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Tree ring and water deficit indices as indicators of drought impact on black truffle production in Spain

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Abstract

Black truffle is a highly-prized fungus that constitutes an important economic resource in many Mediterranean forests of southern Europe. Black truffle production in these forests and in cultivated truffle orchards is subject to important interannual variations linked to climatic factors, among which summer water shortage is particularly relevant in dry regions such as Mediterranean Spain. Thus, in recent years irrigation has become widespread in Spanish black truffle orchards, although no long-term proxies of black truffle production exist and scientific guidelines for the irrigation of these orchards are lacking. In this study, we explore the possibilities and limitations of pine tree-ring width data (seasonal wood production) and agroclimatic indices to predict black truffle production in Spain, while gaining more in-depth knowledge on water requirements of truffle production. For this purpose, we used the national annual estimates of black truffle production and compared these values with several tree-ring width series, monthly climatic factors, and water balance-based indices. We found a strong linkage between summer water availability, black truffle production and Mediterranean pine latewood formation, with summer drought intensity reducing both black truffle and *Pinus pinaster* latewood production. The adjusted latewood index for *P. pinaster* and the accumulated water deficit from April to September were robust predictors of black truffle production. The predictive skills of these two variables were further confirmed with a calibration-verification technique. These variables can be used as climatic proxies of black truffle production in agronomic or silvicultural studies, in a sector where no large-scale network for monitoring soil water content exists, and many truffle orchards are small and have low possibilities for modernisation.

Key words: dendroecology; *Tuber melanosporum*; *Pinus pinaster*; fungal fruiting; latewood; water deficit.

1. Introduction

Truffles are an important economic resource in many Mediterranean forests of southern Europe, and the highly prized black truffle (*Tuber melanosporum*) is nowadays cultivated in many countries around the world (Benucci et al., 2012; Reyna and Garcia-Barreda, 2014). The black truffle is an ectomycorrhizal fungus that lives in obligate symbiosis with many tree and shrub species, among which *Quercus ilex* and *Quercus faginea* are the most common in dry Mediterranean regions of Spain, one of the countries producing more truffles worldwide (Garcia-Barreda et al., 2019; Taschen et al., 2015).

Climatic factors have a major influence on black truffle production, particularly during summer (Büntgen et al., 2012; Le Tacon et al., 2014). The fruit bodies (FBs) of black truffle develop in the soil from late spring to the following winter (Montant et al., 1983; Pacioni et al., 2014), obtaining all the carbohydrates for its growth from the host tree (Le Tacon et al., 2013). Thus, radial growth of host tree species as *Q. ilex* has been used to investigate the influence of climate on truffle production (Büntgen et al., 2012; Garcia-Barreda et al., 2020). *Quercus ilex* radial growth responds to summer climatic conditions but also to climate of other seasons, mainly from the previous autumn to the spring (Granda et al., 2013; Gutiérrez et al., 2011). In contrast, the radial growth of pine species –often coexisting with *Q. ilex*– is very sensitive to summer drought, and its seasonal components (earlywood – hereafter EW– and latewood – hereafter LW) allow a more refined analysis of wood production as related to climate (Griffin et al., 2011; Camarero et al., 2015). In spite of pines not producing black truffle FBs in meaningful amounts, *Pinus nigra* LW production has been used as a proxy for summer climatic conditions in wild truffle stands (Garcia-Barreda and Reyna, 2013; García-Montero et al., 2007). However, the predictive skills of this variable have not been compared to those of oak host trees or to those of other Mediterranean pine species with a natural area of distribution overlapping with black truffle in Spain (Garcia-Barreda et al., 2012).

The seasonal patterns of pine radial growth and its sensitivity to summer drought could help to better understand water requirements of black truffle over the several-months period of FB development. The summer of Mediterranean Spain is characterised by warm, dry conditions; exacerbated in recent decades by the regional increase in drought severity, spring temperatures and spring atmospheric evaporative demand (AED) (Garcia-Barreda et al., 2020; Vicente-Serrano et al., 2017a, 2014). In summer, most black truffle primordia have already been formed and the FBs are growing, although many of them degenerate before having fully developed (Montant et al., 1983; Pacioni et al., 2014). Thus, in recent years, irrigation has become widespread in Spanish truffle orchards (Garcia-Barreda et al., 2020), not only in summer but also in spring and early autumn; although no clear guidelines exist except for the empirical criteria proposed by Olivier et al. (2013). In this context, pine radial growth could help to account for climate-driven fluctuations of truffle production in agronomic or silvicultural studies of nearby rainfed *truffières* (Garcia-Barreda and Reyna, 2013), but for irrigated *truffières* it would be more convenient to develop agroclimatic indices that easily allow to integrate the irrigation amount, such as those based on precipitation (Helluy 2020). Tree-ring width records have frequently been used as a proxy to reconstruct past yields of agricultural and forestry crops (Huhtamaa and Helama, 2017; Maxwell and Knapp, 2012; Therrell et al., 2006). When reliable historical records of yields are available, they can be compared with the predicted values to validate these reconstructions and demonstrate the predictive skill of the proxy. Anomalies in the agreement between predicted and actual values for given time intervals have been interpreted in the light of socio-economic changes (Huhtamaa and Helama, 2017).

In this study, we explored the linkages between black truffle production and the radial growth (EW and LW production) of several pine species coexisting with black truffle in its natural habitat, and we examined which were the main climatic conditions involved in this relationship. These pine species do not produce black truffle FBs. However, we hypothesised that pine ring-width indices with higher responsiveness to summer conditions would show a stronger relationship with black truffle production than ring-width indices of truffle host tree species (*Quercus*), due to the differences in the seasonal pattern and sensitivity to summer drought of the radial growth. After that, based on the linkages between truffle production and pine radial growth, we aimed at proposing agroclimatic indices built with precipitation and AED, as a step towards developing indicators for climate-driven fluctuations of truffle production in irrigated plantations. We hypothesised that, among the proposed agroclimatic indices, those more clearly accounting for water deficit intensity and frequency would show a stronger relationship with black truffle production, in line with the fact that part of the FB growth happens during the driest period of the year, this hampering FB survival (Montant et al., 1983; Pacioni et al., 2014). Finally, we assessed the predictive skill of the most promising ring-width indices and agroclimatic indices with calibration-verification trials.

2. Materials and methods

2.1. Black truffle record

The annual estimates for black truffle production in Spain (1970–2018) were obtained from the Spanish Federation of Associations of Truffle Growers. For the statistical analyses, we used data from the fruiting season November 1970 – March 1971 (hereafter called season 1970) to the season November 2012 – March 2013 (i.e. season 2012). During these years, truffle was harvested entirely or mainly in wild forests, with the proportion of cultivated truffles gradually raising from the mid 1990s. Data from season 2013 onwards were excluded because from that year truffle production sharply increased and interannual variability was sharply reduced, mirroring the generalisation of truffle cultivation and the use of irrigation, with Garcia-Barreda et al. (2020) pointing that truffle production from 2013 onwards showed a time trend that could not be satisfactorily explained by climate trends or variability.

We used detrended black truffle production instead of the raw data because black truffle harvests showed a significant time trend during the 1970–2012 period, in consonance with the continued overharvesting and the changes in the structure of truffle-producing forest (Garcia-Barreda et al., 2020, 2018). Truffle production was detrended by applying a general additive model (GAM) with a normal distribution and thin plate regression splines (Wood, 2011). Detrended black truffle record did not show significant autocorrelation.

2.2. Dendrochronological methods

For the purpose of exploring the ability of pine-ring width data to predict black truffle production, the four pine species coexisting with black truffle in its natural habitat in eastern Spain were selected (Garcia-Barreda et al., 2012). These species occupy from low-elevation warm-dry sites (*Pinus halepensis*, *Pinus pinaster*) to mid-to-high elevation cool-wet sites (*P. nigra*, *Pinus sylvestris*) (Camarero et al., 2015). For comparative purposes, the two most common host tree species of black truffle in Spanish wild stands and orchards, *Q. ilex* and *Q. faginea*, were also included (Garcia-Barreda et al., 2019; Reyna and Garcia-Barreda, 2014). In order to enable a sound comparison among species, all the forest stands selected for the dendrochronological analysis were located in a single, relatively small area (a 10-km radius circle), in which all these species naturally coexist subjected to similar regional climatic conditions (Camarero et al., 2015). We decided to locate these stands in Gúdar-Javalambre county (southern Teruel province, eastern Spain), which is the most important black truffle-producing county in Spain, to ensure the representativeness of the forests stands and to optimise the correlations between black truffle production and tree-ring width. Southern Teruel province, together with the surrounding counties in Cuenca, Guadalajara, Castelló and

Valencia provinces, concentrate most black truffle production of Spain (Garcia-Barreda et al., 2019; Reyna and Garcia-Barreda, 2014). Gúdar-Javalambre county is characterized by a Mediterranean climate with a marked summer drought (Fig. S1), with low-elevation stands (*P. halepensis*, *P. pinaster*, *Q. ilex*, *Q. faginea*) showing warmer, drier conditions (mean annual temperature around 12ºC, mean annual precipitation of 400-500 mm) than high elevation stands (*P. nigra*, *P. sylvestris*, with mean annual temperature of 9-10ºC and mean annual precipitation of 500-700 mm).

For each tree species, one chronology or mean site series of tree-ring width data was selected from previously studied forest stands (Table 1). Relatively undisturbed stands, without indications of recent logging, fire, grazing, pests or dieback were selected. Each ring-width chronology was based on at least 15 trees selected among the adult, dominant trees within 0.5-1 ha sampling areas. The mean height of the sampled trees was higher than 7 m in the pine stands and higher than 4 m in the oak stands, whereas the mean diameter at breast height was higher than 35 cm in the pine stands and higher than 8.5 cm in the oak stands (Camarero et al., 2016, 2015). Two cores were extracted at 1.3 m from each tree using Pressler increment borers. Wood samples were sanded until tree rings were clearly visible and then visually cross-dated. Once dated, tree-ring, EW and LW widths were measured to the nearest 0.01 mm along two radii per tree using a binocular scope and a LINTAB measuring device (Rinntech, Heidelberg, Germany). The accuracy of visual cross-dating was checked with the program COFECHA (Holmes, 1983). Tree age at 1.3 m was estimated by counting the number of annual rings in cores that either passed through the stem pith or were close to it so the arc of the innermost rings was visible. In the second case, we fitted a template of concentric circles to the curve of the innermost rings to estimate the number of missing rings.

Tree-ring, EW and LW widths were standardised and detrended by applying a 15-year long cubic-smoothing spline curve and autoregressive modelling to remove the first-order autocorrelation from the individual tree-ring width series. This resulted in pre-whitened or residual ring-, EW- and LW-width indices that were averaged for each site using biweight robust means. Chronology building procedure was carried out using the software ARSTAN v 4.4 (Cook, 1985).

For each of the selected forest stand, chronologies of EW and LW widths were obtained by averaging the values of all the trees sampled. We used residual width indices instead of raw width data because each chronology showed a specific long-term time trend, due to differences in tree age, size, density, site quality and historical factors. In order to remove the dependence of LW on antecedent EW growth, the adjusted latewood-width index (LWa) was calculated as the residuals of the linear regression between EW and LW indices (Meko and Baisan, 2001).

Table 1. Geographical characteristics of the dendrochronological series used in the study (Camarero et al., 2016, 2015). RW: tree-ring width (mean and standard deviation between parentheses). r_{bt}: mean between-trees correlation.

Species	Latitude (N)	Longitude (W)	Elevation (m)	Mean age at 1.3 m (years)	No trees (No cores)	RW (mm)	r_{bt}
P. halepensis	40º17’17’’	0º48’03’’	1095	73	16 (32)	1.75 (0.18)	0.77
P. pinaster	40º16’00’’	0º48’53’’	1070	105	25 (50)	1.13 (0.10)	0.85
P. nigra	40º18’36’’	0º42’58’’	1550	238	26 (52)	0.46 (0.04)	0.71

P. sylvestris	40º19’05’’	0º40’28’’	1770	153	20 (40)	0.92 (0.08)	0.67
Q. ilex	40º18’02’’	0º52’14’’	1135	39 [a]	30 (60)	1.19 (0.75)	0.37
Q. faginea	40º18’02’’	0º52’14’’	1135	43 [a]	16 (32)	0.88 (0.78)	0.63

[a] Age of the shoots. Since these stands are coppice forests, the age of the stumps is higher, although unknown.

2.3. Climate data

Monthly climatic variables (minimum and maximum temperature, total precipitation, and AED) were obtained for the study period (1970–2012) from the dataset described in Vicente-Serrano et al. (2017b). This dataset was generated using the entire climate records of the Spanish Meteorological Agency, which were quality controlled and homogenised. The data was interpolated to a regular grid of 1.21 km^2 and a weekly temporal resolution by means of a universal kriging method. The AED was calculated by means of the FAO-56 Penman-Monteith reference evapotranspiration equation (Allen et al., 1998).

To assess the relationship between climate variables and tree ring indices, for each of the forest stands used in the dendrochronological analysis (Table 1) the climatic variables were obtained from the gridded dataset by selecting the grids including the forest stands.

To evaluate the relationships between climate and truffle production, we used the dataset of natural truffle-producing forests in Garcia-Barreda et al. (2019) (n = 175 sites) as a meaningful representation of the spatial distribution of the black truffle production in Spain during the 1970–2012 period. For each year of the study period, the monthly climatic variables were calculated as the average of the climatic values retrieved for each of the truffle-producing forest in the dataset. Thus, we intended to increase the representativeness of the climatic data, given the spatial distribution of Spanish truffle production and the fact that black truffle production record is not spatially disaggregated (Garcia-Barreda et al., 2019). Monthly climatic variables were also obtained from the same dataset for the 1961–1969 and 2013–2018 periods, for using them in the reconstruction of truffle production. Some climatic indices required the use of climatic data with weekly temporal resolution. In order to calculate them, weekly climatic variables were retrieved from the same dataset, in which each year is composed of 48 “weekly periods” generated to conform to month intervals (Vicente-Serrano et al., 2017b).

2.4. Water balance-based indices

Several indices based on the climatic water balance (precipitation minus AED) were calculated, taking into account black truffle biology, the results of the dendrochronological analyses and previous research on climatic factors affecting truffle production (Baragatti et al., 2019; Olivier et al., 2013). They were computed with a monthly temporal resolution: (i) accumulated water balance (AWB, the sum of the monthly values of water balance), (ii) accumulated water deficit (AWD, the sum of negative monthly values of water balance), (iii) AWB calculated as precipitation minus k × AED, where k is an empirical correction coefficient for AED (we evaluated the following values for k: 0.3, 0.4, 0.5, 0.6, and 0.7; we accordingly call these indices AWB0.3-AWB0.7), and (iv) AWD calculated as in (ii) after applying an empirical correction coefficient (k) to AED (these indices are hereafter called AWD0.3-AWD0.7). These indices were computed over different timescales (from one to seven months) on a nine-month window from April to December. In March-April the growth of the most common tree hosts of black truffle resumes, with the fruiting induction of black truffle happening in May-June. The FB grows and goes through different developmental stages until October, when the FB attains its final size, with FB ripening beginning in November-December (Pacioni et al., 2014; Zarivi et al., 2015).

While AWD more clearly accounts for the intensity and frequency of water deficit periods, AWB allows that wet months compensate for water deficits in previous or subsequent months (Table S1). The batteries of AWB0.3-AWB0.7 and AWD0.3-AWD0.7 indices, in turn, set water deficit thresholds different from AWB = 0. These empirical coefficients are commonly used for crop evapotranspiration assessment and in soil water balance models (Helluy et al., 2020).

Since the climatic indices selected for further insight were based on AWD, we also assessed the suitability of the monthly temporal resolution used. Monthly temporal resolution was compared to weekly, biweekly and bimonthly temporal resolutions for the selected indices. Determining the appropriate timescale (i.e. the duration of the period over which the index is calculated) is key to develop indices built on water balance, but for indices built on water deficit it is also critical to assess the temporal resolution of the climatic data (i.e. the reference time step for calculation), as shown in Table S1.

Another index was finally calculated as a proxy for the frequency of water deficit periods: the number of weeks with negative water balances.

2.5. Statistical analysis

First, detrended truffle production was correlated against pine width indices using bootstrapped Spearman correlations with a 95% confidence interval. We used bootstrapped Spearman correlations instead of Pearson correlations because for several indices a meaningful deviation between both coefficients was found, with samples not meeting the normal distribution or the linearity assumptions. The tree-ring width indices with the highest significant correlations were selected to further explore its possible interest as indicators of truffle production. A Principal Components Analysis (PCA) was used to more clearly show the associations among tree-ring indices.

Then, the response of the selected width indices to climate was compared to the response of truffle production. The selected indices were correlated against monthly values for minimum and maximum temperature, precipitation, AED and climatic water balance on a 14-month window (from September of the year before tree-ring formation until October of the year of ring formation). Likewise, the detrended truffle production was correlated against monthly climatic variables on an 18-month window (from September of the year before FB induction and development until February of the year after FB induction, when late truffles are ripening). All these analyses were performed using bootstrapped Spearman correlations, with a 95% confidence interval. In view of the importance of water availability during summer, the response of truffle production to monthly water balance from April to September was compared to the response of the selected tree-ring indices, using GAMs with a normal distribution and thin plate regression splines (Wood, 2011).

The water balance-based indices were then calculated and correlated with detrended black truffle production using Spearman correlations. The correlation coefficients were used to compare the different indices, timeframes and temporal resolutions.

Finally, to assess the potential of indices to predict black truffle production, we selected the ring-width index showing higher correlation with truffle production, as well as the AWB index and the AWD index with higher correlations. For assessing the predictive skills of the indices, a split-period calibration-verification process was used (Fritts et al., 1990). The black truffle production record for the 1970-2012 period was divided evenly in half, with calibration and verification made on both periods. Several statistics were used to test whether the two calibration models were statistically significant, including the coefficient of efficiency (CE) and the reduction error statistic (RE) (Fritts et al., 1990). Residuals autocorrelation was tested with the Durbin-Watson statistic (DW). Positive values of CE and RE, and values of DW higher than one were considered acceptable, considering the series length (n = 43 years). Then the detrended black truffle production was reconstructed for the 1961–2018 period with the estimated regression parameters and the estimated root mean square error (RMSE).

For the 1970-2012 period, the values predicted by the various indices were compared to the actual production, and interpreted with the support of the slope of the regression between predicted and actual values. The reconstruction of truffle production for the 1961-1969 period was used to discuss truffle production trends for a decade when only non-official data are available, whereas the reconstruction of truffle production for the 2013-2018 period was compared to the actual production to discuss the hypothesis that during this period truffle production could not be satisfactorily explained by variations in climatic conditions (Garcia-Barreda et al., 2020).

Correlation analysis, PCA, GAMs and calibration-verification were performed with the statistical software R (R Core Team, 2019) and packages *mgcv* and *treeclim* (Wood, 2011; Zang and Biondi, 2015).

3. Results and discussion

3.1. Linkages among truffle production, pine radial growth and climate

Detrended truffle production showed significantly positive correlations with *P. pinaster* and *P. nigra* LWa, *P. halepensis* EW, and *Q. ilex* and *Q. faginea* ring-width indices, with the former showing the highest values (Fig. 1a). The high correlation coefficients of *P. pinaster* and *P. nigra* LWa point that they could play a useful role as proxies of black truffle production, even ahead of oak species, despite pine species do not have a relevant participation in black truffle fruiting (García-Montero et al., 2007). The correlation coefficients of *P. pinaster* and *P. nigra* in our study are similar to those found in previous local and regional studies (Büntgen et al., 2012; Garcia-Barreda and Reyna, 2013), thus supporting the representativeness of the forest stands selected for the ring-width chronologies. The PCA explained 63% of the variability in the analysed ring-width indices with the first two components. These PCA components clearly separated the EW width indices of the four pine species from their corresponding LWa, with *Quercus* ring-width indices tending to group with pines EW, and truffle production tending to group with pines LWa (Fig. 1b). The EW of the four analysed pine species –and the ring width of the analysed oak – is mainly related to wet and cool conditions during the previous autumn up to spring (Camarero et al., 2015; Granda et al., 2013; Montserrat-Martí et al., 2009), whereas the LW of pine species is mainly related to cool, wet conditions during summer, with *P. pinaster* and *P. nigra* showing particularly higher responsiveness (Camarero et al., 2015; Pasho et al., 2012).

Supporting these results, the strongest relationships of climate with *P. pinaster* LWa and truffle production were observed for the summer period. The *P. pinaster* LWa responded negatively to maximum temperatures of July and August, negatively to AED from July to September, positively to precipitation from June to September, and positively to water balance from July to September (Fig. 2). Similarly, detrended truffle production responded negatively to maximum temperatures of July and August, negatively to AED from July to September, and positively to precipitation and water balance from June to August (Fig. 3). The correlations with climate for *P nigra* LWa were similar to those of truffle production, although with lower responsiveness to climatic conditions (Fig. S2).

These results point to analogous July-August climatic signals on *P. pinaster* LWa and black truffle production as the cause of the association between these variables. From June to October, black truffles are in developmental stages of intense growth, with the most important weight increase occurring in August-September (Montant et al., 1983; Zarivi et al., 2015). Similarly, September is a month of intense LW formation in Mediterranean pine species

(Camarero et al., 2010). This highlights the importance of water availability during summer, underpinning the use of water balances for predicting black truffle production. The relationship between summer precipitation and truffle production is one of the most thoroughly examined issues in truffle cultivation (Ricard et al., 2003). However, previous research does not agree on which is the more appropriate timeframe for assessing the impact of water availability; and multiple combinations of months between May and September have been used (Baragatti et al., 2019; Büntgen et al., 2019, 2012; Le Tacon et al., 2014; Ricard et al., 2003).

The response of black truffle production and *P. pinaster* LWa to monthly water balances, analysed with GAMs, highlights that both variables followed similar patterns from June to September (Fig. 4). Both *P. pinaster* LWa and detrended truffle production showed a significant, positive and almost linear relationship with July water balance for the observed range of water balance ($F = 14.7$, $P < 0.001$ and $F = 18.6$, $P < 0.001$, respectively; Fig. 4d). In August, the relationship was also significant, positive and linear for truffle production ($F = 19.7$, $P < 0.001$), whereas for *P. pinaster* LWa was only positive for values of water balance higher than -100 mm ($F = 5.3$, $P = 0.009$, Fig. 4e). In September, the response to water balance was significant, positive and linear for *P. pinaster* LWa ($F = 9.5$, $P = 0.004$), whereas for black truffle production the trend was not significant and the best GAM fit pointed to a non-linear response ($F = 2.9$, $P = 0.08$, Fig. 4f). In June, truffle production and *P. pinaster* LWa responses were not significant ($F = 1.9$, $P = 0.15$ and $F = 1.7$, $P = 0.18$, respectively), with the best GAM fits showing similar patterns –a positive linear trend for values of water balance lower than -25 mm (Fig. 4c). For April and May, all the relationships were not significant ($F < 2.0$ and $P > 0.20$ in all cases, Fig. 4).

The parallelism in the responses of black truffle production and *P. pinaster* LWa to summer water balances supports the hypothesis that the association between these variables is due to water availability during the summer dry period (Fig. 5), with black truffle apparently being more responsive to drier conditions in August and *P. pinaster* LWa more responsive to wetter conditions in September (Fig. 4e-f).

However, in the case of black truffle the differences in the range of water balance between the different months (Fig. 4) could be covering up the existence of water balance – truffle production relationships in these months. Black truffle develops in the soil from late spring to the subsequent winter (Montant et al., 1983; Pacioni et al., 2014). The GAMs show that the water balance for the truffle-producing area (calculated as an average of 175 geographically dispersed points) was in July and August lower than -75 and -45 mm for 95% of the years. These are the months for which black truffle showed higher (and linear) responsiveness (Fig. 4d-e). Water deficit situations in July-August are general throughout the different truffle-producing zones of Spain (Garcia-Barreda et al., 2019). Furthermore, in June and September truffle production apparently shows lower responsiveness to values of monthly water balance higher than -20 and -50 mm, respectively (Fig. 4c, 4f). At the other extreme, in April (for which the water balance – truffle production relationship was not significant), values lower than -70 mm were rare (Fig. 4a). If regional climate becomes warmer and drier in the future, as predicted (Vicente-Serrano et al., 2017a), or if a truffle orchard presents warmer and drier conditions than those shown in Fig. 4, the strength or the pattern of these monthly water balance – truffle production relationships could shift. Indeed, Büntgen et al. (2012) found that the relationship between summer precipitation and truffle production was stronger in the drier Spain regions that in France and Italy, two countries where summer precipitation is higher.

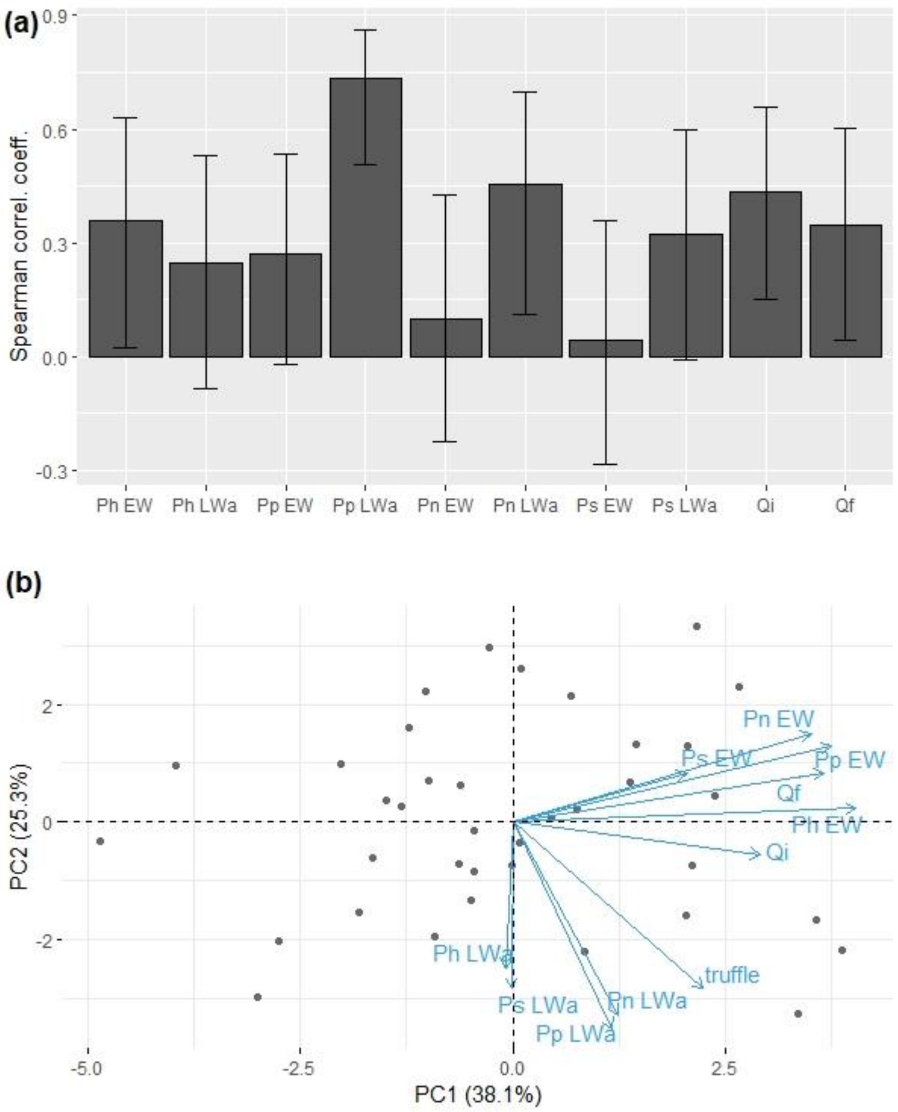

Figure 1. Spearman correlations (and 95% bootstrap confidence intervals) between detrended black truffle production and several ring-width indices, for the 1970–2012 period (a). PCA biplot for the (scaled) annual truffle production (truffle) and ring-width indices during the study period (b). Abbreviations: EW, earlywood; LWa, adjusted latewood; Ph, *Pinus halepensis*; Pn, *Pinus nigra*; Pp, *Pinus pinaster*; Ps, *Pinus sylvestris*; Qf, *Quercus faginea*; Qi, *Quercus ilex*.

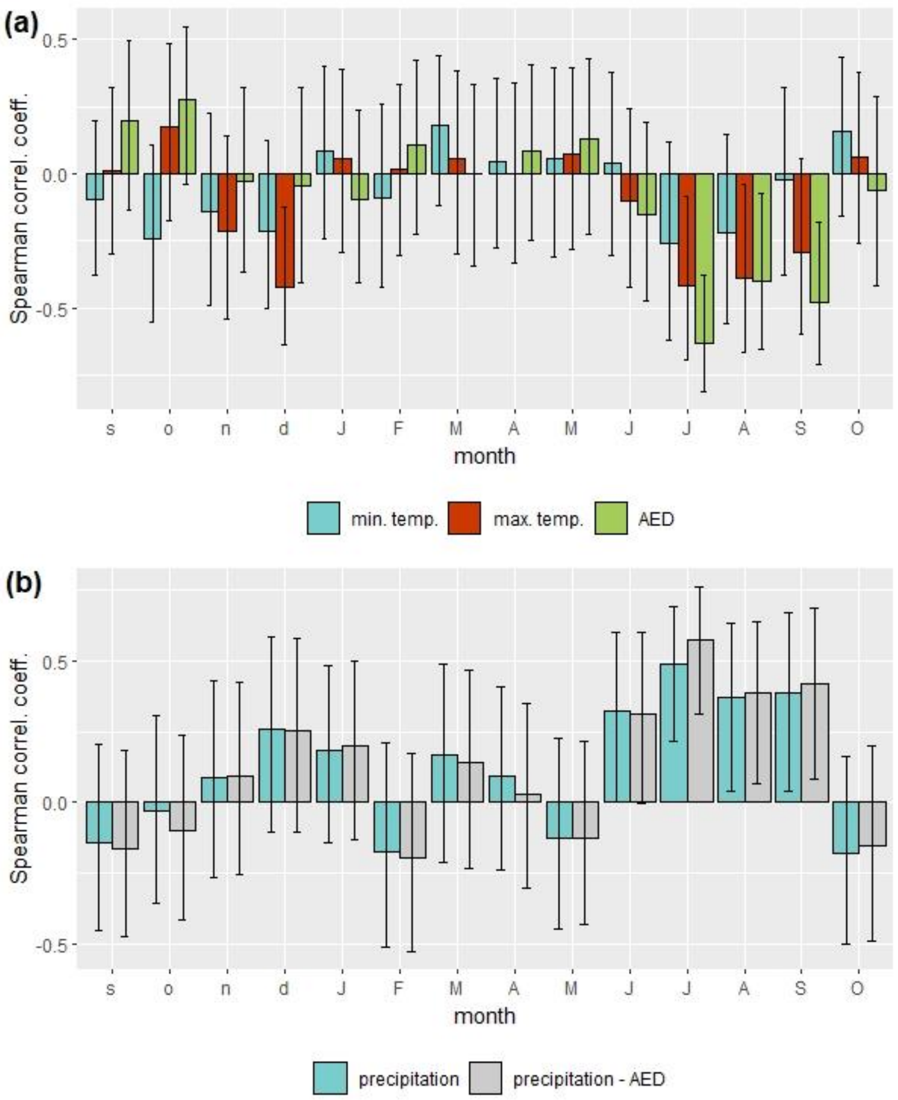

Figure 2. Spearman correlations (and 95% bootstrap confidence interval) between *P. pinaster* adjusted latewood (LWa) width index in Gúdar-Javalambre and climatic variables: monthly minimum temperature, maximum temperature and AED (a), monthly precipitation and water balance (b). Months from the year before ring growth are shown in lowercase, and months from the year of ring growth are shown in capitals.

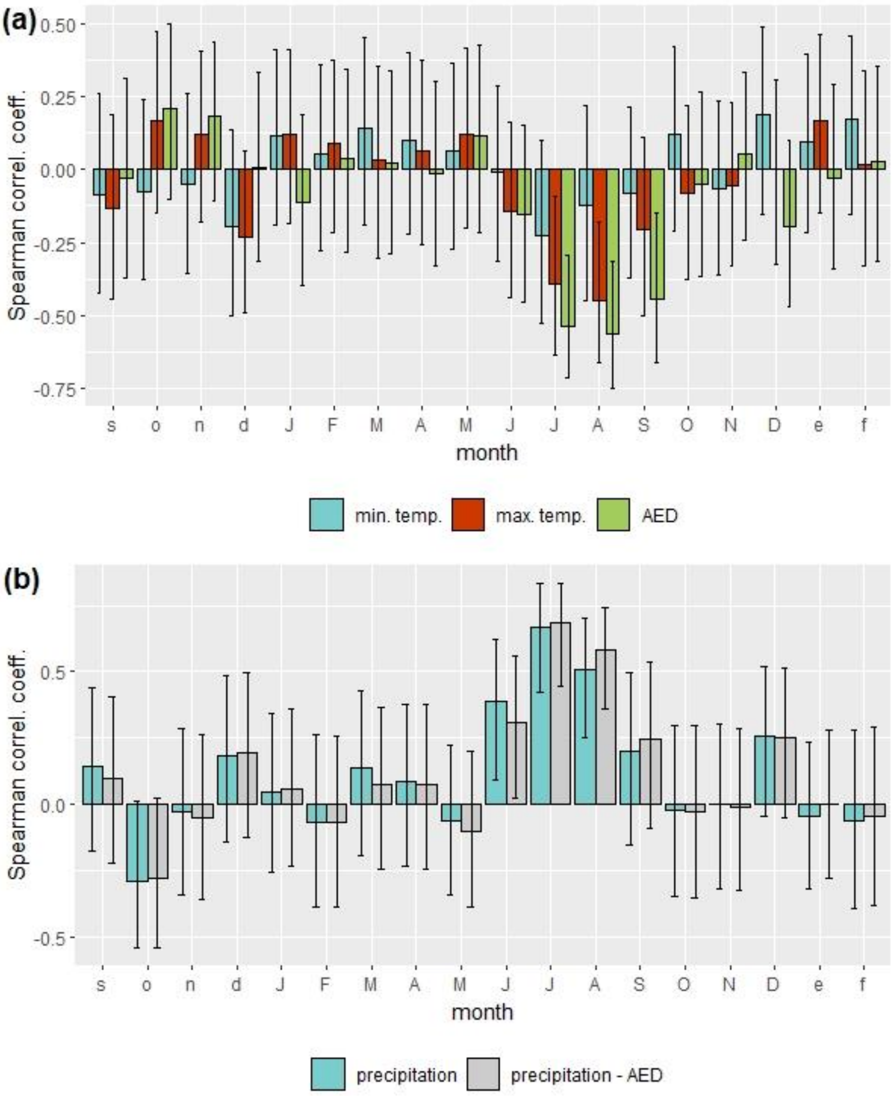

Figure 3. Spearman correlations (and 95% bootstrap confidence interval) between detrended black truffle production and climatic variables: monthly minimum temperature, maximum temperature and AED (a), monthly precipitation and water balance (b). Months from the year before fruiting induction and the year after fruiting induction are shown in lowercase, whereas months from the year of fruiting induction and development are shown in capitals.

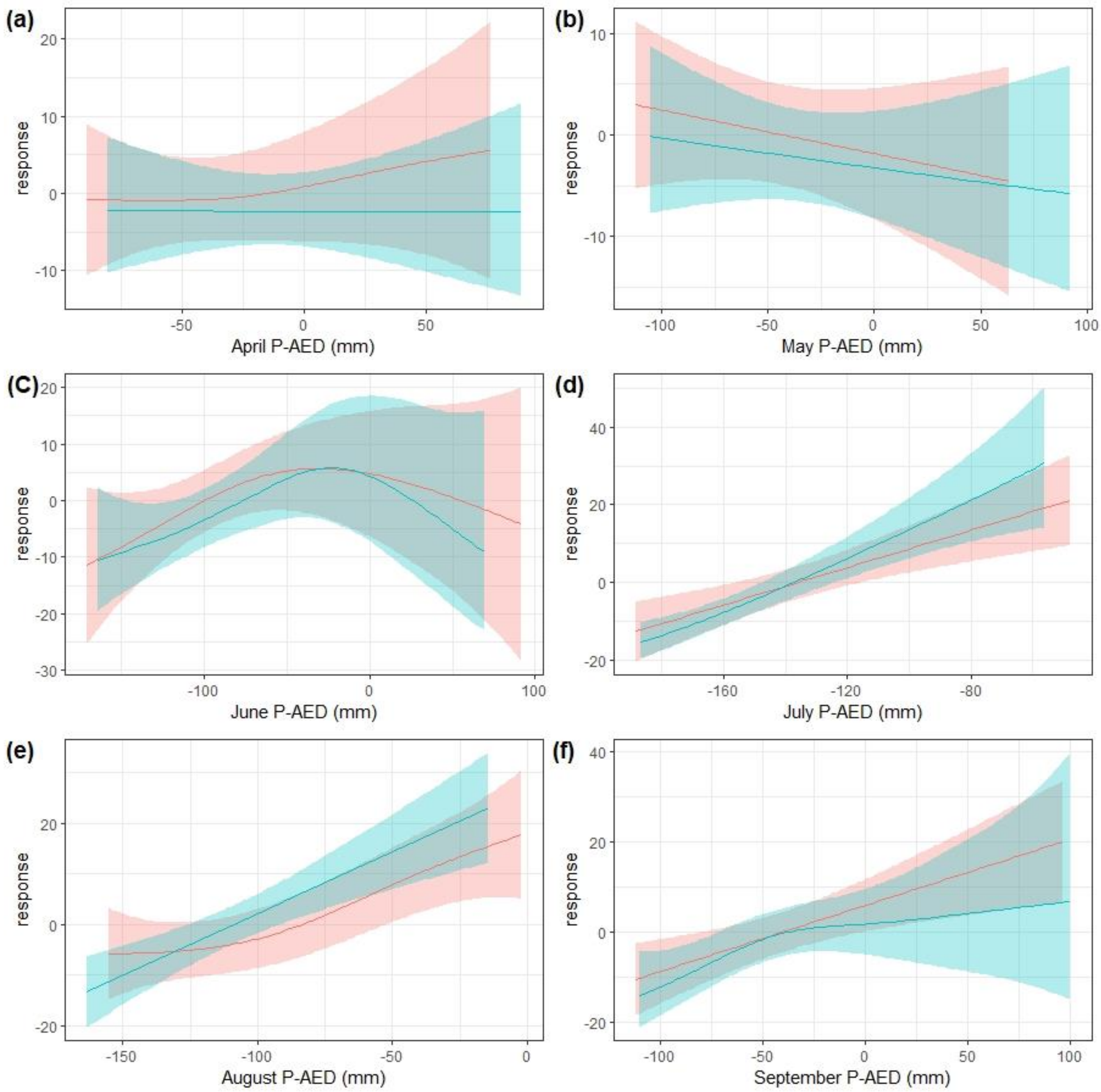

Figure 4. Predicted relationships (mean and 95% confidence band, according to GAMs) of detrended black truffle production (in blue) and *P. pinaster* adjusted latewood production (LWa, in red) with the monthly water balance (P-AED, precipitation minus atmospheric evaporative demand) for the April to September period. The scale of *P. pinaster* LWa is modified (×40) in order to obtain similar band widths for both curves.

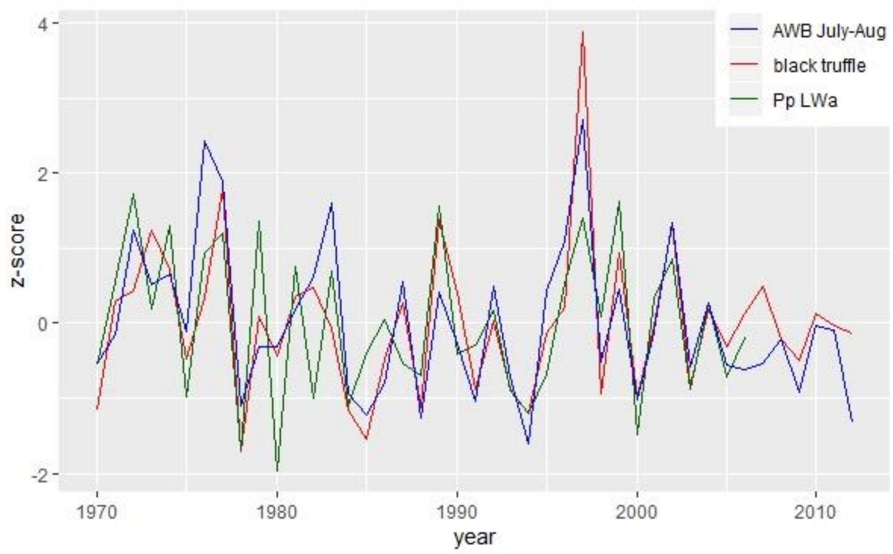

Figure 5. Temporal patterns for the standardised values (z scores) of detrended black truffle production, *P. pinaster* adjusted latewood (Pp LWa) and accumulated water balance (AWB) from July to August during the study period (1970–2012).

3.2. Water balance-based indices

Among the indices calculated with a monthly temporal resolution, those built on water deficit achieved higher correlations with detrended truffle production than those built on water balance (Fig. 6, Figs. S3-S4). The maximum correlations are clearly higher than the correlations found in previous studies –from 0.39 to 0.72 in Büntgen et al. (2019, 2012) and Baragatti et al. (2019)– indicating that water deficit indices can be a robust indicator for truffle production.

The higher correlations achieved by AWD in comparison to AWB agree with the results of Baragatti et al. (2019) and suggest that black truffle production has a lower responsiveness to values of monthly precipitation exceeding the corresponding AED. This supports the hypothesis that months with water surplus cannot compensate (at least not fully) for previous or subsequent months with water deficit, in consonance with the hypothesised relationship between truffle orchard yield and the minimum soil water potential reached during summer (Le Tacon et al., 1982). Moreover, the higher correlations achieved by AWD0.4 in comparison to AWD point to lower responsiveness of truffle production to values of monthly precipitation higher than 40% AED, thus supporting the hypothesis that truffle yield can be optimised at water balances lower than the reference evapotranspiration. This is in line with the fact that mycorrhizal proliferation of black truffle is maximised with a certain degree of climatic water deficit (Olivera et al., 2014).

The highest AWD0.4 values were achieved for the July-September, July-October and July-August periods, with the April-September and April-October periods also achieving remarkable values (Fig. 6c). According to the Akaike information criterion (AIC), the goodness of fit of the AWD0.4 – truffle production relationship (analysed with GAMs, after square-root transforming the detrended truffle production to meet the normality assumption) was equivalent for the July-September (AIC = 109.5) and July-October (110.9) periods, with

July-August (115.3), April-October (121.3), and April-September (121.5) showing worse fits. Similarly, previous studies selected various timeframes for assessing the influence of water availability on black truffle production: July-September (Büntgen et al., 2012), May-August (Le Tacon et al., 2014), June-August, June-September and May-September (Baragatti et al., 2019; Büntgen et al., 2019). The differences among studies could be due to differences in climatic conditions, soil hydraulic properties or host tree species between the different studied areas. They could also be related to differences in the spatial scale of the studies. When the climatic variables or the crop yields are aggregated over a large geographical area, they are less likely to reflect extreme situations, and this could alter the strength and the pattern of the relationship. For instance, Baragatti et al. (2019) found lower correlations at the regional than at the orchard scale. This is particularly relevant for variables showing high spatial variability, such as precipitation or drought severity in Mediterranean Spain (De Luis et al., 2009; Vicente-Serrano et al., 2017a).

The disparate responsiveness of black truffle to water deficit and surplus situations, the likely scale dependence of the climate – truffle production relationships, as well as the irregularity and high spatial variability of Mediterranean climate make AWD0.4 of April-September or April-October a reasonable candidate for predicting truffle production at the orchard scale, since these timeframes cover the sequence of developmental events leading to truffle fruiting. In fact, Olivier et al. (2013) proposed May-September as the recommended period for irrigation of black truffle plantations, and many Spanish plantations are customarily irrigated from April to October. On the other hand, AWD0.4 of July-August or July-September could be enough for predicting black truffle production at a regional scale, due to the high responsiveness of black truffle production to the range of water balance occurring during these months in Spain. For the July-August period, a reasonable alternative to AWD0.4 could be the most parsimonious AWB, which shows a relatively similar value of correlation with black truffle production.

The AWD0.4 index calculated at weekly, biweekly or bimonthly temporal resolutions showed similar patterns to the AWD0.4 index calculated at monthly resolution, although with lower correlations (Fig. S5). This indicates that monthly data are more appropriate for evaluating black truffle production, at least at the regional scale of the study, and suggests that within the monthly temporal resolution the effects of episodic (weekly or biweekly) water deficits on truffle production can be compensated with water surplus in previous or subsequent weeks. On this matter, Olivier et al. (2013) suggested that black truffle FBs in France withstand about 30 days without meaningful rainfall, depending on the climatic conditions and soil water holding capacity, thus recommending to take the decision of when to irrigate on the basis of the previous month precipitation.

Finally, the number of weeks with precipitation lower than $0.4 \times$ AED followed a pattern similar to those of previous indices, showing the strongest (negative) correlations with black truffle production for the July-August and June-October periods (Fig. S6). However, correlations were lower than those of AWD0.4. While AWD indices account for both frequency and intensity of water deficit events, this index accounts only for its frequency.

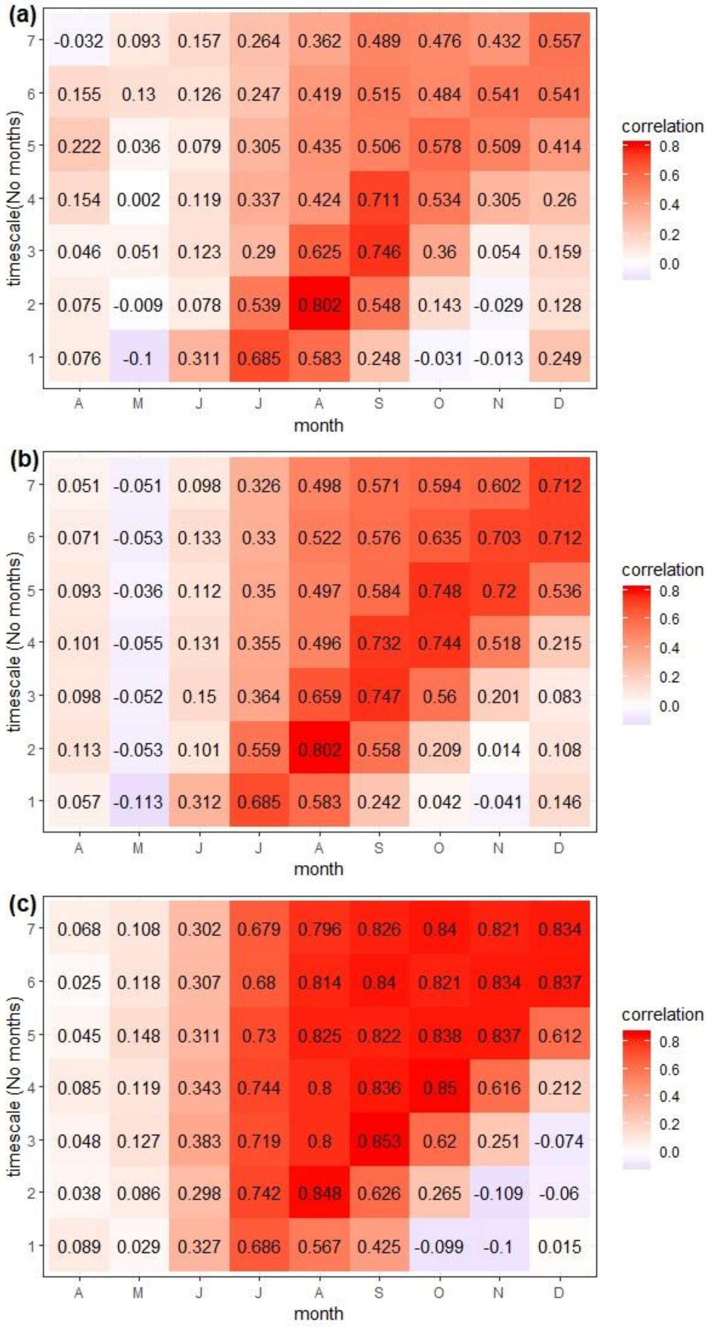

Figure 6. Contour plots of Spearman correlation between detrended truffle production and water deficit indices calculated with monthly climatic parameters: AWB (a), AWD (b), and AWD0.4 (c). The timescale indicates the duration of the period over which the index is calculated, with the time interval finishing in the month indicated on the horizontal axis. Significance thresholds: r = 0.300 for P = 0.05, r = 0.386 for P =0.01, and r = 0.478 for P = 0.001.

3.3. Predictive skill of tree ring and climate indices

The process of calibration-verification was applied to *P. pinaster* LWa, AWB July-August and AWD0.4 July-September. The AWD0.4 April-September was also selected because it showed a high correlation despite the differences in the time interval. The four indices showed a significant relationship with truffle record, verified reasonably well against data of the alternate time period according to the RE and CE statistics (Table 2, Figs. S7-S10), and showed higher R^2 values than calibration-verification trials from previous studies (Büntgen et al., 2019). For the four indices, the predicted black truffle production for the 1970-2012 period agreed reasonably well with the actual values. However, in all cases the indices tended to predict more extreme production values than the observed ones, as pointed out by the slope of the regression between predicted and actual values being lower than one (Fig. 7, Table 2). This was particularly marked for *P. pinaster* LWa and for the 1970-1985 period.

The reconstruction for the 2013–2018 period predicted in all cases values less extreme than the actual ones, particularly for 2016-2018 (Fig. 7); although it was not possible to use *P. pinaster* LWa to make predictions because no chronologies were available for this period. The fact that predictions for 2013-2018 were consistently less extreme than the actual values, whereas for 1970-2012 they tended to be more extreme, supports the hypothesis that the recent increase in Spanish black truffle production cannot simply be explained by propitious climatic conditions and would be related to the generalisation of truffle cultivation and widespread use of irrigation in truffle orchards (Garcia-Barreda et al., 2020). This underlines how important it might be for future truffle research to develop indicators in which irrigation amounts can be easily integrated, such as AWB and AWD.

For the period 1961–1964, the reconstructed black truffle production pointed that the detrended production was similar to that in the 1970–1974 period (-4.0 to +6.5 t, depending on the model), whereas for the period 1965–1969 it pointed to a lower detrended production (-

4.8 to -27.3 t, Fig. 7). Although there is no official record for this period, estimates by Reyna (2012) pointed that average annual truffle production was 47 t in 1960–1964, 72 t in 1965–1969, and 60 t in 1970–1974. Reyna (2007) attributed the productive increase during the 1950s and the1960s to the fact that truffle harvesting was still spreading in Spain, and every year new *truffières* were discovered. The decrease in the early 1970s indicated by Reyna (2012) estimates cannot be solely explained by climate (which was more propitious in the early 1970s), according to our reconstruction. The most likely cause for the decrease is that wild *truffières* already showed the first signs of overharvesting.

Table 2. Calibration-verification statistics (DW: Durbin-Watson statistic; RE: reduction error statistic, CE: coefficient of efficiency, Slope: slope of the regression between predicted and actual values of truffle production)

Calibration	R^2 (P-value)	DW	Verification	RE	CE	Slope (st. err.)
P. pinaster LWa						
1970–2006	0.48 (0.010)	–	–	–	–	0.36 (0.06)
1970–1988	0.41 (0.030)	3.22	1989-2006	0.54	0.54	
1988–2006	0.62 (0.010)	2.71	1970-1987	0.37	0.37	
AWB July-August						
1970-2012	0.61 (0.010)		-	-	-	0.62 (0.08)
1970-1991	0.54 (0.010)	1.76	1992-2012	0.67	0.65	
1991-2012	0.75 (0.010)	1.61	1970-1990	0.47	0.43	
AWD0.4 July-September						
1970–2012	0.65 (0.010)	–	–	–	–	0.60 (0.08)
1991–2012	0.63 (0.010)	1.98	1970-1990	0.60	0.60	
1970–1991	0.74 (0.010)	1.98	1992-2012	0.27	0.24	
AWD0.4 April-September						
1970-2012	0.60 (0.010)		-	-	-	0.60 (0.08)
1970-1991	0.71 (0.010)	1.50	1992-2012	0.40	0.39	
1991-2012	0.55 (0.010)	2.36	1970-1990	0.62	0.60	

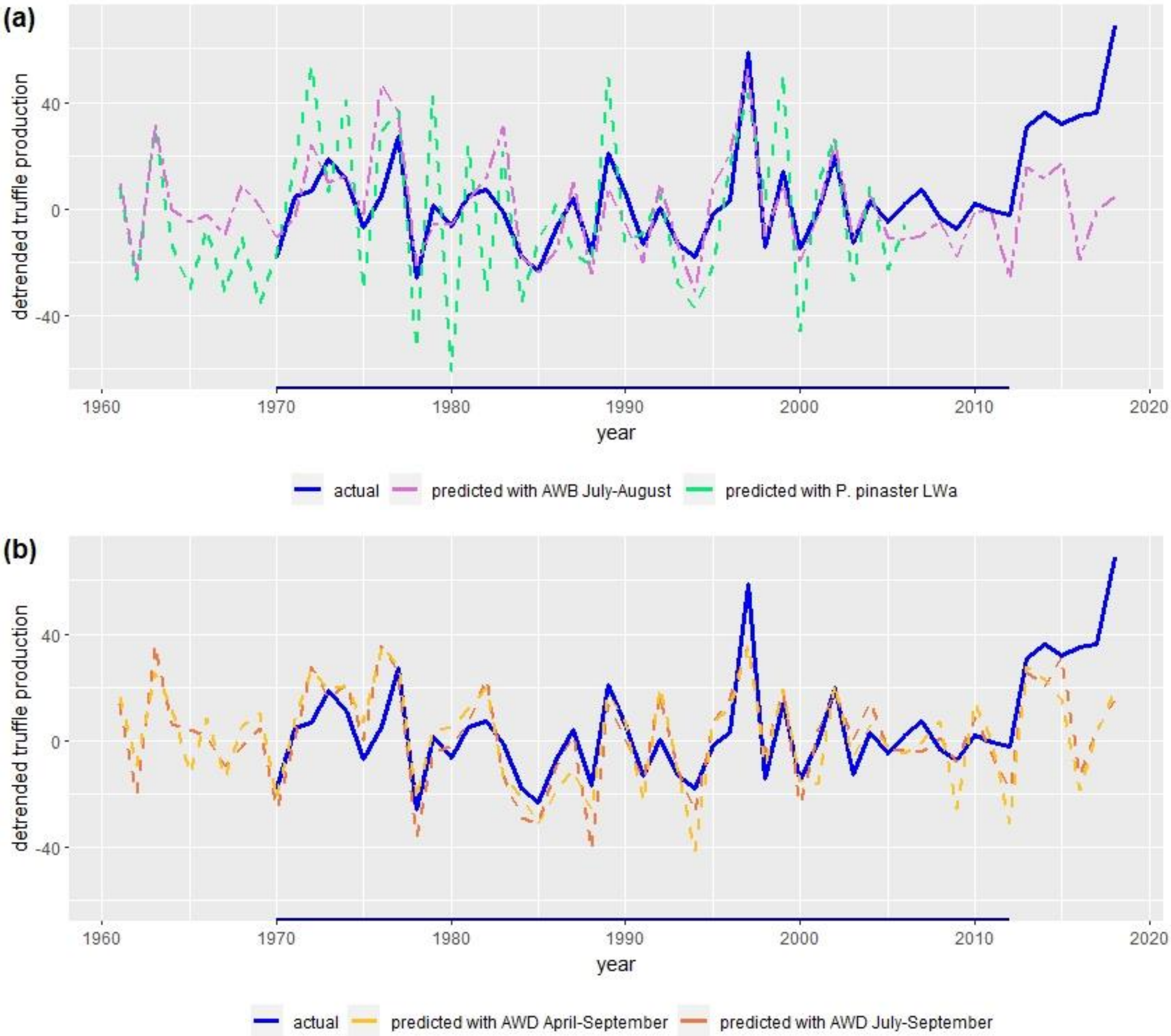

Figure 7. Predicted values of detrended black truffle production for the 1961-2018 period according to the calibration-verification trials (dashed lines), as compared to actual production (solid line) in 1970–2012 (the 1970-2012 period is emphasised in the x-axis). (a) Predicted values using *P. pinaster* LWa and July-August AWB. (b) Predicted values using July-September AWD0.4 and April-September AWD0.4.

4. Conclusions

To conclude, we found a strong linkage between summer water availability, black truffle production and Mediterranean pine latewood formation. Specifically, summer drought reduces black truffle production and *P. pinaster* latewood production. *Pinus pinaster* LWa and AWD0.4 for several timeframes in the April-October window were robust proxies of black truffle production. This is of great practical interest for truffle cultivation in dry regions as Mediterranean Spain, because no large-scale network for monitoring soil water content exists in truffle-producing regions, and many truffle orchards in Spain are of small size and have low possibilities for modernisation. Climatic signals of July-August were responsible for the association between *P. pinaster* LWa and black truffle production, with both variables showing similar response patterns to monthly climatic balances from June to September. The *P. pinaster* adjusted latewood (LWa) could be an interesting climatic proxy for black truffle production in agronomic or silvicultural studies of nearby rainfed *truffières,* since it provides a specific response to summer conditions, when black truffle production is more responsive to climatic factors. On the other hand, the AWD0.4 index showed a stronger relationship with black truffle production than AWB and AWD, indicating the important role of water deficit situations on the assessment of climate – black truffle relationships. These indices, based on precipitation and AED, have the advantage of easily allowing the integration of irrigation amounts, so they are of particular interest to the study of irrigated *truffières*. The strongest relationship of AWD0.4 with black truffle production was obtained for a monthly temporal resolution, suggesting that weekly or biweekly water deficit events could be compensated to a considerable extent with water surplus during the previous or subsequent weeks. On the other hand, our results did not provide a clear response about which is the best timeframe to assess the influence of water deficit on black truffle production, with several timeframes showing a high potential (July-September, July-October, July-August, April-September and April-

October). The predictive skills of these proxies were confirmed with a calibration-verification technique. However, our results were based on truffle productions that were not obtained in controlled conditions of water stress, and climate – truffle production relationships were averaged over a large geographical area. It would be interesting to corroborate the robustness of the proposed climate indices in studies at the orchard and tree scales, in order to fine-tune them. It would also be interesting to evaluate the proposed proxies in other black truffle-producing regions with wetter climates, such as those in France and Italy, in order to assess their generalisability.

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Declaration of Competing Interest

The authors have no competing interests to declare.

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