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Agro-climatic zoning of Spanish forests naturally producing black truffle

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Abstract

Black truffle is a highly appreciated edible fungus that grows wild in southwestern Europe, although its cultivation has recently spread to other continents. In Spain the widespread exploitation of wild truffles began only after 1950, whereas plantations play a relevant role in truffle production from the late 1990s. However, most plantations continue to apply empiric practices not taking into account local environmental conditions. The identification of environmental factors driving black truffle distribution and yield could help to optimise cultivation practices, but no agro-climatic zoning is currently available for this fungus in Spain. This study characterises the climate of Spanish forests naturally producing black truffle, defines an agro-climatic zoning for the fungus and examines the climatic patterns across its spatial distribution. The examined forests presented climatic ranges coherent with the available experts' surveys, except for an extended low end in annual precipitation. The clustering identified three agro-climatic zones, with dry environments tending to be dominant. The principal components analysis indicated that the examined forests tended to cluster along water availability and temperature gradients. Only in one of the zones mean precipitations during the vegetative period were similar to those characterising optimum years for black truffle fruiting, thus suggesting that plantations could benefit from practices increasing soil water content. Similarly, the results suggested that in two of the zones plantations could benefit from practices increasing soil temperature in winter and early spring. The study provides a basis for large-scale planning of truffle cultivation and identification of research priorities in Spain.

Keywords: *Tuber melanosporum*, black truffle, agro-climatic variables, Spain

1. Introduction

The black truffle (*Tuber melanosporum* Vittad.) is a highly appreciated edible fungus worldwide due to its organoleptic quality. It is ectomycorrhizal and typically grows wild in open *Quercus* forests in transitional stages between the Mediterranean and the oceanic climates (Bencivenga et al., 1990; Ricard et al., 2003; Le Tacon, 2016). Its natural distribution is limited to southwestern Europe, being a multi-million euro industry in France, Italy and Spain (Reyna and Garcia-Barreda, 2014).

Although *T. melanosporum* was originally a product merely harvested in the wild, the high demand aroused the interest in its cultivation, achieved for the first time in France during the 19th century. In recent decades truffle cultivation has become an economic alternative for many regions in southwestern Europe and has spread to other continents (Olivier et al., 2002; Reyna and Garcia-Barreda, 2014). In Spain the widespread exploitation of wild truffle harvests began only in the second half of the 20th century, with plantations playing an increasingly dominant role in truffle production from the late 1990s (Garcia-Barreda et al., 2018). Spain is home to many of the southernmost natural locations of *T. melanosporum*, with relatively dry, warm climate and soils with relatively low levels of organic matter (Garcia-Barreda et al., 2007). This could be particularly relevant in view of climate change projections suggesting higher temperatures and lower precipitations, thus increasing irrigation requirements (Büntgen et al., 2012).

Truffle cultivation is not completely domesticated yet and, in most cases, practices applied by growers are empiric, mimicking natural ecosystems or guided by classic handbooks, and frequently not taking into account local conditions (Reyna and Garcia-Barreda, 2014). Management of wild truffle populations and optimisation of truffle cultivation require to identify key environmental factors driving the distribution and yield of the fungus (Ágreda et al., 2016). Agro-climatic zoning studies allow to delimit homogeneous environments from the perspective of a particular crop, thus providing the basis for land use planning, agronomics and identification of research challenges (Yamada and Sentelhas, 2014). However, no systematic agro-climatic zoning for *T. melanosporum* is currently available for the whole of Spain or any other European country. Most of the previous climatic studies are merely descriptive, providing climatic limits based on experts' surveys and commonly used parameters (Pacioni, 1987; Ricard et al., 2003; Garcia-Barreda et al., 2007). Other approaches have simply created regional suitability maps on the basis of the aforementioned parameters (Colinas et al., 2007; Alonso Ponce et al., 2010; Serrano-Notivoli et al., 2015; Serrano-Notivoli et al., 2016). Finally, other approaches assess the correlation between interannual meteorological variability and truffle yields in a particular location (Bardet and Fresquet, 1995; Le Tacon et al., 2014). Despite the limited knowledge about the actual wild truffle presence, yields and their relationship with climatic parameters, the agro-climatic characterisation of wild locations could guide the efforts to identify the key environmental factors for truffle cultivation and harvesting.

In this study we aim (i) to define an agro-climatic zoning for the Spanish forests naturally producing *T. melanosporum*, and (ii) to examine variations in climate among the defined agro-climatic zones. As a secondary aim, the climate of Spanish truffle-producing forests is characterised. The implications of the results on land use planning, evaluation of the climatic requirements of the fungus, management of plantations and adaptation to local conditions are discussed. The truffle-producing locations were selected according to a criterion of good performance of the fungus instead of the traditional limits of existence. This approach benefits from the fact that in most Spain truffle was not exploited until recently and that harvesting became widespread throughout the country in a relatively short time. The selected climatic parameters included both commonly used parameters and parameters more directly related to the physiology of the sporocarp development and the host tree.

2. Material and methods

2.1. Selection of wild truffle stands

The Spanish truffle stands we selected for the study met the following two criteria: (i) forests naturally producing *T. melanosporum*, and (ii) the fungus had been commercially harvested at some point over the period 1990-2017. We assumed that these stands are more likely to present higher occurrence and fruiting yield of the fungus and thus better chances for the establishment of new truffle-producing stands. Sites where simply the occurrence of mycorrhizas has been cited were not considered, as well as stands where fruiting has been cited but without any record of commercial harvesting. The selected 175 wild truffle-producing stands are located in eastern Spain (Fig. 1a).

We collected data about forests with commercial lease agreements for black truffle from regional operating plans and from calls to public auctions. Yet our dataset is probably not complete and with varying degree of completion among regions. No information on private forests is publicly available, so only forests under public administration were included. Only lease agreements for the period 1990-2017 were considered. In most Spain harvesting of wild truffle began between the late 1940s and late 1960s, in all cases with a commercial aim (Garcia-Barreda et al., 2018), so the selected forests continued to provide commercial yields after at least 20 years of intensive harvesting. In addition, information for previous periods is scarce and fragmentary. In municipalities with more than three commercially harvested forests, up to a maximum of three stands were included, avoiding those with distance lower than 3 km.

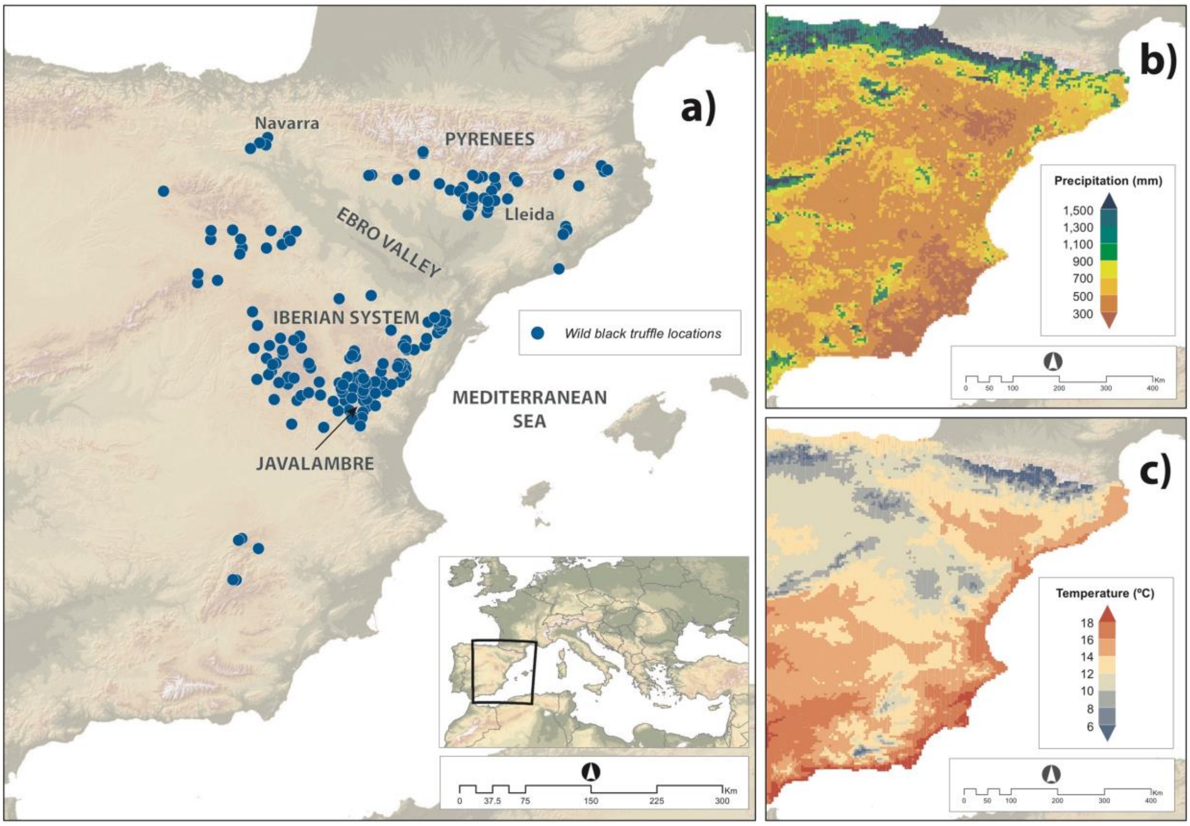

Fig. 1. Location of the selected wild productions of black truffle (a). Mean annual precipitation (b) and mean annual temperature (c) for the period 1961-1990.

A single geographical location corresponding to a truffle-producing stand was selected to represent each forest. In this way we intended to avoid potential issues related to the variability in the surface area of the forests selected, which ranged from less than 100 ha to more than 3,000 ha, and to the distribution pattern of truffle fruiting in wild stands. Truffle-producing stands typically take up only a small portion of the forest surface, showing a clumped distribution (Garcia-Barreda and Reyna, 2013). On the other hand, this approach does not mean that all the truffle production of the forest was concentrated in that stand. No information is available on the truffle yield of Spanish public forests or on the density of truffle-producing stands.

Given the particularities of truffle ecology and harvesting (Garcia-Barreda et al., 2018), we chose to avoid the use of absence data. It is often not possible to reliably corroborate absence points or the factor originating the absence (e. g. unsuitable climate, soil, hosts or forest density, postglacial expansion pattern or intensive harvesting). Truffles grow below ground and their symbiotic phase is microscopic. In edge populations, reproductive structures occur only occasionally (i. e. in years with very favourable weather). In productive regions, particularly in private forests, location and yield of the truffle stands are often withheld to avoid poaching and competition. Bearing in mind these constraints, we feel that our approach is still necessary because it focuses on the variations in climate throughout the natural distribution area and their potential implications on yield and management of truffle plantations.

2.2. Climate data

We selected 16 agro-climatic parameters related to the natural distribution area of *T. melanosporum* or to sporocarp productivity (Bardet and Fresquet, 1995; Ricard et al., 2003; Garcia-Barreda et al., 2007; Le Tacon et al., 2014) (Table 1). The agro-climatic parameters were chosen based on their representativeness of (1) the general mean climatic characteristics (e.g. mean annual temperature and precipitation, temperatures of the coldest and warmest month), or (2) periods which are related to key physiological stages of black truffle sporocarp development (Montant et al., 1983; Montant and Kulifaj, 1990; Coquelin et al., 2007). In this regard, several parameters were constrained to bimonthly aggregations to better represent the key periods for sporocarp formation (late spring), survival and rapid growth (summer) and ripening (winter), which are likely to happen during an extended period comprising more than one month (Montant et al., 1983; Montant and Kulifaj, 1990; Coquelin et al., 2007).

Table 1. Descriptive statistics for the agro-climatic parameters characterising the selected wild truffle stands (n=175).

Parameter (units)	Abbreviation	Mean	Min.	Max.	5th perc.	95th perc.
Mean annual temperature (ºC)	T_ANN	11.1	7.2	14.7	9.1	13.0
Mean temperature of coldest month (ºC)	T_COLD	3.5	0.1	7.8	1.4	5.6
Mean temperature of warmest month (ºC)	T_WARM	20.5	16.9	23.6	18.7	22.5
Mean temperature April-May (ºC)	T_4_5	10.5	6.2	14.1	8.4	12.6
Mean daily temperature range May-June (ºC)	T_DTR_5_6	12.5	8.7	14.8	11.2	13.9
Annual percentage of days with minimum temperature lower than -5ºC (%)	DAYS_ICE	3.7	0.1	10.7	0.8	8.1
Mean annual precipitation (mm)	P_ANN	646	290	1672	386	1124
Mean precipitation March-April (mm)	P_3_4	111	44	337	57	196
Mean precipitation May-June (mm)	P_5_6	129	69	305	79	232
Mean precipitation July-August (mm)	P_7_8	63	19	221	32	148
Mean precipitation September-October (mm)	P_9_10	119	52	301	68	215
Aridity intensity [1]	ARID	0.23	0	0.63	0	0.44
Potential available water March-April (mm) [2]	AW_3_4	-16	-54	117	-49	33
Potential available water May-June (mm) [2]	AW_5_6	-71	-120	33	-102	1
Potential available water July-August (mm) [2]	AW_7_8	-145	-202	-48	-190	-82
Potential available water September-October (mm) [2]	AW_9_10	-24	-68	83	-56	38

[1] Calculated as a non-dimensional ratio between the dry and the humid area in climate diagrams (Walter and Lieth, 1960).

[2] Calculated as the difference between precipitation and reference evapotranspiration according to Hargreaves and Samani (1982).

These parameters were calculated for the climatological period 1961-1990, in which Spanish wild *T. melanosporum* production was at its maximum. Before 1960 truffle harvesting was not widespread in Spain and no information on the national production or the productive status of forests is available. Besides, before 1960 climatic data are scarce and irregular in almost all Spain. On the other hand, after 1990 Spanish wild truffle production was sensibly lower likely due to a combination of habitat deterioration, overexploitation and climate change (Büntgen et al., 2012; Garcia-Barreda et al., 2018).

Precipitation information was collected from the SPREAD dataset (Serrano-Notivoli et al., 2017a), a 5x5 km spatial resolution daily precipitation dataset that has been widely used for high resolution climate analysis in Spain (Serrano-Notivoli et al., 2018a; Serrano-Notivoli et al., 2018b). We extracted the values of the grid points nearest to the pair of coordinates of each truffle stand. Temperature data series were estimated for the same locations of the nearest grid points of SPREAD. The estimates were based in the same methodology as precipitation (Serrano-Notivoli et al., 2017b), estimating daily temperature values through a multivariate logistic regression using altitude, latitude and longitude of the original observations as predictor variables.

2.3. Data analysis

Agro-climatic parameters were clustered using a *k-means* method to provide a first insight about the grouping of truffle stands based on their climatic characteristics. Following Hartigan and Wong (1979) the scaled variables (using mean and standard deviation) were disaggregated into *k* groups so that the sum of squares in the within-cluster was minimized. The number of clusters was chosen according to criteria provided by the NbClust package (Charrad et al., 2014). Differences among clusters were tested through analysis of variance and post-hoc comparison tests with Bonferroni correction.

It was not possible to validate the clustering with previous climatic or production zoning of *T. melanosporum*, because none exists. However, in order to better describe the clusters, their correspondence with three Spanish phytoclimatic and natural vegetation maps was essayed: (1) the phytoclimatic classification by Allué-Andrade (1990); (2) the most abundant truffle-producing tree according to the actual vegetation map by MAGRAMA (2006); and (3) the potential vegetation according to Rivas-Martínez (1987). The relationship between these classifications and the clusters was assessed with the Adjusted Rand Index (ARI), which measures the agreement between two divisions from -1 (no agreement) to 1 (perfect agreement).

Finally, a Principal Components Analysis (PCA) was used to explore the variability explained by climatic parameters and to analyse the relationships between these parameters and *k-means* clusters.

3. Results and discussion

3.1. Agro-climatic characterisation and zoning of wild truffle stands

In the analysed truffle stands the agro-climatic parameters varied within typical values for Mediterranean climate, with mild winters (3.5 ºC of mean temperature in the coldest month and a mean of 3.7% days reaching temperatures under -5 ºC) and dry, warm summers (20.5 ºC of mean temperature in the warmest month) (Table 1).

The values for several parameters spanned over wide ranges, with marked temperature and precipitation gradients from the Mediterranean coast and the bottom of the Ebro valley to the Pyrenees and the main ranges of the Iberian System (Fig. 1b, 1c). Most temperature parameters showed range widths of about 7 ºC. Annual precipitation ranged from less than 300 mm to more than 1,600 mm, with bimonthly precipitations showing differences between stands of up to 80% (Table 1). The aridity intensity ranged from absence of a dry period (in which monthly precipitation is lower than twice the mean temperature) to typical values for semi-arid climate (Table 1). Potential available water from May to August were clearly negative in almost all truffle stands, whereas in March-April and September-October they were more balanced in coincidence with typical seasonal precipitation peaks of Mediterranean Spain (De Luis et al., 2010).

The resulting area suggested by the selected stands (Fig. 1a) was consistent with previous distribution maps (Reyna and Garcia-Barreda, 2009). Mean annual temperature and mean temperature of the coldest and warmest months showed ranges similar to those cited for wild *T. melanosporum* areas in France, Italy and Spain; whereas annual precipitation showed a wider range, particularly in the lower end (Pacioni, 1987; Ricard et al., 2003; Garcia-Barreda et al., 2007).

The *k-means* clustering resulted in three well-differenced groups. Cluster 1 included 46% of the stands and represented warm and dry climate conditions (Table 2). Most truffle stands in this cluster are located near the Mediterranean coast or in the southernmost areas, whereas the remaining are scattered throughout northern areas, mixed with the two other groups (Fig. 2). This cluster reaches the southernmost natural locations of *T. melanosporum* (Olivier et al., 2002). Cluster 2 included 21% of the stands and was characterised by a wetter and colder climate (Table 2). Truffle stands in this cluster are mainly located in the foothills of the Pyrenees and in the slopes of the main ranges of eastern Iberian System (Fig. 2). Cluster 3 included 33% of the stands. It represented a cold and dry climate, typical from inland areas of northern Spain where the influence of maritime airflow from the Atlantic and the Mediterranean is minimum (Table 2). Truffle stands in this cluster are mostly located in central and western Iberian System (Fig. 2).

Table 2. Mean characteristics (and 95% confidence interval) for the agro-climatic parameters in each cluster (parameter abbreviations in Table 1). Letters indicate significant differences ($\alpha = 0.05$) among clusters.

Cluster	1	2	3
N	81	36	58
T_ANN	12.1 (12.0, 12.3) a	10.3 (10.0, 10.6) b	10.1 (9.9, 10.3) b
T_COLD	4.8 (4.6, 4.9) a	2.7 (2.5, 3.0) b	2.4 (2.1, 2.6) b
T_WARM	21.3 (21.1, 21.5) a	19.6 (19.3, 19.9) b	19.9 (19.7, 20.1) b
T_4_5	11.6 (11.3, 11.8) a	9.8 (9.5, 10.1) b	9.5 (9.2, 9.7) b
T_DTR_5_6	12.1 (11.9, 12.3) b	12.0 (11.7, 12.2) b	13.3 (13.1, 13.5) a
DAYS_ICE	1.7 (1.3, 2.1) c	4.5 (4.0, 5.1) b	6.0 (5.6, 6.4) a
P_ANN [1]	504 (479, 532) c	1000 (924, 1082) a	572 (537, 609) b
P_3_4 [1]	82 (77, 88) c	169 (154, 186) a	101 (94, 109) b
P_5_6 [1]	101 (96, 105) c	204 (190, 218) a	116 (110, 122) b
P_7_8 [1]	46 (43, 50) b	116 (104, 129) a	44 (41, 48) b
P_9_10 [1]	104 (99, 110) b	184 (170, 198) a	91 (86, 97) c
ARID	0.30 (0.27, 0.32) a	0.05 (0.01, 0.09) b	0.26 (0.23, 0.29) a
AW_3_4	-33 (-37, -29) c	22 (16, 29) a	-17 (-22, -12) b
AW_5_6	-86 (-90, -83) b	-20 (-26, -15) a	-80 (-85, -76) b
AW_7_8	-149 (-154, -145) b	-99 (-106, -93) a	-166 (-172, -161) c
AW_9_10	-31 (-35, -27) b	19 (13, 25) a	-41 (-46, -37) c

[1] Variable log-transformed

The southeastern Iberian System exhibited a combination of the three clusters with clear climatic gradients: (i) warm and dry (cluster 1) near the coast; (ii) cold and dry (cluster 3) in inland areas and (iii) cold and wet (cluster 2) in higher areas of Javalambre mountain range. On the other hand, there were at least two areas apparently contradicting expected climatic patterns. The Pre-Pyrenees in Lleida province showed a combination of the three clusters in a small space. This could be due to the east-west orientation of local mountain ranges leading to contrasting results in north and south exposures. The northwesternmost stands, in Navarra region, were dominated by cluster 1 despite the marked influence of the Atlantic on the regional climate, predominantly characterised by high precipitations and smooth temperatures. This could suggest that in regions with these conditions *T. melanosporum* seeks for more xeric local climatic characteristics.

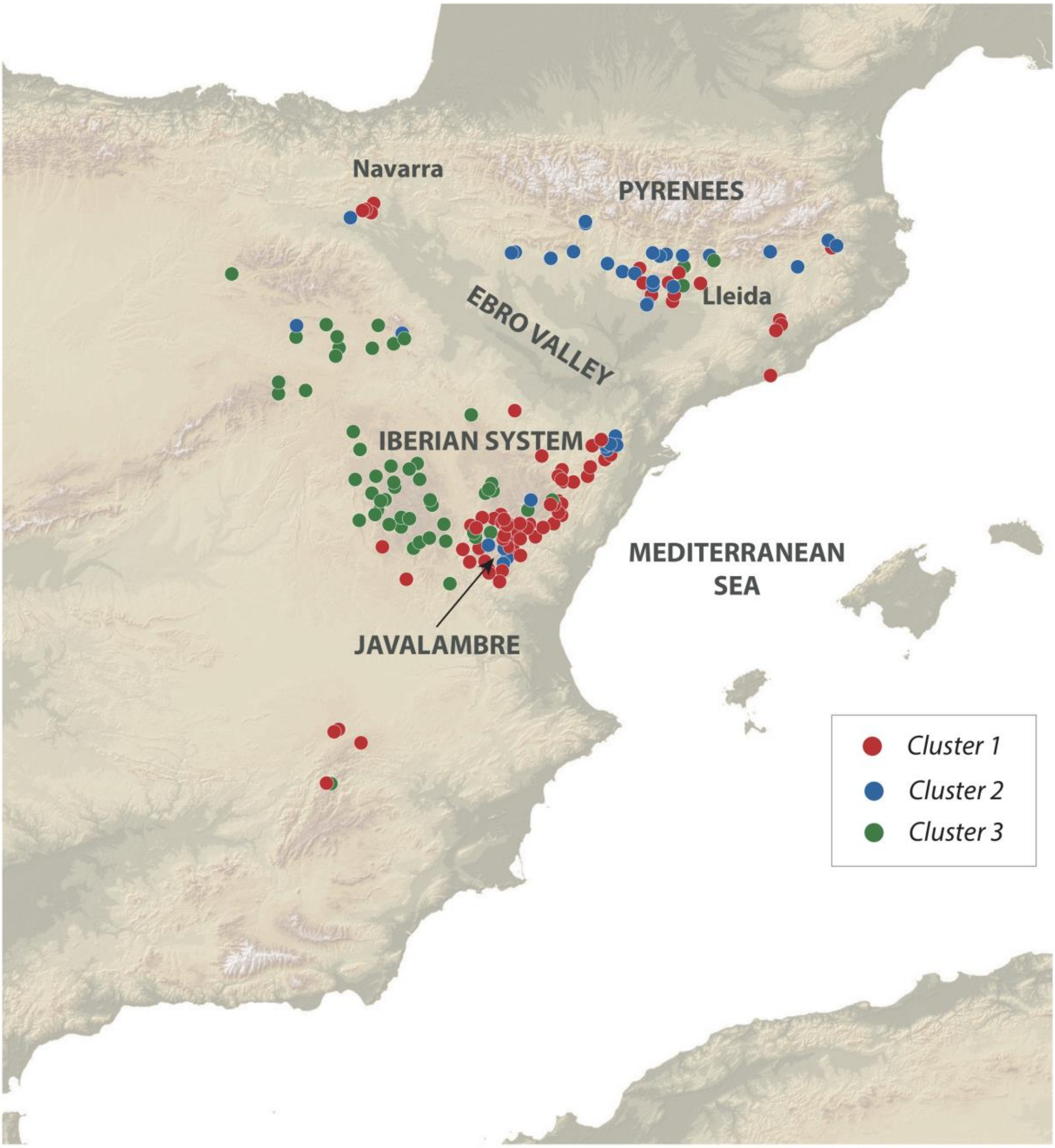

Figure 2. Location of the truffle stands in eastern Spain, coloured by cluster (cluster 1 in red, cluster 2 in blue and cluster 3 in dark green).

The k-means clusters did not show high ARI values (clearly different from a random distribution) with any of the three phytoclimatic and natural vegetation classifications with which they were compared: Allué-Andrade phytoclimate (0.08), Rivas-Martínez potential vegetation (0.19) and actual truffle-producing vegetation (0.06). Almost 90% of the truffle stands were included in phytoclimates VI(IV)$_1$ (52%), VI(VII) (22%) or VI(IV)$_2$ (13%), nemoral phytoclimates with minimal or absent summer drought period. In more than 90% of the stands the potential vegetation was dominated by *Quercus ilex* L. (43% of supramediterranean type and 17% of mesomediterranean type), *Quercus faginea* Lam. (18%) or *Juniperus thurifera* L. (14%). In more than 90% of the stands the most abundant truffle-producing host tree was *Q. ilex* (77%) or *Q. faginea* (18%). *Quercus ilex* is the most widespread *Quercus* species in Spain, being present from sea level to more than 2,000 m of altitude, whereas *Q. faginea* is particularly frequent in Mediterranean-continental climates.

The PCA analysis explained 83% of the data variability with the two first components. The first component corresponded to a water availability gradient and the parameters with the highest loadings were AW_5_6 (0.32), P_5_6 (0.32), P_ANN (0.31) and AW_3_4 (0.30), although the remaining precipitation, potential available water and aridity parameters all showed loadings between 0.25 and 0.29. Spring precipitation and potential available water (March-April, May-June) showed high correlations among them and with annual precipitation (all of them higher than 0.80), while showing slightly lower correlations with summer and autumn parameters (Fig. 3).

The second component corresponded to a temperature gradient and the parameters with the highest loadings were DAYS_ICE (-0.41), T_COLD (0.41), T_ANN (0.39) and T_4_5 (0.39). These parameters showed high correlations among them, being all of them higher than 0.83 (Fig. 3).

These two PCA components allowed to clearly separate the three *k-means* clusters. Stands in the Pre-Pyrenees and Iberian System ranges (cluster 2) were differentiated from the rest by relatively high precipitations and more positive potential available water throughout March to October (Fig. 3, Table 2).

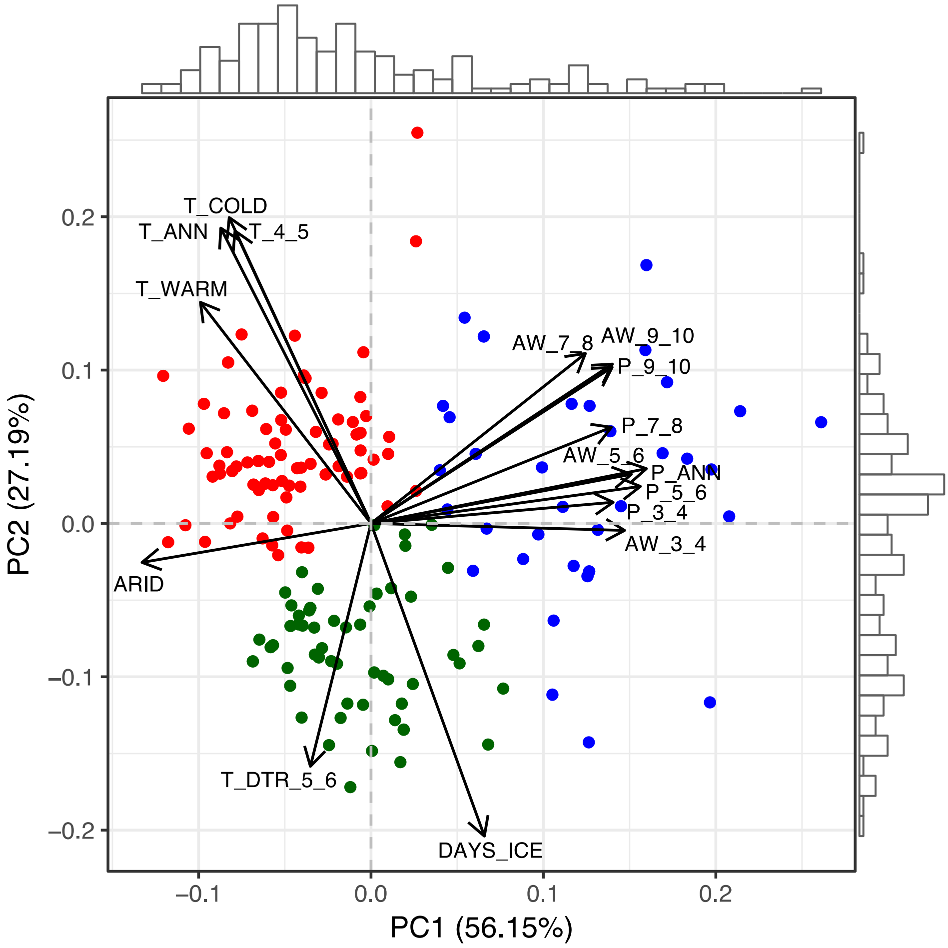

Figure 3. Biplot of the two first (scaled) components of the PCA. The left and bottom axes show PCA scores. Colours indicate *k-means* clusters (cluster 1 in red, cluster 2 in blue and cluster 3 in dark green). Parameter abbreviations as presented in Table 1.

Based on our current knowledge of *T. melanosporum* sporocarp development, May-June is likely the period in which sporocarps are formed, whereas August is likely the period in which they show rapid weight increment (Montant et al., 1983). Climatic studies commonly considered water deficit and precipitation throughout late spring and summer as the parameters explaining most interannual variability in truffle harvest (Ricard et al., 2003; Le Tacon et al., 2014).

Delmas (1976) and Bardet and Fresquet (1995) also pointed out the importance of early spring precipitation, although it could be supplemented by high soil water content linked to late winter precipitation, according to Olivier (2008). In most truffle-producing areas of Spain, bud breaking of *Q. ilex* commonly happens in April. Le Tacon et al. (2013) found that sporocarps depended on carbon transfer from host trees throughout their development, thus suggesting a relevant role of host physiology on truffle yield. On the other hand, autumn precipitation is not considered critical in France, except for long periods of soil flooding or drought (Olivier, 2008).

Stands near the Mediterranean coast (cluster 1) and inland areas (cluster 3) comprise relatively drought-prone areas, with cluster 1 being differentiated from cluster 3 by relatively high temperatures in winter and spring and low frequency of frosts (Fig. 3, Table 2). Soil and air temperatures in April-May, the period of bud breaking and sporocarp induction, have been positively related to truffle harvest (Kulifaj, 1994 in Ricard et al., 2003; Coquelin et al., 2007). A similar relation was pointed out for summer temperature, the period of rapid sporocarp growth (Montant and Kulifaj, 1990; Bardet and Fresquet, 1995), although Ricard et al. (2003) and Olivier (2008) noticed that exceptionally long spells of hot weather could damage truffle harvest. Sporocarp ripening, characterised by spore maturation and aroma development, typically happens from late autumn, along with soil temperature drop (Montant and Kulifaj, 1990). On the other hand, Delmas (1976) and Le Tacon et al. (2014) indicated the negative effect of winter frosts below -5 to -10ºC on truffle harvest.

3.2. Implication on truffle cultivation

Agro-climatic zoning allowing to delimit homogeneous environments from the perspective of a particular crop provides a useful approach to regional planning, agronomics and identification of research challenges (Yamada and Sentelhas, 2014).

Regional planning for truffle cultivation has generally been based on suitability maps assessing climate, soil and topography potential. In Spain climate suitability has mostly been evaluated through annual precipitation, summer precipitation, mean annual temperature and mean temperature of the warmest and the coldest months (Colinas et al., 2007; Alonso Ponce et al., 2010; Serrano-Notivoli et al., 2015; Serrano-Notivoli et al., 2016). Our results showed that for eastern Spain, where truffle naturally grows, annual precipitation and temperature were highly correlated with most of the climatic parameters related to sporocarp development (Fig. 3), thus confirming their usefulness and suggesting that they alone could provide a coarse estimate for climate suitability. The most useful parameters to supplement them, according to our results, could be July-August (or September-October) potential available water, aridity intensity and mean temperature of the warmest month, due to their correlation with annual parameters not being especially high despite their high loading values (Fig. 3).

In the last 20 years in Spain, truffles from plantations have an increasingly dominant position compared to those harvested in the wild (Garcia-Barreda et al., 2018). Truffle cultivation is not completely domesticated yet and many practices are empiric, mimicking natural ecosystems or following classic handbooks (Reyna and Garcia-Barreda, 2014). One of these empirically developed practices is irrigation, which has become key in many regions due to recent climate trends and future projections for precipitation (Millán et al., 2005; Büntgen et al., 2012; Le Tacon, 2016).

In the three clusters, precipitation and potential available water followed very similar patterns of seasonal variation, with values of cluster 2 being markedly higher than the rest and hence with much lower aridity intensity (Table 2). This demonstrates that in the wild *T. melanosporum* is able to grow in contrasting drought-stress environments, with the higher occurrence of truffle stands in relatively dry clusters apparently suggesting that the fungus prefers these environments. However, differences among clusters in water availability run in parallel with differences in vegetation cover and growth, with wetter climates leading to improved vegetation performance (Vicente-Serrano et al., 2006; Alcaraz-Segura et al., 2010). Canopy closure negatively affects habitat suitability for truffle fruiting (Garcia-Barreda and Reyna, 2013) and this could explain that in dry regions conditions of low canopy cover linked to high habitat suitability are more frequent and persist longer term.

This interaction of water availability and canopy closure is not such a big deal in most plantations, which use to be intensively pruned (Olivier et al., 2002; Ricard et al., 2003). Cluster 2 was the only one showing mean precipitations similar to those characterising the summer period and the early spring of optimum years, according to data reviewed by Ricard et al. (2003) for the former and to Bardet and Fresquet (1995) for the latter. Thus, in plantations located in areas with a climate analogous to clusters 1 and 3 irrigation could be used to improve potential available water taking cluster 2 as a reference. This would imply maintaining a slightly positive potential available water in March-April and September-October, allowing a slight water deficit in May-June and a more important water deficit (about 50 mm per month) in July-August (Table 2, Fig. 4). In an average year, it would mean 25-30 mm of irrigation each month from March to October. Le Tacon et al. (1982) already showed that sporocarp yield could be increased with irrigation, whereas Olivera et al. (2014) showed that the spread of *T. melanosporum* mycorrhizas in young plantations was favoured by a certain degree of water deficit. Büntgen et al. (2015) found that in an adult truffle plantation tree-ring growth was enhanced by medium –instead of high– irrigation intensity.

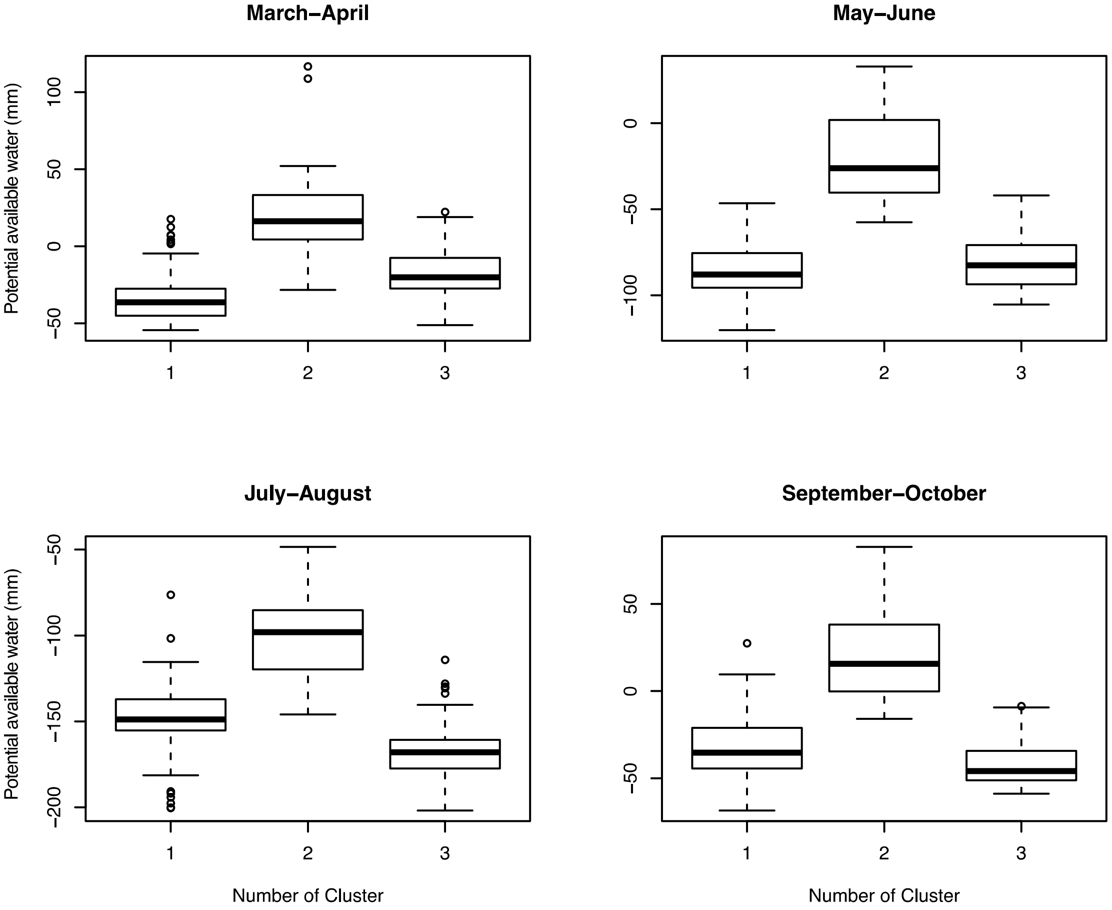

Fig. 4.Variability of mean bimonthly potential available water within clusters

Managing soil temperature for truffle cultivation is more difficult than managing water content. Pruning and management of soil cover (e. g. grass cover, mulching) are the most useful tools (Ricard et al., 2003), although others such as soil tilling could also have a non-negligible influence. Stands in cluster 1, which showed mean temperatures in the upper range within *T. melanosporum* natural area (Garcia-Barreda et al., 2007), were the only ones showing mean April-May temperature close to those pointed by Kulifaj (1994) in Ricard et al. (2003) for optimum years. Cluster 1 also showed a relatively low frequency of severe frosts, similar to the average value found by Le Tacon et al. (2014) in Richerenches region. The remaining clusters showed higher mean frequencies, which in Richerenches were related to years of low yield. Thus our results suggest that plantations located in areas with a climate analogous to clusters 2 and 3 could benefit from practices increasing soil temperature in early spring and winter (in any case, monitoring of soil temperature would be necessary to address this issue in greater detail).

However canopy and soil cover have an effect not only on temperature but also on water content. During the growing season, higher temperatures intensify potential evapotranspiration, with prolonged droughts jeopardising sporocarp survival (Montant and Kulifaj, 1990; Olivier, 2008). This raises serious concerns about the interest of increasing soil temperature in non-irrigated plantations, especially in highly variable, drought-prone climates. On the other hand, it would be interesting to assess the potential of soil temperature management as a tool to boost truffle yields in irrigated plantations with climates analogous to clusters 2 and 3. In all cases these practices should not compromise potential available water or autumn temperature drop and should not be responsible of extreme and prolonged soil temperatures in summer (Montant and Kulifaj, 1990). Büntgen et al. (2015) found that a combination of warm spring and wet summer favoured tree-ring growth in an irrigated truffle plantation.

Some caution is required in extrapolating these results to the field. Climate change projections suggest lower precipitation and higher temperatures in southwestern Europe (Büntgen et al., 2012). For clusters 1 and 3 this means that in the short-medium term mean precipitation could drop below or near the lower end of the range in natural areas, increasing irrigation requirements. For cluster 1 it could also mean that mean temperatures could rise near the upper end of the natural range, with temperatures of the warmest month rising even above. This would require a rearrangement of cultural practices in plantations (Le Tacon, 2016). Wild populations in these areas would likely be highly vulnerable to climate change. Conservation strategies should take into account the existing genetic diversity (García-Cunchillos et al., 2014).

Another limitation of the study is that the agro-climatic zoning approach is not a manipulation experiment and lacks direct measures of the soil environment. Thus, we focused on identifying ecological patterns and research challenges. Irrigation research with precise soil temperature and water content measurements would be needed to fully understand irrigation needs and develop scientific irrigation programs (Le Tacon, 2016). Analysis of the correlation between interannual meteorological variability and truffle yields could be an intermediate step to approach this issue (Le Tacon et al., 2014).

4. Conclusions

To our knowledge this is the first agro-climatic zoning for *T. melanosporum* cultivation in Europe and the first agro-climatic characterisation of Spanish forests naturally producing *T. melanosporum* that combines data from the whole country. Despite the lack of complete and reliable information about wild truffle presences and absences, the methodology has proved useful as a large-scale approach to truffle cultivation in Spain. Our results support the suitability of the climatic parameters and ranges commonly used in potentiality maps for Spanish regions. Three agro-climatic zones have been identified with a cluster analysis: (1) warm and dry climate conditions near the Mediterranean coast; (2) wet and cold conditions in the foothills of the Pyrenees and Iberian mountain ranges; and (3) cold and dry climate of the inland areas. The study provides insight into the climatic factors likely limiting harvests in the various zones and highlights the relevance of adapting cultural practices to environmental conditions. This can help truffle growers to plan ahead cultural itineraries and foresee climatic challenges. It can also help researchers to focus experimentation on the most likely limiting factors and to model the response of wild and cultivated truffles to climate change. Assessing the influence of these factors on each stage of the fruiting body development with manipulation experiments in which soil temperature and water content are precisely monitored could greatly improve the management of truffle plantations.

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