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

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Summary

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

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

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

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

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

Introduction

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

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

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

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

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

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

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

Materials and Methods

Truffle orchard

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

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

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

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

Tree-ring analyses

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

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

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

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

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

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

Results

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

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

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

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

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

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

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

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

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

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

Discussion

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

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

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

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

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

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

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

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

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References

Abrantes J, Campelo F, García-González I, Nabais C. 2013. Environmental control of vessel traits in *Quercus ilex* under Mediterranean climate: relating xylem anatomy to function. *Trees* **27**: 655–662.

Alla AQ, Camarero, JJ. 2012. Contrasting responses of radial growth and wood anatomy to climate in a Mediterranean ring-porous oak: implications for its future persistence or why the variance matters more than the mean. *European Journal of Forest Research* **131**: 1537-1550.

Averill C, Turner BL, Finzi AC. 2014. Mycorrhiza-mediated competition between plants and decomposers drives soil carbon storage. *Nature* **505**: 543–545.

Barbero M, Loisel R, Quézel P. 1992. Biogeography, ecology and history of Mediterranean *Quercus ilex* ecosystems. *Vegetatio* **99/100**:19–34.

Bonet JA; De Miguel S, Martínez de Aragón J, Pukkala T, Palahí M. 2012. Immediate effect of thinning on the yield of *Lactarius* group *deliciosus* in *Pinus pinaster* forests in North-Eastern Spain. *Forest Ecology and Management* **265**: 211–217.

Bonet JA, Fischer CR, Colinas C. 2006. Cultivation of black truffle to promote reforestation and land-use stability. *Agronomy for Sustainable Development* **26**: 69–76.

Bonet JA, Oliach D, Fischer CR, Olivera C, Martínez de Aragón J, Colinas C. 2009. Cultivation methods of the black truffle, the most profitable mediterranean non-wood forest product; a state of the art review. In: Palahi M, Birot Y, Bravo F, Gorriz E, eds. *Modelling, valuing, and managing Mediterranean forest ecosystems for non-timber godos and services*. EFI proceedings 57: 57–71.

Büntgen U, Franke J, Frank D, Wilson R, Gonzales-Rouco F, Esper J. 2010. Assessing the spatial signature of European climate reconstructions. *Climate Research* **41**: 125–130.

Büntgen U, Egli S, Camarero JJ, Fischer EM, Stobbe U, Kauserud H, Tegel W, Sproll L, Stenseth NC. 2012. Drought-induced decline in Mediterranean truffle harvest. *Nature Climate Change* **2**: 827–829.

Büntgen U, Martinez-Peña F, Aldea J, Rigling A, Fischer EM, Camarero JJ, Hayes MJ, Fatton V, Egli S. 2013. Declining pine growth in Central Spain coincides with increasing diurnal temperature range since the 1970s. *Global and Planetary Change* **107**: 177–185

Callot G. 1999. *La truffe, la terre, la vie.* Paris. Ed. INRA.

Campelo F, Nabais C, Gutiérrez E., Freitas H, García-González I. 2010. Vessel features of Quercus ilex L. growing under Mediterranean climate have a better climatic signal than tree-ring width. *Trees* **24**:463–470.

Ceruti A, Fontana A, Nosenzo C. 2003. European species of the genus Tuber. An historical revision. In: Ceruti A, Fontana A, Nosenzo C, eds. *Le specie europee del genere Tuber. Una revision storica*, 467.

Chevalier G, Grente J, Pollaksek A. 1973. Obtention de mycorhizes de different Tuber par synthèse à partir de spores en conditions gnotoxéniques et à partir de cultures pures du mycélium en conditions axéniques et gnotoxéniques. *Annals of the Phytopathology* **5**: 107–108

Chevalier G, Grente J. 1979. Application pratique de la symbiose ectomycorrhizienne: production à grande échelle de plants mycorrhizés par la truffe (*Tuber melanosporum* Vitt.). *Mushroom Science.* **10**: 483–505.

Coll LL, Camarero JJ, Martínez de Aragón J. 2012. Fine root seasonal dynamics, plasticity, and mycorrhization in 2 coexisting Mediterranean oaks with contrasting aboveground phenology. *Ecoscience* **19**: 238–245.

Cook ER, Briffa KR, Meko DM, Graybill DA, Funkhouser G. 1995. The `segment length curse´ in long tree-ring chronology development for palaeoclimatic studies. *The Holocene* **5**: 229–237.

Cook ER 1985. *A time series analysis approach to tree-ring standardization.* New York, Lamont-Doherty Geological Observatory.

Cook ER, Peters K. 1981. The smoothing spline: A new approach to standardizing forest interior tree-ring width series for dendroclimatic studies. *Tree-Ring Bulle* **41**: 45–53.

Cook ER, Peters K. 1997. Calculating unbiased tree-ring indices for the study of climatic and environmental change. *The Holocene* **7**: 361–370.

Corcuera L, Camarero JJ, Gil-Pelegrin E. 2004. Effects of a severe drought on *Quercus ilex* radial growth and xylem anatomy. *Trees* **18**:83–92.

Delmas J. 1978. Tuber spp. In: Chang ST, Hayes WA, eds. *The biology and cultivation of edible mushrooms*, London, UK: Academic Press, 645–681.

Domínguez Núñez JA, Serrano JS, Barreal JAR, González JAS. 2006. The influence of mycorrhization with *Tuber melanosporum* in the afforestation of a Mediterranean site with *Quercus ilex* and *Quercus faginea*. *Forest Ecology and Management* **231:** 226–233.

Esper J, Cook ER, Krusic PJ, Peters K, Schweingruber FH. 2003. Tests of the RCS method for preserving low-frequency variability in long tree-ring chronologies, *Tree Ring Research* **59**: 81–98.

Fischer, CR and Colinas, C. 2013. Effect of irrigation treatments on soil water potential and truffle productivity in a Quercus ilex – Tuber melanosporum orchard. In Proceedings 1st International Congress of Truffficulture. March 2013, Teruel, Spain

Fonti P, von Arx G, García-González I, Eilmann B, Sass-Klaassen U, Gärtner H, Eckstein D. 2010. Studying global change through investigation of the plastic responses of xylem anatomy in tree rings. *New Phytologist* **185**: 42–53.

Gärtner H, Nievergelt D. 2010. The core-microtome: A new tool for surface preparation on cores and time series analysis of varying cell parameters. *Dendrochronologia* **28**: 85–92.

Guerin-Laguette A, Cummings N, Hesom-Williams N, Butler R, Wang Y. 2013. Mycorrhiza analyses in New Zealand truffières reveal frequent but variable persistence of *Tuber melanosporum* in co-existence with other truffle species. *Mycorrhiza* **23**: 87–98.

Gutiérrez E, Campelo F, Camarero JJ, Ribas M, Muntán E, Nabais C, Freitas H. 2011. Climate controls act at different scales on the seasonal pattern of *Quercus ilex* L. stem radial increments in NE Spain. *Trees: Structure and Function* **25**: 637–646.

Hall IR, Yun W, Amicucci A. 2003. Cutivation of edible ectomycorrhizal mushrooms. *Trends in Biotechnology* **21**: 433–438.

Huikka K, Hårmå E, Markkola A, Rautio P, Roitto M, Saikkonen K, Ahonen-Jonnarth U, Finlay R, Tuomi J. 2003. Severe defoliation of Scots pine reduces reproductive investment by ectomycorrhizal symbionts. *Ecology* **84**: 2051–2061.

Kues U, Martin F, 2011. On the road to understanding truffles in the underground. *Fungal Genetics and Biology* **48**: 555–560.

Letho T, Zwiaszek JJ. 2011. Ectomycorrhizas and water relations of trees: a review. *Micorrhiza* **21**: 71–90.

Le Tacon F, Delmas J, Gleyze R, Bouchard D. 1982. Influence du régime hydrique du sol et de la fertilisation sur la fructification de la truffe noire du Périgord (*Tuber melanosporum* Vitt.) dans le sud-est de la France. *Acta Oecologica* **3**: 291–306.

Le Tacon F, Zeller B, Plain C, Hossann C, Bréchet C, Robin C. 2013. Carbon Transfer from the host to *Tuber melanosporum* mycorrhizas and ascocarps followed using a 13C pulse-labeling technique. *PLoS ONE* **8**: e64626.

Le Tacon F, Marçais B, Courvoisier M, Murat C, Becker M. 2014. Climatic variations explain annual fluctuations in French Périgord black truffle wholesale markets but does not

explain the decrease in black truffle production over the last 48 years. *Mycorrhiza* **24**: 115–125.

Limousin JM, Longepierre D, Huc R, Rambal S. 2010. Change in hydraulic traits of Mediterranean *Quercus ilex* subjected to long-term throughfall exclusion. *Tree Physiology* **30**: 1026–1036.

Martínez de Aragón J, Fischer C, Bonet JA, Olivera A, Oliach D, Colinas C. 2012. Economically profitable post fire restoration with black truffle (*Tuber melanosporum*) producing plantations. *New Forests* **43:** 615–630.

Molinier V, Bouffaud ML, Caste T, Mounier A, Colombet A, Recorbet G, Frochot H, Wipf D. 2013. Monitoring the fate of a 30-year-old truffle orchard in Burgundy: from *Tuber melanosporum* to *Tuber aestivum*. *Agroforestry Systems* **87**: 1439–1449.

Montserrat-Martí G, Camarero JJ, Palacio S, Pérez-Rontomé C, Milla R, Albuixech J, Maestro M. 2009. Summer-drought constrains the phenology and growth of two co-existing Mediterranean oaks with contrasting leaf habit: implications for their persistence and reproduction. *Trees: Structure and Function* **23**: 787–799.

Nara K, Nakaya H, Hogetsu T. 2003. Ectomycorrhizal sporocarp succession and production during early primary succession on Mount Fuji. *New Phytologist* **158**: 193–206.

Nardini A, Salleo S, Tyree MT, Vertovec M. 2000. Influence of the ectomycorrhizas formed by *Tuber melanosporum* Vitt. on hydraulic conductance and water relations of *Quercus ilex* L. seedlings. *Annals of Forest Science* **57**: 305–312.

Olivera A, Fischer CR, Bonet JA, De Aragón JM, Oliach D, Colinas C. 2011. Weed management and irrigation are key treatments in emerging black truffle (*Tuber melanosporum*) cultivation. *New Forests* **42**: 227–239.

Olivera A, Bonet JA, Oliach D, Colinas C. 2014a. Time and dose of irrigation impact *Tuber melanosporum* ectomycorrhiza proliferation and growth of *Quercus ilex* seedling hosts in young black truffle orchards. *Mycorrhiza* **24**: 73–78.

Olivera A, Bonet JA, Oliach D, Colinas C. 2014b. Low summer soil temperature and moisture favours root tip colonization of *Quercus ilex* by *Tuber melanosporum In* Book of Abstracts, 1st International Conference on Truffle Research´14, Vic-Barcelona, Spain, 9-12 Mar. 2014.

Olivera A, Bonet JA, Palacio L, Colinas C. 2014c. Adequate weed suppression strategies can promote *T. melanosporum* mycelial expansion and seedling growth in newly established black truffle plantations. *Annals of Forest Science*. in press.

Olivier JM, Savignac JC, Sourzat P. 2012. *Truffe et trufficulture.* Périgueux. France Ed. Fanlac.

Osborn TJ, Briffa KR, Jones PD. 1997. Adjusting variance for sample-size in tree-ring chronologies and other regional-mean time-series. *Dendrochronologia* **15**: 89–99.

Palenzona M. 1969. Sintesi micorrizica tra *Tuber aestivum* Vitt., *Tuber brumale* Vitt., *Tuber melanosporum* Vitt. e semenzali di *Corylus avellana*. *Allionia* **15**: 121–131.

Plett, JM, and Martin, F. 2011. Blurred boundaries: lifestyle lessons from ectomycorrhizal fungal genomes. *Trends in Genetics* **27**: 14–22.

Samils N, Olivera A, Danell E, Alezander SJ, Colinas C. 2003. Aportación de la truficultura al desarrollo socioeconómico: resultados de los estudios realizados en el municipio de Sarrión (Teruel). *Vida rural* **181:** 54–60.

Samils N, Olivera A, Danell E, Alexander I, Fischer CR, Colinas C. 2008. The socioeconomic impact of truffle cultivation in rural Spain. *Economic Botany* **62**: 331–340.

Shaw PJA, Lankey K, Jourdan A. 1996. Factors affecting yield of *Tuber melanosporum* in a *Quercus ilex* plantation in southern France. *Mycological Research* **100**: 1176–1178.

Reyna S, Garcia-Barreda S. 2014. Black Truffle cultivation: a global reality. *Forest Systems* in press.

Valladares F, Gianoli E, Gómez JM. 2007. Ecological limits to plant phenotypic plasticity. *New Phytologist* **176**: 749–763.

Villar-Salvador P, Castro-Díez P, Pérez-Rontomé C, Montserrat-Marti G. 1997. Stem xylem features in three *Quercus (Fagaceae)* species along a climatic gradient in NE Spain. *Trees* **12**: 90–96.

von Arx G, Dietz H. 2005. Automated image analysis of annual rings in the roots of perennial forbs. *International Journal of Plant Sciences* **166**: 723–732.

von Arx G, Carrer M. 2014. ROXAS-A new tool to build centuries-long tracheid-lumen chronologies in conifers. *Dendrochronologia*, doi: org/10.1016/j.dendro.2013.12.001.

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

	Fungi Ecology		Dendro Data				Ring Width		Vessel Count		Vessel Size		Max Vessel Size	
Site (code)	Irrigation (intensity)	Productivity (yield)	Series (no)	Start (>5)	End (>5)	MSL (years)	RW (mm)	Lag1 (r)	VC (no)	Lag1 (r)	VS (μm2)	Lag1 (r)	MVS (μm2)	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

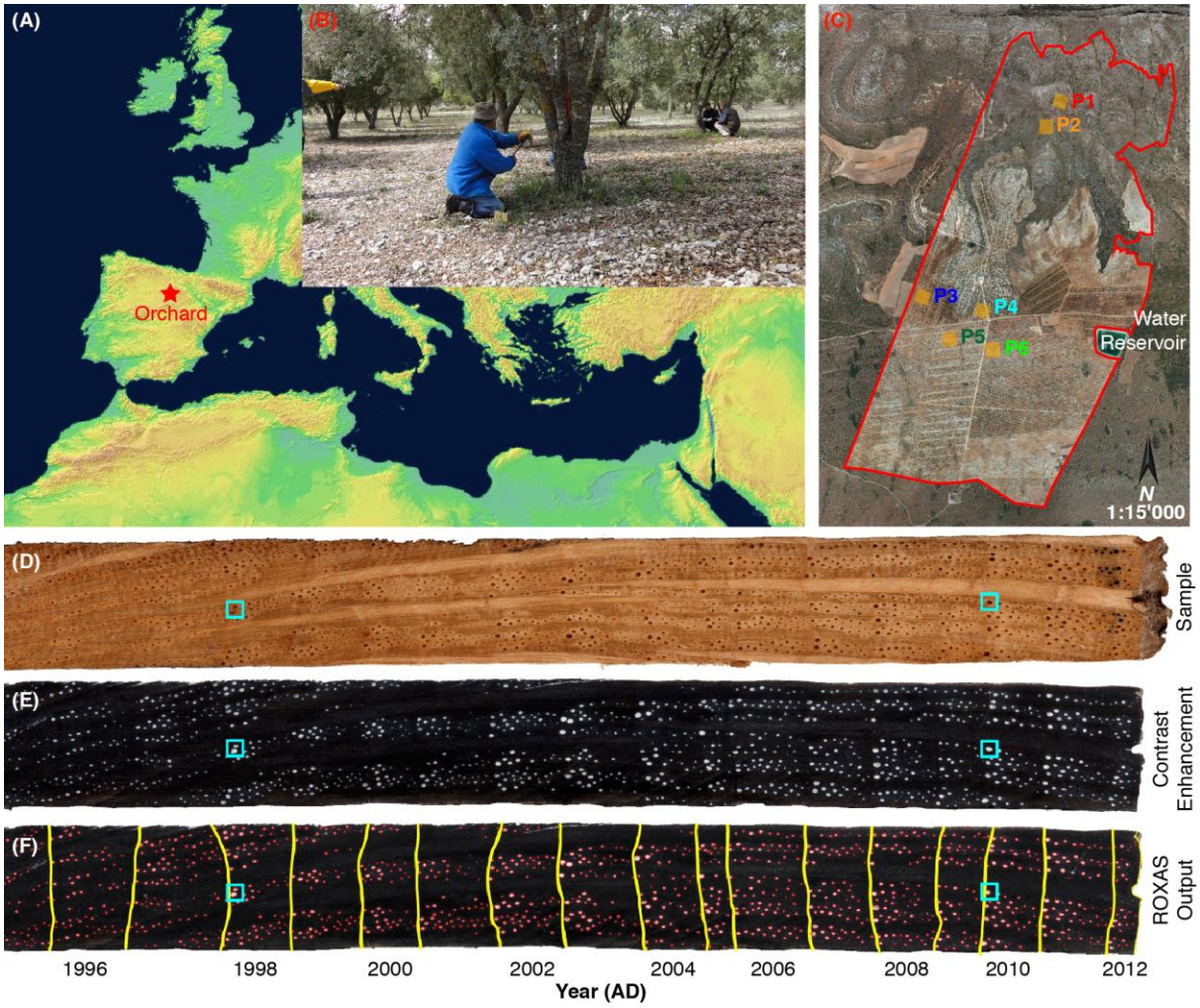

Fig. 1. (a) Location of the world's largest Périgord Black truffle (*Tuber melanosporum*) plantation "Los Quejigares" within the Spanish Province of Soria. (b) Sample collection in (c) the 600 ha large plantation situated between 1100 and 1400m asl at the southern slope of the Sierra de Cabrejas, ~20km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

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

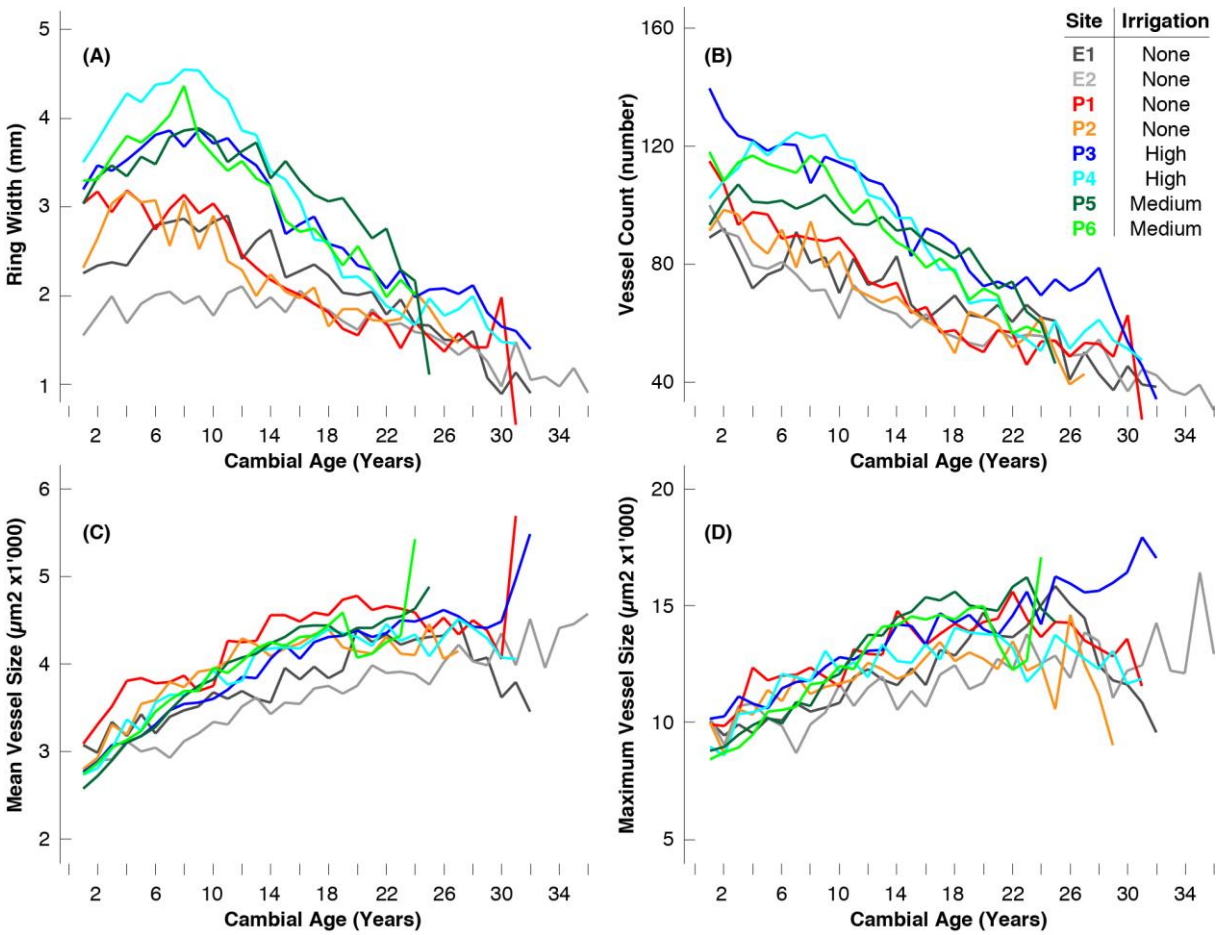

Fig. 2. Biological age-trends of the 295 individual oak samples calculated for (a) RW, (b) VC, (c) VS and (d) MVS, and classified into six sectors within the plantation (P1-P6) plus two reference sites outside the plantation (E1-E2). The resulting Regional Curves (RCs) are truncated at a minimum replication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

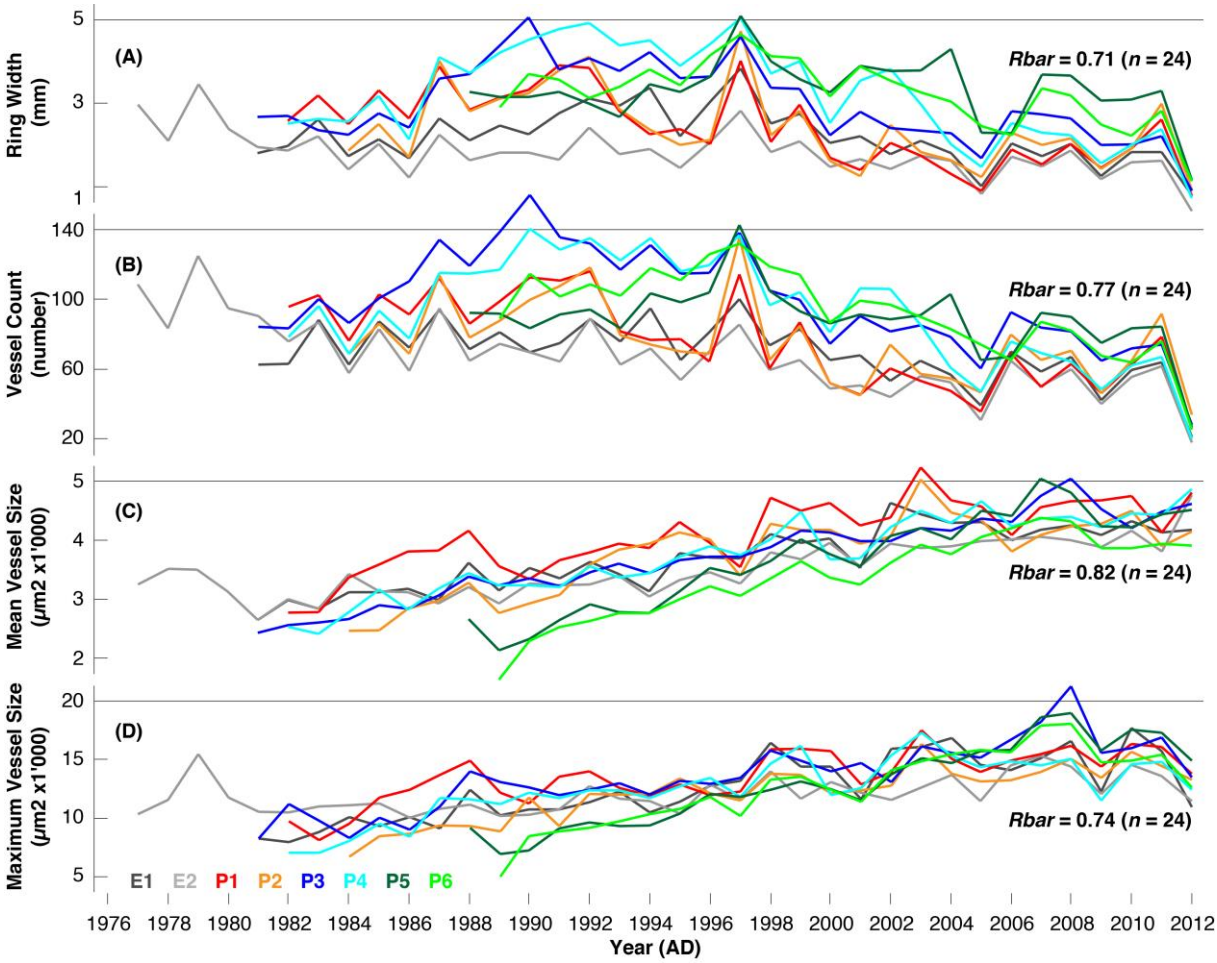

Fig. 3. Temporal variation in (a) RW, (b) VC, (c) VS and (d) MVS, with all data being classified into six sectors within the plantation (P1-P6) plus two reference sites outside the plantation (E1-E2; see Fig. 2). Each of the raw chronologies contains a high fraction of biological induced age-trend, because no detrending was applied at this stage. The *Rbar* values show the degree of parameter-specific coherency between 1989 and 2012, the period common to all records.

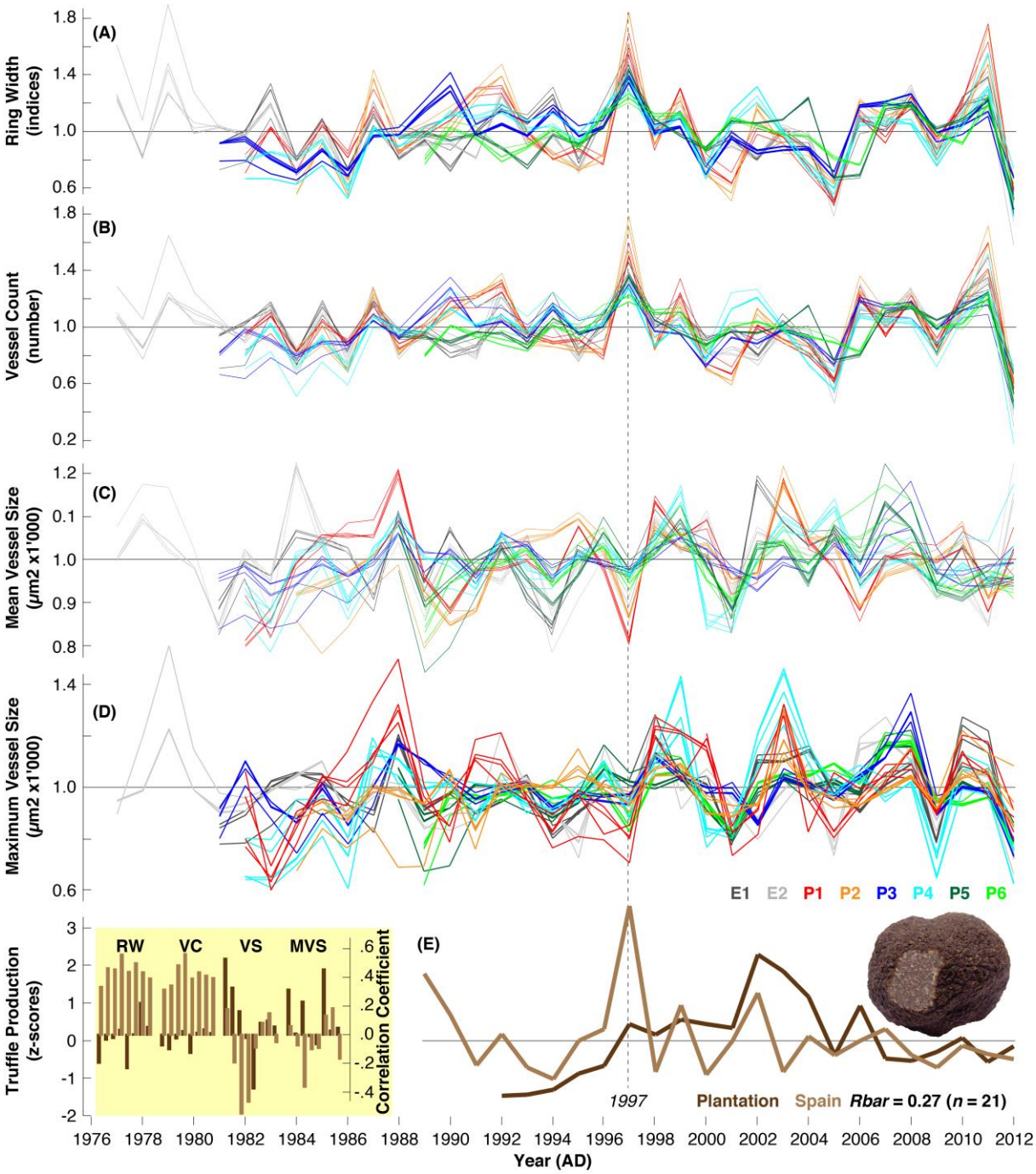

Fig. 4. Chronologies of (a) RW, (b) VC, (c) VS and (d) MVS after the application of different detrending techniques (individual 30-year splines and RCS), index calculations (with and without power-transformation) and chronology versions (standard and residual), with the resulting time-series being classified into six sectors within the plantation (P1-P6) plus two reference sites outside the plantation (E1-E2). See supporting information for more details on parameter- and site-specific growth coherency (Figs. S5-S8). (e) Anomalies of truffle production averaged over the plantation and Spain, together with their correlation (*Rbar*). The inset shows correlating coefficients between truffle production and the RW, VC, VS and MVS

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

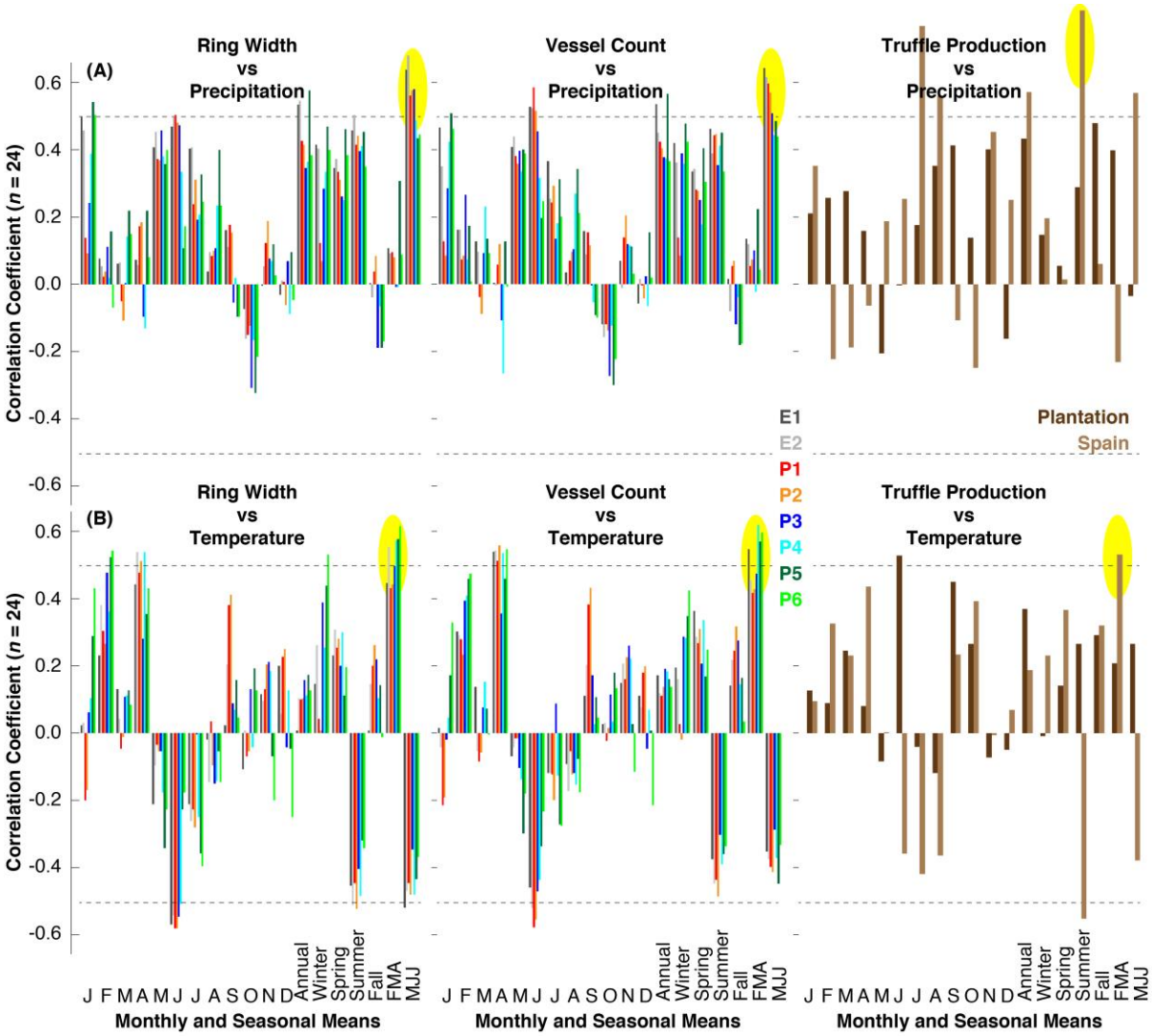

Fig. 5. Correlation coefficients of monthly and seasonal resolved (a) precipitation totals and (b) temperature means computed against the mean RW and VC chronologies from the eight sectors, and records of truffle harvest averaged for the plantation (dark brown) and entire Spain (light brown). The horizontal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

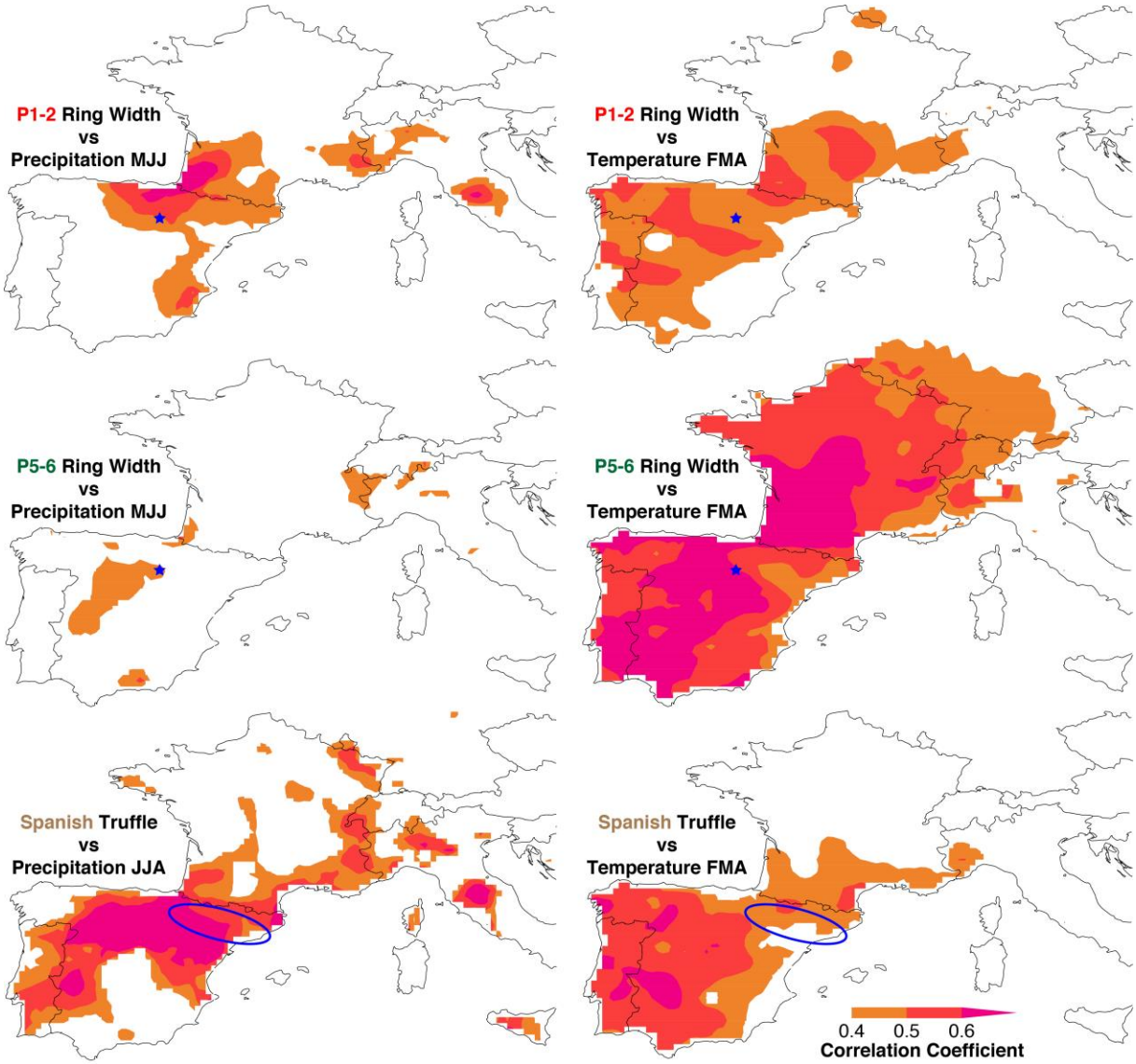

Fig. 6. Spatial correlation fields of the mean RW chronologies from non and medium irrigated sectors (P1-P2 and P5-P6), as well as the Spanish truffle production computed against a European-wide high-resolution gridded dataset of surface temperature and precipitation indices. Blue star indicates location 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.

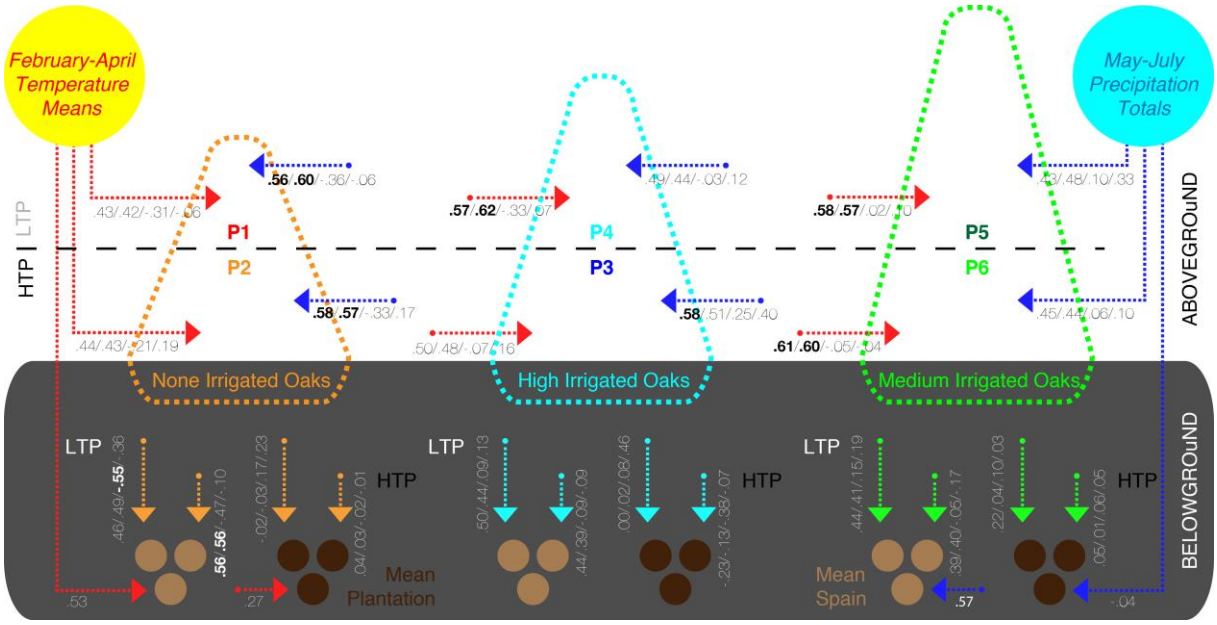

Fig. 7. The most important 48 correlation coefficients obtained between early spring (February-April) temperature means as well as early summer (May-July) precipitation totals (from Soria) and the four different oak chronologies (RW/VC/VS/MVS) from the six sectors in the plantation (main diagram), with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