then examined with a scanner (Epson Expression 10000 XL, Seiko Epson Corporation,
195 Japan) to produce high-resolution images (2400 dpi). Digital photographs were analyzed
196 using the image analysis tool ROXAS (von Arx & Dietz, 2005; von Arx & Carrer, 2014).
197 This tool automatically recognizes ring borders and vessels to calculate various statistics (Fig.
198 1d-f), from which we selected ring width, vessel count, mean vessel size (transversal area)
199 and maximum vessel size (averaged over the three largest vessels per ring), with the different
200 parameters herein abbreviated to RW, VC, VS and MVS, respectively.

201 While RW is a good indicator for growth integrating an array of environmental factors
202 that occurred during the entire growing season, the vessel-related parameters possibly contain
203 additional information on water transport capacity and water limitation. In the semi-ring to

204 diffuse porous *Q. ilex*, VC will be closely related to the whole-ring water transport capacity
205 and may scale up with RW if vessel density does not change with RW (e.g., Campelo *et al.*,
206 2010). VS values provide a robust estimate of the cross-sectional lumen of most vessels. The
207 importance of the widest vessels (MVS) lies in the fact that according to Hagen-Poiseuille's
208 law the efficiency of an ideal tube increases with the fourth power of its radius; the widest
209 vessels therefore contribute over-proportionally and most to overall hydraulic capacity (Fonti
210 *et al.*, 2010). Previous work associated with this species, has shown that the widest vessels
211 respond most sensitively to fluctuations in early spring precipitation (Campelo *et al.*, 2010).

212 To remove ontogenetic, i.e., non-climatic, geometric- and hydraulic-induced growth
213 trends (so-called age-trends) from the parameter-specific raw measurement series (RW and
214 VC as well as VS and MVS, respectively), two conceptually different detrending techniques
215 were applied at the sector level: Cubic smoothing splines with a 50% frequency-response cut-
216 off at 30 years (SPLs; Cook & Peters, 1981) and the regional curve standardization (RCS;
217 Esper *et al.*, 2003). Combined evidence from these methods, either emphasizing high-
218 frequency extremes (SPL) or lower-frequency trends (RCS), is most suitable for growth-
219 climate response analyses and subsequent climate reconstructions. Different ways of tree-ring
220 index calculation (ratios or residuals after power-transformation) were utilized to further
221 account for possible end-effect problems in the resulting time-series (Cook *et al.*, 1995; Cook
222 & Peters, 1997). The final set of chronologies, comprising standard and residual chronologies
223 obtained from the ARSTAN software (Cook, 1985), was calculated for each of the four tree-
224 ring parameters using bi-weight robust means. Artificial variance changes inherent to the
225 chronologies were temporally stabilized (Osborn *et al.*, 1997).

226 Growth-climate response analyses were calculated between the parameter-specific average
227 sector chronologies (RW, VC, VS and MVS), as well as monthly and seasonal precipitation
228 totals and temperature means recorded at the meteorological station in Soria ~20 km nearby
229 (Fig. S1). High-resolution 0.25° gridded climate indices over the European landmass were
230 used for spatial field correlations (E-OBS v8.0; Haylock *et al.*, 2008), facilitating the
231 evaluation of geographical patterns in the obtained growth-climate relationships (Büntgen *et*
232 *al.*, 2010).

233 Annual values of *T. melanosporum* harvest (weight of all fruit bodies collected between
234 November and February), estimated for the whole orchard (600 ha) and entire Spain (FETT;
235 Federacion Española de Asociaciones de Truficultores), were correlated against the various
236 host chronologies as well as the meteorological station measurements from Soria and the
237 European grid-box indices.

238 **Results**

239 The final dataset of 295 *Q. ilex* samples, fulfilling highest cross-dating criteria for all four
240 tree-ring parameters (RW, VC, VS and MVS), is replicated by >5 series per sector back to
241 1989 (Fig. S2). Each sector is represented by a minimum of 17 series and a maximum of 63
242 series. Mean segment length (MSL) and average growth rate (AGR) values of each individual
243 sample of the eight different sites reveal a clear relationship between total increment and
244 lifespan (Fig. S3). MSL of the natural woodland oaks is slightly higher (29-30 years) in
245 comparison to the planted trees (22-27 years). Lowest AGR is found for the woodland sites
246 (E1-E2) and the non-irrigated orchard sectors (P1-P2). Overall higher AGR values are
247 characteristic for both, the medium as well as intensively irrigated sectors (P3-P6). A more
248 detailed view on the sector-specific growth levels and trends further underlines the positive
249 effect of irrigation on radial increment. After aligning each individual oak measurement series
250 by cambial age (i.e., the innermost ring per core sample) and averaging at the sector-level, it
251 becomes evident that stem thickening is most pronounced at tree ages between six and ten
252 years (Fig. 2a). This juvenile RW-increase is followed by a continuous, near linear, decline.
253 Medium and highly irrigated oaks reveal comparable growth levels, well above the
254 corresponding RW-values of both, the non-irrigated planted as well as natural reference oaks.
255 A similar picture is reflected by the VC series (Fig. 2b), whereas the positive age-trends of VS
256 and MVS reveal no differences among the sectors (Fig. 2c-d). All relevant information of the
257 four tree-ring parameters, separated into the eight sampling sites, is summarized in Table 1.

258 Interannual to decadal-long variation in the raw measurement series is dominated by the
259 parameter-specific negative (RW and VC) and positive (VS and MVS) age-trends (Fig. 3a-d),
260 which inflate their inter-series correlation coefficients that range between 0.71 and 0.82
261 (R_{bar}). Reduced parameter-specific coherency is found during the juvenile growth period,
262 whereas more agreement characterizes all chronologies after ~2000 AD. Increased RW and
263 VC in the medium and intensively irrigated sectors contrasts with the overall lower values of
264 the non-irrigated natural and planted oaks. Almost no level offset between the sector-specific
265 data is a key feature of both, the raw VS and MVS chronologies. Some tendency for slightly
266 smaller vessels is, however, indicated for the first portion of the medium irrigated
267 chronologies until ~1995 (Fig. 3c-d), which possibly reflects ontogenetic effects of these
268 relatively young trees.

269 After age-trend removal, the ensemble of sector-specific RW and VC chronologies is
270 almost identical (Fig. 4a-b), whereas more disagreement exists within and between the VS
271 and MVS chronologies (Fig. 4c-d). Year-to-year variability in all four tree-ring parameters

272 appears strongest in the non-irrigated trees, particularly when considering the greatest and
273 most consistent annual extremes as, for instance in 1997 and 2011. VC is significantly
274 positively correlated with RW, whereas VS correlates negatively with VC and RW. Wider
275 rings contain more, though generally smaller vessels, while the size of the largest vessels
276 (MVS) was found to be quite variable and thus less robust as an anatomical parameter. Vessel
277 density remains unaffected from thicker or thinner rings (Fig. 1f). The largest peaks in MVS
278 are seen in the highly irrigated sectors and since ~2000 AD. More details regarding the high
279 level of sector-specific chronology coherence is separately provided for each tree-ring
280 parameter (RW, VC, VS and MVS) in the corresponding sections of the supporting
281 information (Figs. S4-S7). The most distinct growth anomaly occurred in 1997, when RW and
282 VC reached maximum but VS minimum values (note also the reverse pattern during the 2005
283 dry year). A similar though slightly less pronounced pattern struck in 2011. Lowest RW and
284 VC indices terminate the chronologies in 2012 AD (Fig. 4a-b). Those extreme years –
285 prominent in the RW and VC data – are not reflected by the VS and MVS records (Fig. 4c-d),
286 which further tend to differ between each other.

287 Interannual to decadal-long changes in the estimated local *T. melanosporum* fruit body
288 production at the plantation as well as countrywide for Spain describe decreasing variance
289 from ~1997 AD to present (Fig. 4e). Surprisingly little agreement is found between the local
290 and countrywide yields from the plantation and Spain ($r = 0.27$). Comparison between the
291 different tree-ring chronologies and the *T. melanosporum* production estimates shows
292 significant positive (negative) relationships between Spanish truffle yields and RW and VC
293 (VS) over the past decades (Fig. 4 inset). Most significant correlation coefficients of 0.56 and
294 -0.55 are obtained between the Spanish yields and RW, VC and VS, respectively (Fig. S8).
295 Although MVS does not show a clear relationship to truffle harvest, there are still some
296 consistent peaks, e.g., 1999, 2003, 2008 and 2009 (Fig. 4). Most striking is the Spanish truffle
297 boost in 1997, which coincides with anomalously high RW and VC but low VS values. This
298 year is further characterized by an exceptionally warm spring from February-April and
299 outstanding summer precipitation surplus between May and July (Fig. S1).

300 The most obvious response pattern of the sector-specific RW and VC chronologies against
301 monthly and seasonal resolved precipitation totals is the highest correlation with the May-July
302 summer period (Fig. 5a). Moreover, Spanish truffle yields also reveal the highest correlation
303 with summer precipitation totals. In contrast, truffle harvest and oak growth both correlate
304 significantly positively with February-April temperature means (Fig. 5b). A more detailed
305 view clearly emphasizes increased summer precipitation-sensitivity of the non-irrigated oaks

306 within and outside the plantation (P1-P2 and E1-E2). In turn, stem increments from the
307 medium and highly irrigated oak sectors (P3-P6) reveal increased spring temperature-
308 sensitivity. Interestingly, almost all correlation coefficients based on the various precipitation
309 totals are positive, while both, significantly positively and negatively associations are found
310 with temperature. In this regard, some of the observed patterns likely reflect the inverse
311 natural relationship between precipitation and temperature. Although years of elevated *T.*
312 *melanosporum* fruit body production coincide with cold summers, the associated wet
313 conditions ultimately appear beneficial for fungi productivity. Comparison of the various VS
314 and MVS chronologies from the eight different sampling sites against the wide range of
315 monthly and seasonal climate indices mainly denotes randomly distributed, non-significant
316 correlation coefficients (Figs. S9-S10).

317 Spatial field correlations further provide robust relationships between totals of May-July
318 summer precipitation and oak RW chronologies from the non-irrigated sectors (P1-P2) (Fig.
319 6), whereas most significant positive correlations with February-April spring temperature
320 means are found for medium irrigated RW data. Less distinct patterns are indicated between
321 spring temperature and non-irrigated oak growth, as well as summer precipitation and
322 medium-irrigated RW. Interannual variation in radial increments of oaks that obtain a
323 medium dose of water from the summer irrigation system is mainly driven by springtime
324 temperature variability over most of the Iberian Peninsula and large parts of southwestern
325 France, and thus earlier or later onsets of the vegetation period. In contrast, growth rates of
326 the non-irrigated oaks are mainly determined by changes in summer precipitation originating
327 from the Bay of Biscay. Almost similar spatial patterns derive from the correlations with
328 average Spanish truffle harvest (Fig. 6). Not only are significantly positive correlations found
329 with summer precipitation totals over northern Spain, but also with spring temperature means
330 at the Iberian scale.

331 In summary, non-irrigated *Q. ilex* trees have smaller rings, whereas medium, instead of
332 intense irrigation caused thickest stem increments (Fig. 7). Although stimulating the overall
333 growth level and slightly dampening the interannual growth variability, medium to intense
334 irrigation was, however, not enough to prevent from drought-induced ring width depressions
335 in almost all cases. RW and VC chronologies of the non-irrigated oaks correlated significantly
336 positively with May-July precipitation ($r = 0.56-0.60$), whereas most positive correlations
337 with February-April temperatures were obtained from the medium-irrigated RW and VC
338 chronologies ($r = 0.57-0.61$). Tree-ring data from sectors with generally higher or lower
339 truffle production showed similar relationships with climate. RW and VC chronologies from

340 non-irrigated but highly productive oaks correlated significantly positively with Spanish
341 truffle harvest ($r = 0.56$), which in turn denoted a clear dependency on summer precipitation
342 and spring temperature ($r = 0.57$ and 0.53). Surprisingly little statistical evidence for
343 physiological host-fungus interactions and/or similar ecological responses have been found
344 for the VS and MVS chronologies, as well as the annual *T. melanosporum* yields estimated
345 for the plantation.

346

347 **Discussion**

348 This study, exploring fungi-host associations in the world's largest *T. melanosporum* orchard
349 in Spain, provides conceptual advancement at the yet little explored interface of mycology
350 and dendroecology (Fig. S11). Mounting evidence suggests irrigation at medium intensity to
351 be most beneficial for host tree growth in the plantation. Above average temperatures between
352 February and April trigger an earlier onset of the vegetation period, whereas precipitation
353 surplus from May-July prolongs the growing season. Changes in temperature prior to
354 February and in precipitation after July are irrelevant for tree growth because it is either too
355 cold before or too dry afterwards. Combined warm spring and wet summer conditions,
356 however, not only enhance RW but likely also the complex interplay with its belowground
357 truffle symbionts. A favorable climate possibly enhances carbohydrate supply for optimal
358 mycelium development and mycorrhizal colonization of the host's fine roots.

359 Despite our newly obtained findings, it is noteworthy to mention that data and
360 methodological constraints are manifold. The smallest vessels are, for instance, possibly
361 missed in the scanned cores because of limited image resolution, or their number and size
362 might be biased by reduced plasticity. In field observations, vessel diameter of *Q. ilex*
363 declined in response to lower water availability across a geographical gradient (Villar-
364 Salvador *et al.*, 1997) or in response to a severe drought (Corcuera *et al.*, 2004). However, a
365 throughfall exclusion did not result in any change in VS with water availability but caused an
366 increase in lumen fraction, accompanied by a reduction in the transpiring leaf area, in the dry
367 treatment of the experiment (Limousin *et al.*, 2010). Vessels are generally formed within a
368 two- to four-week interval. Assuming VS generally decreases from the early- to the late-
369 formed portion of the ring, any positive and negative deviations from the usual trend in one of
370 the intra-annual zones are smoothed-out in the mean value. VS values are therefore not
371 cumulative in the same way as RW. VS indices were not significantly related to temperature
372 and precipitation in this study. In contrast, Abrantes *et al.* (2013) found positive correlations
373 of holm oak VS and MVS with year-round precipitation and negative correlations with spring

374 temperature. It also juxtaposes to other studies about ring-porous deciduous Mediterranean
375 oak species that confirmed relationships between VS and climatic conditions (e.g., Alla &
376 Camarero, 2012). Our results suggest a rather small phenotypic plasticity of VS to external
377 drivers. Such behavior may be indicative for a species that evolved in a region with very
378 predictable summer droughts and a relatively short growing season imposed by continental
379 conditions. In this situation, phenotypic plasticity bears costs that can readily be saved by a
380 rigid genetic fixation of this trait (Valladares *et al.*, 2007).

381 Particular interest emerges from the positive (negative) correlations between RW, VC,
382 (VS, MVS) and Spain-wide truffle production versus the local plantation yield in 1997/98.
383 With nearly 80 t of fruit bodies from natural and planted oak woodlands (Reyna & Garcia-
384 Barreda, in press), this season had the highest *T. melanosporum* production in Spain over the
385 last 40 years, likely triggered by warm temperatures in February, March and April, followed
386 by precipitation surplus in May, June and July (Fig. S2). The truffle production in our
387 plantation, however, does not reflect a similarly high level of harvest for that year, possibly
388 due to reduced drought stress during spring and summer as irrigation treatments continued to
389 be applied despite the wet and cold summer conditions (Fig. S12). The importance of episodic
390 drought stress in the development of the full potential of the mycorrhizal symbiosis of *T.*
391 *melanosporum* has been demonstrated from a truffle orchard (Olivera *et al.*, 2014).
392 Continuous irrigation of *Q. ilex* trees between May and October resulted in lower root tips
393 colonized by *T. melanosporum*. The same study also revealed that mitigating all
394 evapotranspiration loss through irrigation does not favor mycorrhizal development. In fact,
395 from preliminary data from productive truffle plantations, Fischer & Colinas (2013) suggest
396 that water stress may also be important for the development of *T. melanosporum* fruit bodies.
397 Water potential below -3MPa caused a decrease in truffle production, whereas allowing the
398 water potential to drop from -0.5 to -1MPa for 2-3 weeks appears to favor black truffle
399 production as opposed to weekly irrigations that eliminate drought stress. Le Tacon *et al.*
400 (1982) also showed a positive effect of episodic medium irrigation (only three applications of
401 ~40 mm each between July and September) on black truffle yields in a *Quercus lanuginosa*
402 plantation in southeastern France. Overall, truffle production does not seem to be limited by
403 water-transport efficiency of the host trees. This is supported by negative (absent)
404 relationships between fruit body harvest and VS (MVS). Furthermore, *T. melanosporum* is a
405 very competitive fungal symbiont in Mediterranean climates characterized by periodic
406 summer droughts.

407 The high correlation of VC with truffle harvest is likely a mere consequence of wider RW,
408 which we observed at intermediate irrigation levels. To achieve optimal hydrological
409 conditions, farmers should avoid overwatering by adapting their irrigation regimes to
410 accommodate for specific periods of natural water deficit in summer, allowing seasonal
411 climatic and plant metabolic perturbations with a certain level of system oscillation while
412 avoiding prolonged or extremely low water potentials. Precluding unnecessary overwatering
413 also prevents wasting water. Irrigation should avoid keeping soil moisture at or above field
414 capacity for extended periods.

415 The development of the ectomycorrhizal symbiosis between *Q. ilex* seedlings and *T.*
416 *melanosporum* often depends on the environmental conditions under which it occurs, and
417 determining how mycorrhiza formation of *T. melanosporum* in *Q. ilex* is driven by
418 fluctuations in soil temperature and moisture during the warm season is a still pending issue.
419 For instance, Olivera *et al.* (2014b) observed interactions between soil temperature and
420 moisture on the amount of *T. melanosporum* ectomycorrhizal formation per inoculated
421 seedling. In their experiment cooler conditions were the most favorable for developing
422 truffles, even with medium-low soil moisture. High soil moisture, however, only increased the
423 capacity of competitor fungi to form mycorrhizas, regardless of soil temperature (Olivera *et*
424 *al.*, 2014b). According to this study and our findings, strategies to manage substrate
425 temperatures should be implemented in nurseries or when establishing truffle orchards in
426 particularly warm sites.

427 The equilibrium between the host and the mycorrhizal fungi represents most likely a
428 dynamic relationship with a multitude of factors that drive the direction of nutrient transfer
429 (Plett & Martin, 2011) and could potentially be optimized to increase truffle production.
430 Increasing inputs (i.e., irrigation, fertilization) does not necessarily have a positive effect on
431 the quantity of black truffle mycorrhiza, as recently demonstrated by Bonet *et al.* (2006) and
432 Olivera *et al.* (2014b). It seems logical that improving tree growth could have positive
433 benefits for the ectomycorrhizal system (Le Tacon *et al.*, 2013), because the carbohydrates
434 derived from the host's photosynthesis will sustain mycorrhizal species. Both photosynthetic
435 rate (Huikka *et al.*, 2003; Nara *et al.*, 2003) and basal area increment (Bonet *et al.*, 2012) are
436 positively correlated with sporocarp production of ectomycorrhizal fungal partners. However,
437 from our knowledge, only Shaw *et al.* (1996) reported a positive relationship between truffle
438 fruit body production and tree basal diameter in a young *T. melanosporum* plantation in
439 southern France, but further research is needed in order to confirm this.

440 To further enhance truffle research, we prioritize eight research avenues: i) Perform in situ
441 excavations of well defined soil units between the putative period of increased fine root
442 production and mycelia formation in summer and fruit body harvesting in winter to expose
443 intra-annual dynamics of the fungus lifecycle. ii) Install continuous high-resolution
444 (dendrometer) measurements of radial stem growth, including sap flow for comparisons with
445 observations of fruit body and mycelial growth to ultimately detect linkages between the
446 phenology and net primary productivity of mycorrhizal fungi webs and their host partners. iii)
447 Trace symbiotic carbon, nutrient and water (host-fungi/fungi-host) pathways and fluxes
448 including actual rainfall and accumulated reservoir water via isotopic labeling to reconstruct
449 the continuum between plant growth and ectomycorrhizal fungus energy capture and
450 partition. iv) Perform field and greenhouse experiments with model host-fungus pairings to
451 quantify the power abiotic factors may have in the reciprocal transfer of nutrient, phosphorus,
452 water and carbon in order to predict environmental effects on symbiosis functioning. v)
453 Utilize the advent of bioinformatic sensor technologies, such as metagenomic and/or
454 metatranscriptomic analyses or biochemical assays to gauge belowground functional hyphal
455 activity for evaluation against intra-annual ring width patterns. vi) Relate long-term truffle
456 inventories to dendroecological, wood anatomical and meteorological records to disentangle
457 direct and indirect climatic drivers of the productivity and phenology of fruit body production.
458 vii) Adjust orchard management strategies to assess the effects of diverse age classes and
459 stand structures, open versus close canopies, as well as more or less intense irrigation doses
460 with different seasonal timings. viii) Consider natural and planted truffle sites of different host
461 species and age classes along elevational and climatological gradients to gain further insight
462 into their ecological plasticity.

463

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474

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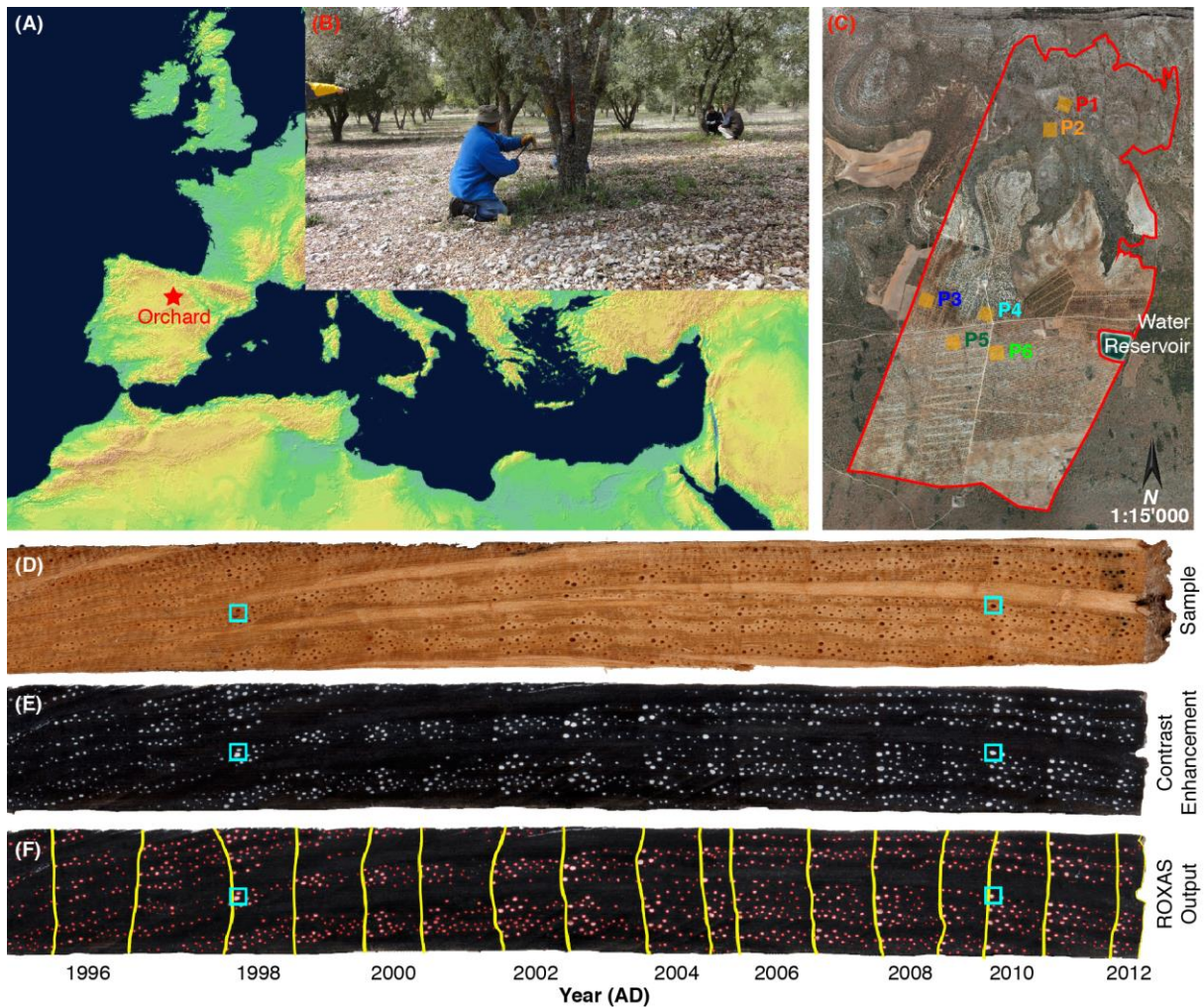
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633 **Table. 1.** Inventory and metadata of all 295 individual oak samples classified into six
634 sectors within the plantation (P1-6) plus two reference sites outside the plantation
635 (E1-2). Information on fungi ecology contains rough estimates of irrigation intensity
636 (non, medium, high) and truffle yield (low, high), whereas information on
637 dendroecology comprises precise measurements of the four different tree-ring
638 parameters RW, VC, VS and MVS. The yellow shadings enhance visual comparison.

Site (code)	Fungi Ecology		Dendro Data				Ring Width		Vessel Count		Vessel Size		Max Vessel Size	
	Irrigation (intensity)	Productivity (yield)	Series (no)	Start (>5)	End (>5)	MSL (years)	RW (mm)	Lag1 (r)	VC (no)	Lag1 (r)	VS (μm^2)	Lag1 (r)	MVS (μm^2)	Lag1 (r)
E1	None	--	17	1981	2012	29	0.23	0.46	82	0.37	3'829	0.34	12'468	0.30
E2	None	--	30	1977	2012	30	0.18	0.26	66	0.25	3'575	0.34	11'624	0.14
P1	None	Low	29	1982	2012	27	0.24	0.35	77	0.33	4'241	0.27	13'235	0.21
P2	None	High	27	1984	2012	24	0.24	0.20	75	0.21	3'959	0.37	12'261	0.14
P3	High	High	45	1981	2012	26	0.31	0.56	100	0.52	3'947	0.52	13'546	0.31
P4	High	Low	45	1982	2012	26	0.33	0.59	95	0.54	4'117	0.43	13'063	0.23
P5	Medium	Low	63	1988	2012	22	0.35	0.24	95	0.31	3'890	0.53	12'951	0.43
P6	Medium	High	39	1989	2012	22	0.33	0.45	97	0.46	3'896	0.49	12'606	0.37

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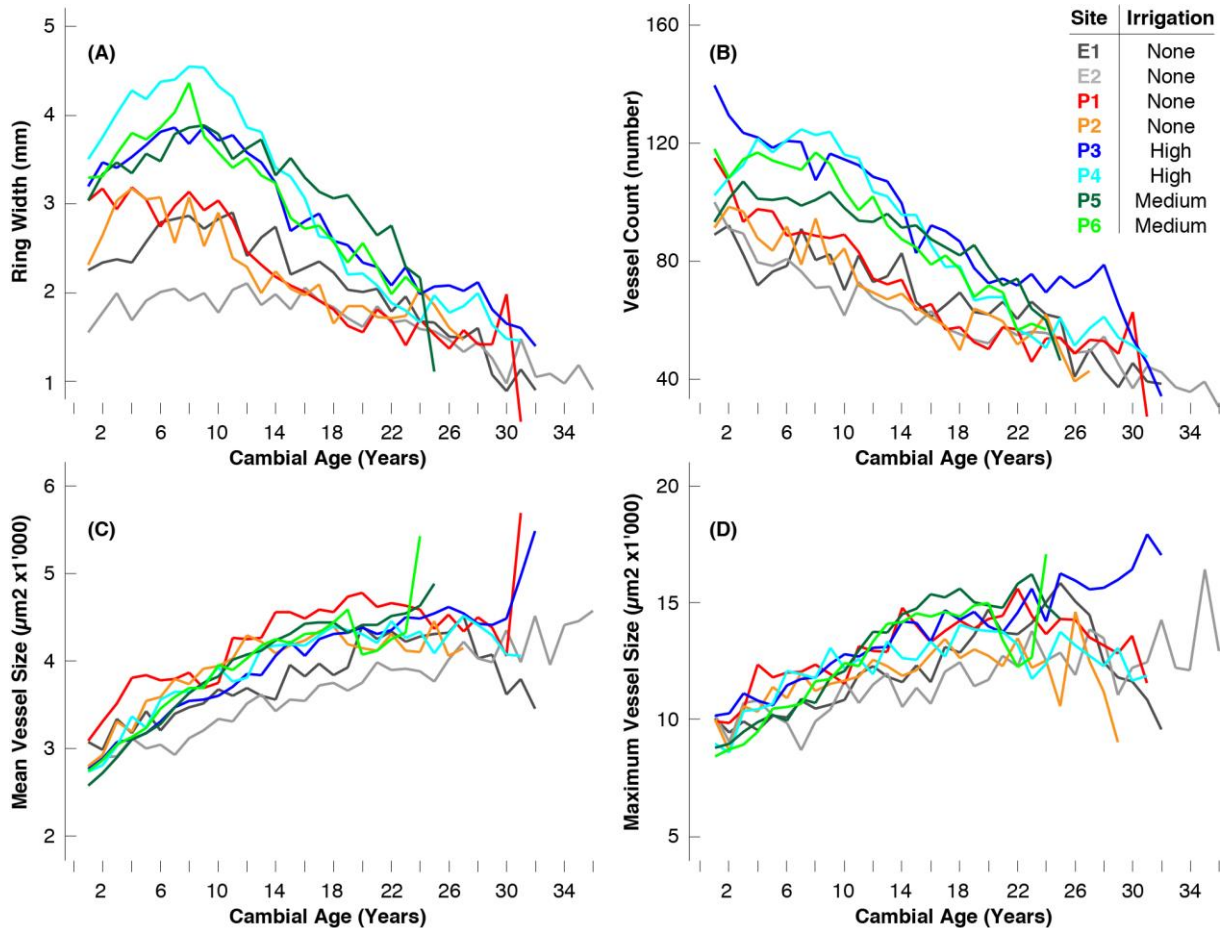
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Fig. 1. (a) Location of the world's largest Périgord Black truffle (*Tuber melanosporum*) plantation "Los Quejigares" within the Spanish Province of Soria. (b) Sample collection in (c) the 600 ha large plantation situated between 1100 and 1400m asl at the southern slope of the Sierra de Cabrejas, ~20km west of the town of Soria, Central Spain (~41°N and ~3°W). Individual holm oak (*Quercus ilex*) trees

648 were sampled in six sectors within the plantation (P1-P6), for which detailed
 649 information on irrigation intensity (non, medium, high) and truffle harvest (low, high)
 650 exists. (d) High-resolution (2400dpi) scan of a holm oak sample (E57b) after surface
 651 preparation with a core microtome (Gärtner & Nievergelt, 2010). (e) The same
 652 sample after contrast enhancement using black staining and white charcoal, and (f)
 653 application of the ROXAS software (von Arx & Dietz, 2005) to determine ring
 654 boundaries (yellow lines) and individual vessels (red circles). The combination of
 655 surface preparation, contrast enhancement and image analysis yielded a wide range
 656 of different tree-ring parameters including ring width, as well as vessel number and
 657 size. Blue squares are simply to enhance visual orientation amongst the three
 658 images.

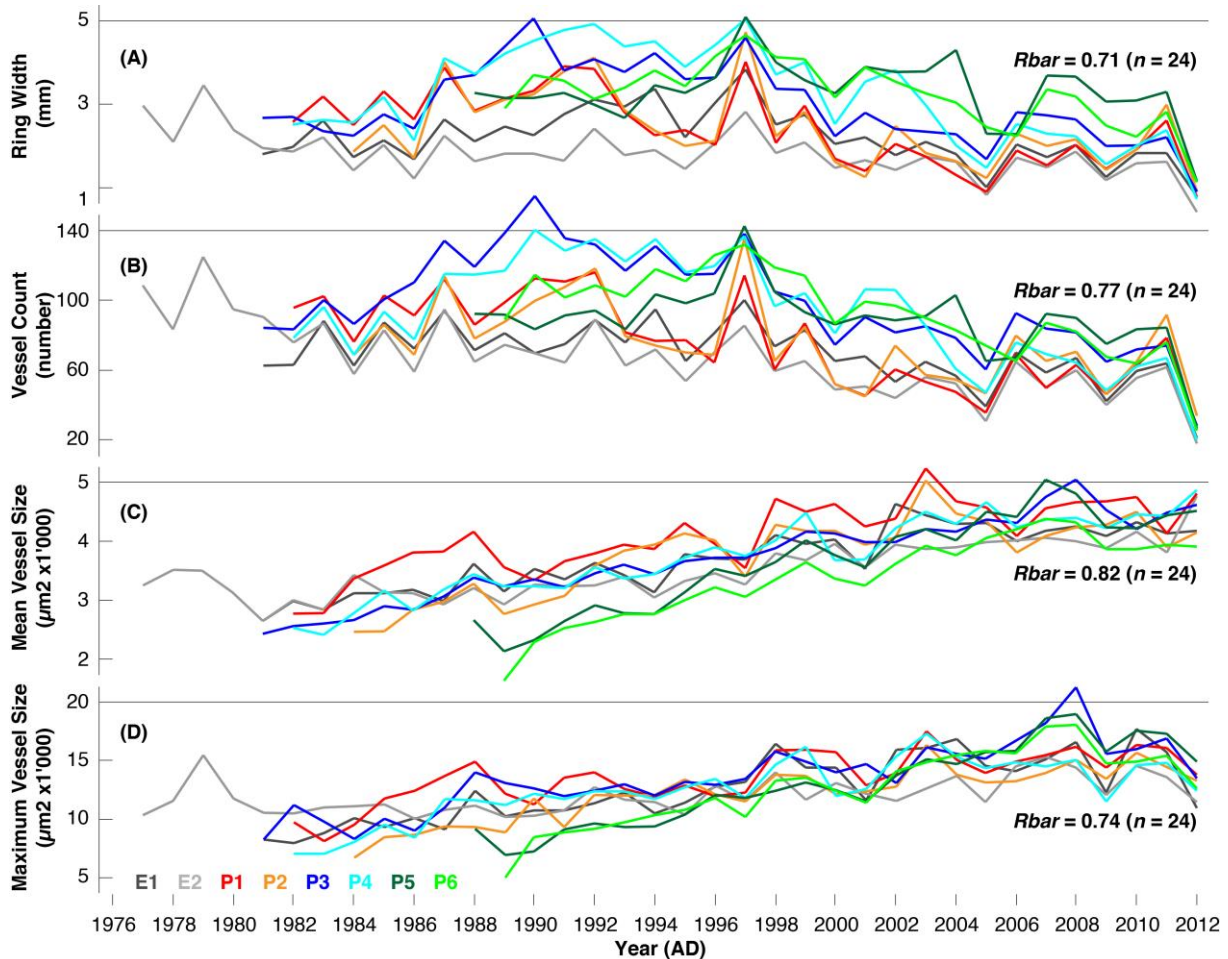
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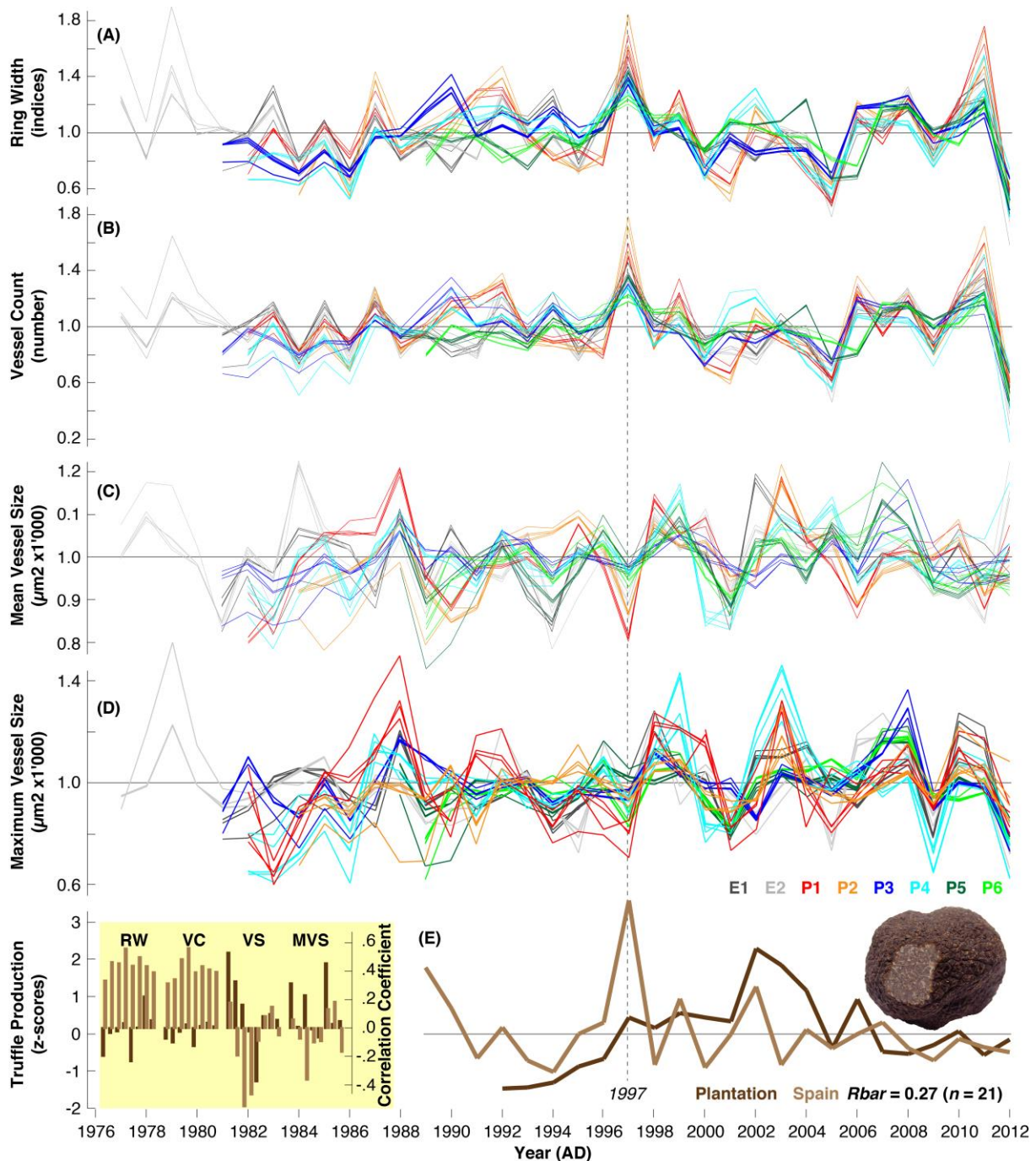


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662 **Fig. 2.** Biological age-trends of the 295 individual oak samples calculated for (a) RW,
 663 (b) VC, (c) VS and (d) MVS, and classified into six sectors within the plantation (P1-
 664 P6) plus two reference sites outside the plantation (E1-E2). The resulting Regional
 665 Curves (RCs) are truncated at a minimum replication of five series.
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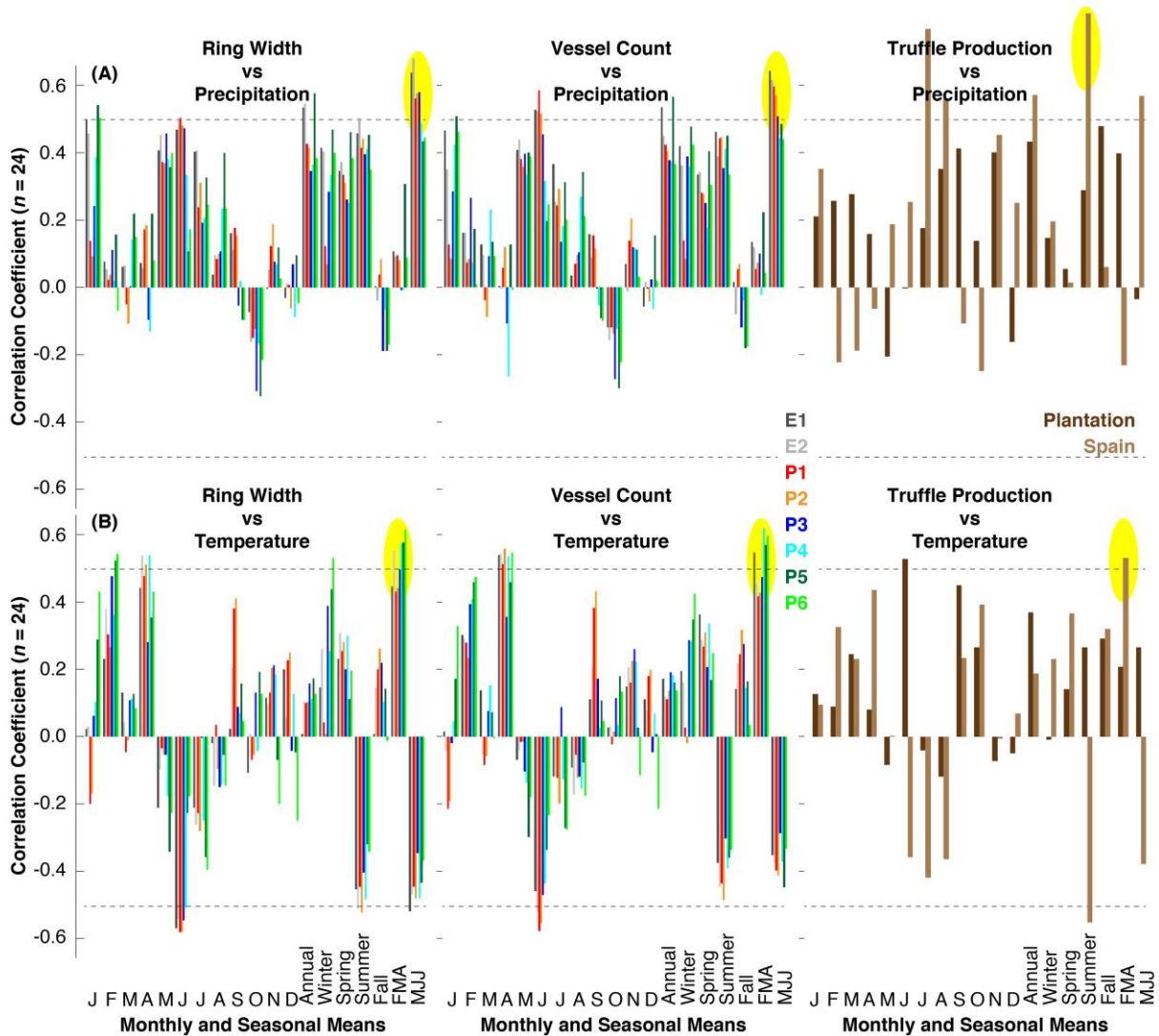


667 **Fig. 3.** Temporal variation in (a) RW, (b) VC, (c) VS and (d) MVS, with all data being
 668 classified into six sectors within the plantation (P1-P6) plus two reference sites
 669 outside the plantation (E1-E2; see Fig. 2). Each of the raw chronologies contains a
 670 high fraction of biological induced age-trend, because no detrending was applied at
 671 this stage. The $Rbar$ values show the degree of parameter-specific coherency
 672 between 1989 and 2012, the period common to all records.
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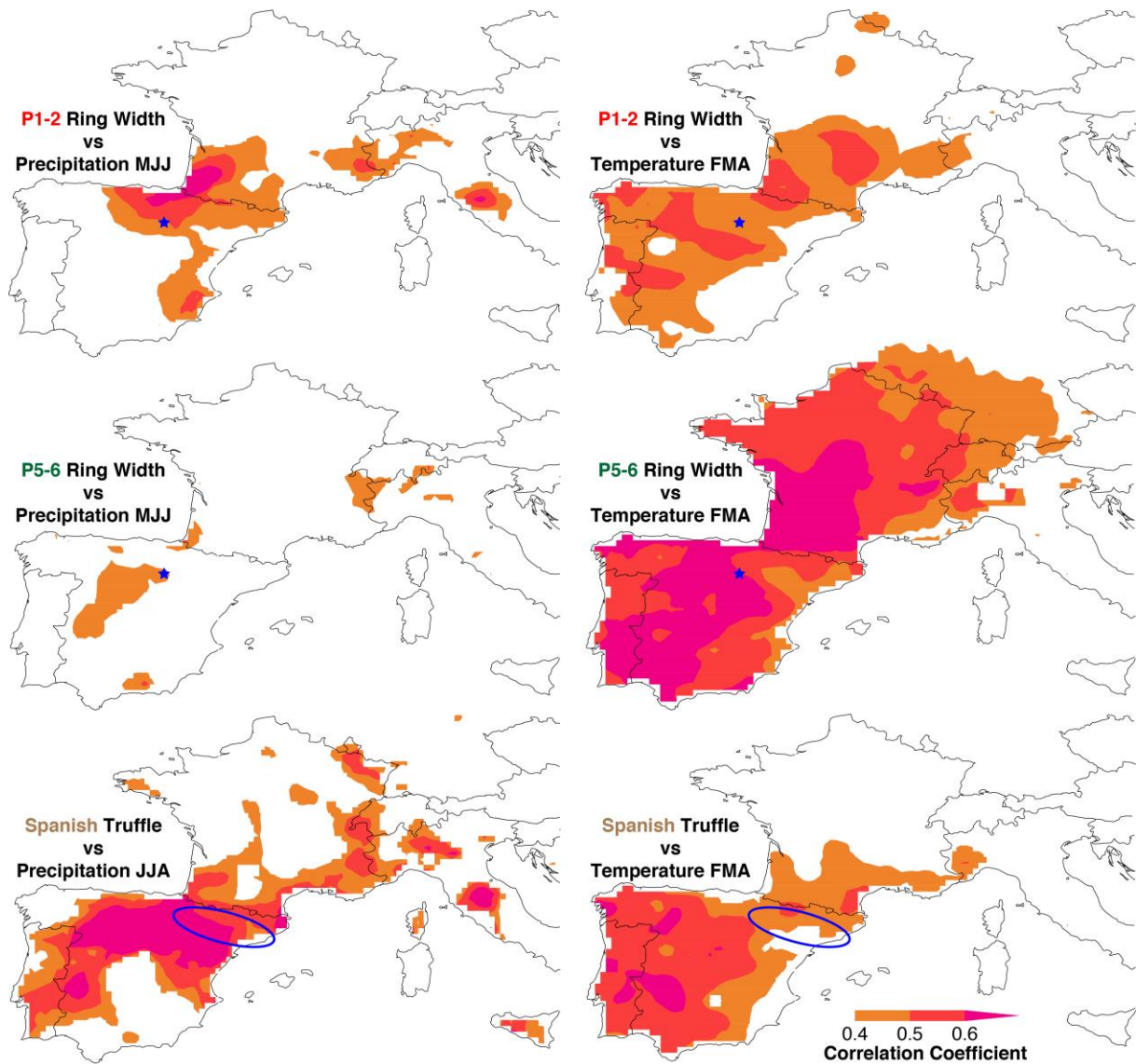
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 676 **Fig. 4.** Chronologies of (a) RW, (b) VC, (c) VS and (d) MVS after the application of
 677 different detrending techniques (individual 30-year splines and RCS), index
 678 calculations (with and without power-transformation) and chronology versions
 679 (standard and residual), with the resulting time-series being classified into six sectors
 680 within the plantation (P1-P6) plus two reference sites outside the plantation (E1-E2).
 681 See supporting information for more details on parameter- and site-specific growth
 682 coherency (Figs. S5-S8). (e) Anomalies of truffle production averaged over the
 683 plantation and Spain, together with their correlation (R_{bar}). The inset shows
 684 correlating coefficients between truffle production and the RW, VC, VS and MVS

685 chronologies of the two reference sites and six plantation sectors (E1-P6 expressed
 686 by the individual vertical bars).
 687



688
 689 **Fig. 5.** Correlation coefficients of monthly and seasonal resolved (a) precipitation
 690 totals and (b) temperature means computed against the mean RW and VC
 691 chronologies from the eight sectors, and records of truffle harvest averaged for the
 692 plantation (dark brown) and entire Spain (light brown). The horizontal dashed lines
 693 refer to the 99% significance levels.

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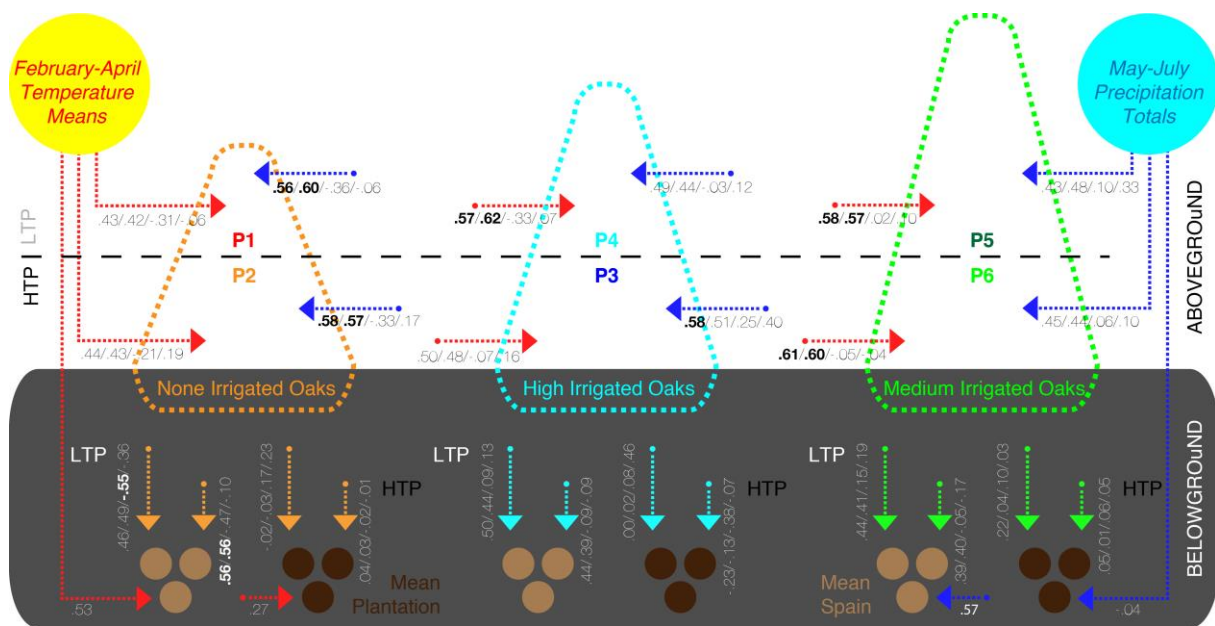
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Fig. 6. Spatial correlation fields of the mean RW chronologies from non and medium irrigated sectors (P1-P2 and P5-P6), as well as the Spanish truffle production computed against a European-wide high-resolution gridded dataset of surface temperature and precipitation indices. Blue star indicates location of the truffle orchard near Soria, Central Spain (~41°N and ~2°W), whereas the blue zone refers to some of the main Spanish truffle habitats.



703
 704 **Fig. 7.** The most important 48 correlation coefficients obtained between early spring
 705 (February-April) temperature means as well as early summer (May-July) precipitation
 706 totals (from Soria) and the four different oak chronologies (RW/VC/VS/MVS) from the
 707 six sectors in the plantation (main diagram), with additional division into high and low
 708 productivity (HTP and LTP), as well as above- and belowground (dark grey)
 709 components. Additional four correlation coefficients between climate variation and
 710 truffle production averaged for Spain and the plantation (right side), as well as 48
 711 correlation coefficients between different oak chronologies and truffle harvest. All 100
 712 pairings either refer to direct mechanistic dependency or common climatic sensitivity.
 713 Correlation coefficients >0.55 are highlighted in bold.