Mycorrhiza (2014) 24 (Suppl 1):S55–S64
DOI 10.1007/s00572-014-0558-7

ORIGINAL PAPER

Soil physical properties influence "black truffle" fructification in plantations

Rafael Alonso Ponce · Teresa Ágreda · Beatriz Águeda · Jorge Aldea · Fernando Martínez-Peña · María Pilar Modrego

Received: 29 August 2013 / Accepted: 9 January 2014 / Published online: 2 February 2014

Abstract Although the important effects of pH and carbonate content of soils on "black truffle" (*Tuber melanosporum*) production are well known, we poorly understand the influence of soil physical properties. This study focuses on physical soil characteristics that drive successful production of black truffles in plantations. Seventy-eight *Quercus ilex* ssp. *ballota* plantations older than 10 years were studied in the province of Teruel (eastern Spain). Soil samples were analyzed for various edaphic characteristics and to locate *T. melanosporum* ectomycorrhizae. The influence of cultivation practices, climatic features, and soil properties on sporocarp production was assessed using multivariate analyses. Low contents of fine earth and silt and high levels of bulk density, clay content, and water-holding capacity appear to promote fructification. Watering is also highly positive for truffle fructification. We develop and discuss a logistic model to predict the probability of truffle fructification in field sites under consideration for truffle plantation establishment. The balance between water availability and aeration plays a crucial role in achieving success in black truffle plantations.

Electronic supplementary material The online version of this article (doi:10.1007/s00572-014-0558-7) contains supplementary material, which is available to authorized users.

R. Alonso Ponce · T. Ágreda · B. Águeda · J. Aldea · F. Martínez-Peña · M. P. Modrego
Centro de Investigación Forestal de Valonsadero. Consejería de Fomento y Medio Ambiente, Junta de Castilla y León, Apdo. de correos 175, 42080 Soria, Spain

R. Alonso Ponce (✉)
40517 Aldeanueva del Monte, Segovia, Spain
e-mail: rafalopon@yahoo.es

Present Address:
F. Martínez-Peña
Research Unit of Forestry Mycology and Trufficulture, Cesefor Foundation, 42005 Soria, Spain

Keywords *Tuber melanosporum* · Fine earth · Silt · Soil aeration · Water availability · Production logistic model

Introduction

The "black truffle" (*Tuber melanosporum* Vittad.) is a hypogeous Ascomycota, fam. Tuberaceae, endemic to Mediterranean calcareous soils in southern Europe and forms ectomycorrhizal associations with the roots of oaks (*Quercus* spp.), hazel (*Corylus avellana* L.), and other broad-leaved trees. In the last four decades, advances in truffle research, including the black truffle, have provided important information about their biology (Rubini et al. 2012; Zampieri et al. 2012), genetic features, such as the whole genome sequencing of *T. melanosporum* (Martin et al. 2010), ecological requirements (Alonso Ponce et al. 2010; Bragato et al. 2010; Lulli et al. 1999), and helpful silvicultural and cultivation practices (García-Barreda and Reyna 2013; Olivera et al. 2011; Valverde-Asenjo et al. 2009). Nevertheless, we are far from having an in-depth knowledge of the biological processes that rule the triggering and maturing of sporocarps.

Regrettably, our increasing knowledge of truffle biology and ecology has been paralleled by a steady and sometimes dramatic decline in the production of black truffles in natural stands due to an interaction of ecological and social factors (Büntgen et al. 2012; Hall and Zambonelli 2012). Given this situation, as well as the high price and demand for truffles, cultivation of black truffles has increased dramatically in Mediterranean countries and elsewhere in the world. Nonetheless, successful large-scale cultivation has not progressed enough to satisfy the rising demand (Samils et al. 2008). In addition, the hasty expansion of commercial truffle production in some areas of Spain has led to discouraging failures or uneven production (Águeda et al. 2011), probably due to an incomplete assessment of the suitability of sites for truffle cultivation.

The importance of cultivation practices such as weed control and irrigation (Bonet et al. 2006; Olivera et al. 2011) or of chemical properties of soils such as pH and carbonates has been reported by several authors (Bencivenga and Granetti 1989; García-Montero et al. 2009; Valverde-Asenjo et al. 2009). Accordingly, soil chemical conditions, cultivation practices, and climate are typically considered when designing a new plantation for truffle production. However, truffle ectomycorrhiza development and sporocarp formation in plantations and natural stands are also influenced by other ecological aspects, including soil physical characteristics (Bencivenga et al. 1988; Bragato et al. 2010; Lulli et al. 1999) and light infiltration or density of tree canopy (García-Barreda and Reyna 2013). Moreover, biological issues such as the genetic and physiological status of the host tree and the fungus (Bruhn et al. 2013), and competing mycorrhizal fungus species should also be taken into account (Reyna 2012). The complexity of these biological and ecological factors and their unknown interactions are likely the reasons for the variability of sporocarp yields in plantations and natural truffle grounds (Kües and Martin 2011).

We believe that overlooking some of the aforementioned variables when selecting potential sites for truffle plantations can result in plantation failure. Hence, we focused this work on the effects of edaphic characteristics, cultivation practices, and their interactions on truffle production in a diverse array of plantations. We chose the province of Teruel, eastern Spain, as the test region for three reasons. First, the area occupied by truffle orchards has dramatically increased in the last two decades (Samils et al. 2008), and unsuccessful plantations can be easily found. Second, the area devoted to truffle cultivation has a somewhat uniform climate, suggesting that factors other than climate are driving truffle productivity. And, third, the local truffle association requested a study to explain the causes of plantation failure or poor production and development of a decision tool to reduce the risk of failure in the future. Decision-making tools for truffle culture have been previously developed for climatic factors (Alonso Ponce et al. 2010; Colinas et al. 2007; Domínguez et al. 2003) but not for other types of ecological variables.

Traditionally, prior to planning a new orchard, the climate of the area and soil chemical properties are taken into consideration (Chevalier and Sourzat 2012), but even when considering these factors, some plantations never produce sporocarps. Thus, we hypothesize that the physical nature of the soil, along with some cultivation techniques, is controlling the success or failure in producing black truffles in plantations. To test this hypothesis, we address three specific questions: (1) Are cultivation practices (watering and ploughing) significantly related with fructification of truffles in plantations? (2) Which edaphic factors are related with the success of truffle fructification? (3) Can we provide truffle plantation managers and owners with a straightforward, reliable, and operational model to improve their ability to predict potential success of field sites under consideration for truffle plantations?

Materials and methods

Study area

This research was conducted in Teruel, which is the southernmost province of the autonomous community of Aragón, in northeast Spain (Fig. 1). This province was chosen because of its large investment in truffle plantations over the last three decades, with a total of more than 5,700 ha dedicated to cultivation of black truffle. Indeed, ecological conditions for much of the Teruel province, particularly the south and the east areas, are suitable for black truffle (Alonso Ponce et al. 2010). The climate of Teruel is characterized by low annual rainfall ranging from 400 to 520 mm and summer rainfall from 110 to 130 mm. Temperature fluctuations are very pronounced throughout the year, with an average annual temperature between 10 and 13 °C, severe winters and short and warm summers. The study area has a particularly steep altitudinal gradient with elevations between 615 and 1,424 m asl (mean 956) without a prevailing aspect, and most of the territory is situated on the high massifs of the southern Iberian System. Mesozoic and Tertiary limestones, dolomites, and marlstones are abundant. According to the World Reference Base (WRB) (FAO 1998), the soils in the area are mainly Cambisols and Leptosols.

Sampling sites and data collection

In 2008, 78 *T. melanosporum* plantations of *Quercus ilex* ssp. *ballota* (Desf.) Samp. over 10 years old were selected (minimum age 10, mean 15, maximum 24); some of the plantations were known as producing black truffles and others not (Fig. 1). Trees were arranged on a grid, with distances ranging from 5 to 7 m. Information about previous land use, tree age, mycorrhizal seedling origin, management during the life of the plantation—shallow ploughing (PL often/rarely/no) and watering (W yes/no)—and the existence or not of truffle production (PR yes/no) in truffle orchards was reported by owners. The size of the plantations ranged from 0.13 to 15.00 ha (mean 1.67).

Each orchard was inspected to determine a location that represented its average features where a research plot could be installed. Plots contained a matrix of 3×3 trees and a variable area depending on the distance between trees. Within the plot, three of the nine trees were randomly selected in order to sample soil and roots in their surrounding area. A pair of soil samples per tree (six per plot) was extracted with a 20-cm long

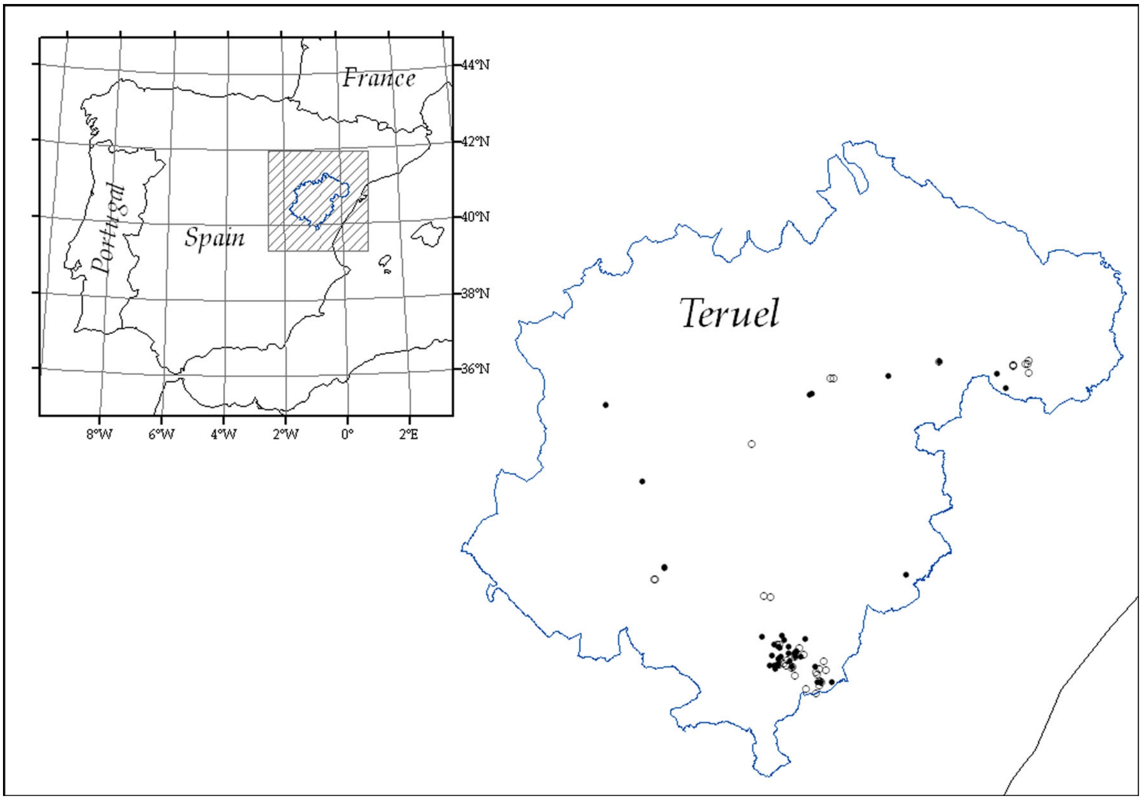

Fig. 1 Study area and sampling locations. *Filled markers* plots with fructification, *empty markers* plots without fructification

by 4-cm wide cylinder. One of the samples per tree (three samples per plot) was used to analyze soil characteristics while the other one was used to examine the presence of ectomycorrhizae of *T. melanosporum* through the inspection of root tips. Following the global method proposed by Giraud (1990), the extraction was done between the end of the crown projection and the edge of the "truffle burn" area, in a south-southeast direction from the host stem (Barry-Etienne et al. 2008).

Additionally, a 30×30×20-cm deep hole was dug outside the truffle burn area, as close as possible to the place where the cylinders were extracted, for estimating bulk density according to the excavation method (ASTM 1958). Given that this sampling method is highly disturbing, it was made outside the truffle burn area in order to lessen the impact on the fungus.

Finally, 15 climatic variables were estimated for each plot using the models of Sánchez Palomares et al. (1999), which are functions of altitude, geographical position, and hydrographical basin (see Supplementary Material, Table S1).

Soil property analysis

Samples devoted to soil variables were air-dried and sieved to obtain the <2-mm fraction (fine earth fraction). Details about the acronyms, units, and references for the laboratory methods of the soil variables can be found in Table 1. All variables but water-holding capacity (WHC) and fine earth (FE) fraction have been measured and reported according to international standards. WHC was estimated through a pedotransfer rule grounded in a model with physical basis (Rubio et al 2002; Domingo et al 2006) expressed in millimeter (l/m^2) of water for the depth of soil considered (20-cm topsoil in our case). It is calculated in the fine earth fraction but adjusted for gravel content; consequently, its value represents the estimated amount of water that the undisturbed soil can hold at field capacity for the considered depth. FE is expressed in terms of percentage, in weight, of all particles less than 2 mm in relation to the natural soil.

Ectomycorrhizae analysis

As stated above, we focused our study on edaphic and management variables. Accordingly, the influence of other factors, such as the quality of the inoculation in the original seedlings, must be reduced as much as possible to lessen the noise in the statistical analysis. Therefore, we assessed the presence or absence of *T. melanosporum* ectomycorrhizae in our soil samples to preclude the latter from subsequent assessments. These samples were stored frozen at −28 °C until further cleanup and study. Following Agerer's (1991) procedures, ectomycorrhizae in every sample were observed under the stereomicroscope, separating nonmycorrhizal and mycorrhizal roots with either *T. melanosporum* or other fungal species. The anatomorphological identification of *T. melanosporum*

Table 1 Edaphic variables, acronyms, units, and references for the laboratory methods used

Variable	Acronym	Unit	Reference
pH in H_2O	PH	pH unit	(Rubio et al. 2002)
Electric conductivity	COND	µS/cm	(MAPA 1994)
Organic matter	OM	%	(Rubio et al. 2002)
Total carbonates	TCARB	%	(MAPA 1994)
Active carbonates	ACARB	%	(MAPA 1994)
Nitrogen content	N	%	(Rubio et al. 2002)
Carbon:nitrogen ratio	CN	–	(Rubio et al. 2002)
Sodium	NA	$mg \cdot kg^{-1}$	(Rubio et al. 2002)
Potassium	K	$mg \cdot kg^{-1}$	(Rubio et al. 2002)
Calcium	Ca	$mg \cdot kg^{-1}$	(Rubio et al. 2002)
Magnesium	Mg	$mg \cdot kg^{-1}$	(Rubio et al. 2002)
Phosphorous	P	$mg \cdot kg^{-1}$	(Rubio et al. 2002)
Free iron oxides	FIOX	$mg \cdot kg^{-1}$	(Soil Conservation 1972)
Cation exchange capacity	CEC	$cmol \cdot kg^{-1}$	(Davis and Freitas 1970)
Fine earth fraction	FE	%	(Rubio et al. 2002)
Sand	SAND	%	(Rubio et al. 2002)
Silt	SILT	%	(Rubio et al. 2002)
Clay	CLAY	%	(Rubio et al. 2002)
Water-holding capacity	WHC	mm	(Rubio et al. 2002)
Bulk density	BD	$Mg \cdot m^{-3}$	(ASTM 1958)

ectomycorrhizae was confirmed according to the description by Rauscher et al. (1995).

Data analysis

First, those plots that showed no presence of *T. melanosporum* after ectomycorrhizal assessment were discarded from subsequent analysis. Thus, 8 of the 78 initial plots were excluded. Next, each plot was classified as productive or nonproductive according to information from the owners.

Second, to evaluate the influence of cultivation practices in PR—our first objective—we performed a log-linear test for independence of the three categorical variables (PR, W, and PL) (García Pérez 2005b). The comparisons among models were evaluated by the significance of the residual deviance (χ^2 test) and the Akaike's information criterion (AIC). PL and/or W was eliminated from further analysis if they did not have a significant relationship with PR.

Third, we verified the effect of the climatic attributes on our target variable (PR), at every level of W or PL (if they were identified as significant in the previous analysis), by two approaches: (a) We executed the robust version of the Welch test (García Pérez 2005a) separately for the 15 climatic variables to identify any significant univariate relationship with PR, and (b) we examined the projection of the 70 plots on the first principal plane of a principal component analysis performed on the 15 climatic variables to separate any multivariate trend. If no significant relationship with PR was identified, as we hypothesized, climate was excluded from the study.

In the fourth step, to answer the second question of our work, the robust Welch test was also used to ascertain those edaphic variables related to truffle production at every level of W or PL, if significant.

Finally, we pursued our third question by fitting a logistic model using PR as the dependent variable and edaphic factors as the independent variables. The logistic model is a type of generalized linear model used to predict a binary response based on one or more predictor variables. In our case, the binary response is the production or truffles (PR yes/no), whose probability of occurrence (p and $q=1-p$) follows a binomial distribution. Logistic regression relates probability p to a set of predictors (in our case, FE, SAND, SILT, etc.) using the logit link function:

$$\text{logit}(p) = \ln\left(\frac{p}{q}\right) = \ln\left(\frac{p}{1-p}\right) = X'\beta$$

where X' is the vector of predictors and β is the vector of the coefficients to be estimated from the data. In this form, the logit(p) is expressed as some given linear combination of the predictors, and hence, it is feasible to fit by linear regression.

Table 2 Data for the log-linear model: number of plots according to production and cultivation practices

		Production=Yes				Production=No			
		Ploughing				Ploughing			
		No	Rarely	Often	Total	No	Rarely	Often	Total
Watering	No	12	5	7	24	7	4	15	26
	Yes	4	2	10	16	0	1	3	4
	Total	16	7	17	40	7	5	18	30

This function can be rewritten as the following:

$$\frac{p}{1-p} = e^{X'\beta}$$

The quotient at the left of the equation is known as the *odds ratio*, which represents how many times it is more probable that the predicted variable (PR) equals yes than no.

Variables entered or left the model by the AIC stepwise selection method and the significance of every parameter by the Wald test. The *best model* approach was used to select the fitted equation, as our goal is a *prediction* model rather than an *exploratory* model. The goodness of fit of the model was evaluated through the Kappa and Gwet statistics (Gwet 2002) and the receiver-operating characteristic (ROC) curve (Hosmer and Lemeshow 2000). Lastly, we calculated the confusion matrix to assess the percentage of success and failure in predicting PR.

All analyses were performed in R2.15 (R Development Core Team 2009) and Rmo (García Pérez 2005a).

Results

Production and cultivation practices

The data used for the log-linear models, i.e., the number of plots according to PR and cultivation practices, watering (W), and ploughing (PL), are presented in Table 2.

Only four of the eight log-linear models for production, watering, and ploughing resulted in a deviance not significantly (p>0.05) different from 0 (Table 3). Comparison between the four accepted models, from the most complex to the simplest, shows that none is significantly better than the simplest one: model 2 (p>0.05). Although model 8 is weakly better than model 5 and model 6, the latter are clearly not significantly better than model 2. Therefore, we can accept that the only significant interaction is between production and watering and that the variable ploughing can be excluded from further analysis. In fact, the Pearson statistic for the contingency table PR×W is 5.97, denoting a strong dependency between those variables (p=0.0145), while between PR and PL is 2.50 (p=0.2856).

Table 3 Log-linear model for production (PR), watering (W), and ploughing (PL) (N=70)

Model	Deviance	Degrees of freedom	p value	AIC
(1) PR,W,PL	15.68	7	0.02821	66.23
(2) PR,W,PL,PRW	9.32	6	0.1564	62.42
(3) PR,W,PL,WPL	12.85	5	0.0249	66.39
(4) PR,W,PL,PRPL	13.13	5	0.0222	67.32
(5) PR,W,PL,PRW,WPL	6.49	4	0.1653	62.57
(6) PR,W,PL,PRW,PRPL	6.77	4	0.1485	63.51
(7) PR,W,PL,PRPL,WPL	10.30	3	0.0162	67.47
(8) PR,W,PL,PRPL,WPL,PRW	1.56	2	0.4581	60.89
Model comparison	Deviance difference	Degrees of freedom	p value	
8~6	5.21	2	0.0739	
8~5	4.93	2	0.0850	
6~2	2.55	2	0.2799	
5~2	2.83	2	0.2432	

Each interaction is denoted by the combination of the respective abbreviations. The performance of each model was evaluated by the significance of the residual deviance (χ^2 test, p) and the Akaike's information criterion (AIC)

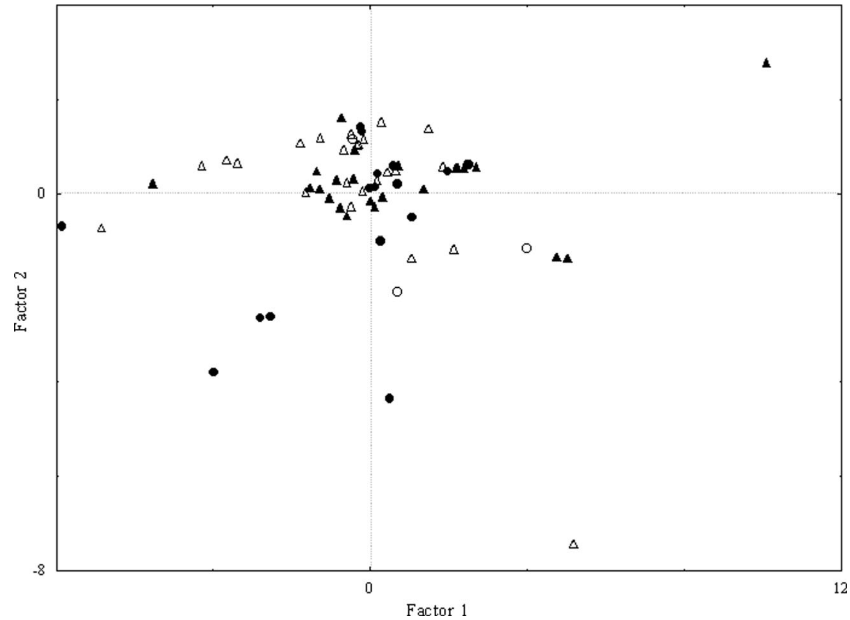

Fig. 2 Projection of the 70 samples on the first PCA principal plane (92.2 % of the variance). *Filled markers* plots with fructification, empty markers plots without fructification. *Triangles* nonwatered plots, *circles* watered plots

Production and climatic features

As hypothesized, none of the climatic variables exhibited significant differences between productive and nonproductive plots, both with or without watering (robust Welch test, $p>0.10$) (values of the climatic variables in every plot can be found in the Supplementary Material, Table 2). Moreover, the projection of the 70 observations on the first principal plane, which absorbed 92.2 % of the variance, did not show any clustering of the plots (Fig. 2).

Production and soil properties

The mean and ranges of the edaphic variables for our samples are summarized in Table 4. These ranges must be borne in mind when interpreting the results below, as no extrapolation should be done beyond the limits of those ranges. Moreover, in Table 5, we show the Spearman's correlation matrix of the 12 edaphic variables displaying informative variability.

Seven of the 20 studied variables were significantly related with the factors PR and W or their interaction (Table 6). Four of them are linked to physical properties of the soil: silt content, fine earth, water-holding capacity, and bulk density. On the other hand, high total and active carbonate contents appear to produce an abatement in truffle production, but the former variable has a significant relationship with the interaction of PR×W. Thus, the interplay between PR and W could be masking the actual effect of the carbonate content on truffle fructification.

Multivariate analysis and predictive models for production success

Since we found a clear relationship between truffle production (PR) and plot watering (W), we performed two separate models for all plots, irrespective of whether they were watered

Table 4 Mean and ranges of the edaphic variables used in the study

	Minimum	Mean	Maximum
pH	8.13	8.37	8.67
COND	78	112	153
OM	0.63	1.89	4.46
TCARB	8.5	35.2	67.6
ACARB	0.5	7.1	14.9
N	0.05	0.14	0.29
CN	4	8	12
Na	4	11	26
K	58	228	449
Ca	3184	4927	7735
Mg	29	106	269
P	2	6	37
FIOX	4736	11607	25414
CEC	5.9	20.8	39.3
FE	26.9	64.0	96.1
SAND	18.7	41.4	74.3
SILT	10.3	31.8	56.3
CLAY	13.9	26.9	45.5
WHC	26.2	59.2	94.4
BD	1.08	1.37	1.65

See Table 1 for variable acronyms and units

Table 5 Spearman's correlation matrix of the 12 edaphic variables showing informative variability

	TCARB	ACARB	N	FEOX	CEC	FE	SAND	SILT	CLAY	WHC	BD
OM	0.17	**0.27**	**0.86**	**0.18**	**0.65**	**−0.16**	**−0.48**	**0.45**	**0.44**	**−0.01**	**−0.26**
TCARB		**0.88**	−0.05	**−0.52**	−0.09	0.22	**−0.42**	**0.71**	−0.14	**0.27**	**−0.41**
ACARB			0.05	**−0.58**	−0.04	**0.31**	**−0.43**	**0.70**	−0.12	**0.34**	**−0.47**
N				**0.45**	**0.68**	**−0.27**	**−0.37**	0.22	**0.59**	−0.13	−0.19
FEOX					**0.50**	**−0.34**	−0.18	**−0.25**	**0.71**	**−0.26**	0.11
CEC						−0.09	**−0.57**	**0.29**	**0.78**	0.06	**−0.31**
FE							**−0.32**	**0.35**	−0.08	**0.66**	**−0.50**
SAND								**−0.81**	**−0.64**	**−0.48**	**0.60**
SILT									0.17	**0.45**	**−0.58**
CLAY										0.09	**−0.27**
WHC											**−0.57**

Significant coefficients ($p<0.05$) are stressed in boldface. See Table 1 for variable acronyms and units

or not, and only for nonwatered plots. We achieved two satisfactory logistic models in both cases (Table 7).

The watering-independent model included three parameters, namely, fine earth, silt content, and water-holding capacity. Plots with lower contents in fine earth and silt and higher water-holding capacity succeeded most frequently in producing truffles. The ROC curve showed that the logistic model predicted production success reasonably well (Area under the curve (AUC)=0.794). Kappa and Gwet statistics reached figures above 0.6, and the probability threshold for both optimum Kappa and Gwet statistics was 0.658. The confusion matrix derived from this value (Table 8) exhibits an 80 % rate of correctly classifying plots, with only 10 % of nonproductive plots misclassified.

The model for nonwatered plots showed that soils with lower fine earth and silt contents and higher total carbonate and clay contents were most likely to produce truffles. Moreover, AUC exceeded 0.85 and Kappa and Gwet statistics 0.70, which represents a reliable performance of the model. The probability threshold for both optimum Kappa and Gwet statistics was 0.574, which generated a confusion matrix (Table 8) showing that 86 % of plots were correctly classified while only 8 % of nonproductive plots were misclassified. The mean values of the selected variables, both in the watering-independent model or in the model for nonwatered plots, split by the two levels of PR, are shown in Table 9.

Discussion

Our results show that some variables of soil physical status play key roles in truffle fructification in plantations. Particularly interesting is that low fine earth and silt contents appear to be promoting fructification, as do high levels of bulk density, clay content, and water-holding capacity. Therefore, we are facing a problem of balance between aeration and water availability, as was also found in *T. melanosporum* plantations in Italy (Lulli et al. 1999).

The negative relationship between SILT and PR is consistent with the assertion by Bonneau and Souchier (1987), who claimed that large amounts of silt particles (2–50 μm) bring about a large

Table 6 *p* values of the robust Welch test for the edaphic variables according to truffle production (PR), plot watering (W), and their interaction

	Factor		
	PR	W	PR×W
pH	0.789	0.344	0.541
COND	0.414	0.386	0.524
OM	0.331	0.927	0.347
TCARB	0.003 (−)	0.716	0.011
ACARB	0.017 (−)	0.956	0.065
N	0.648	0.899	0.607
CN	0.114	0.708	0.269
Na	0.192	0.088	0.636
K	0.894	0.692	0.748
Ca	0.857	0.412	0.288
Mg	0.262	0.199	0.895
P	0.471	0.074	0.262
FIOX	0.156	0.518	0.566
CEC	0.796	0.491	0.605
FE	0.010 (−)	0.734	0.091
SAND	0.169	0.234	0.552
SILT	0.036 (−)	0.678	0.758
CLAY	0.576	0.492	0.656
WHC	0.020 (+)	0.563	0.175
BD	0.011 (+)	0.148	0.159

The symbol + or – indicates the sign of the significant ($p<0.05$) relationships between variable and factor. See Table 1 for variable acronyms and units proportion of capillary water and consequently poorer aeration. Macroporosity (especially due to elongated pores >50 μm) was also significantly higher in fluvial landscapes of Croatia producing *Tuber magnatum* Pico (Bragato et al. 2010). Although the edaphic environments suitable for *T. magnatum* and *T. melanosporum* are distinctly different, they have in common a high degree of aeration in their surface horizons.

The negative relationship between FE and PR also supports the idea of the water availability-aeration offset. Thus, the environment close to gravels and stones is rich in macropores and consequently holds a large amount of gravitational water in the topsoil, which drains rapidly after downpours or irrigation to deeper horizons. Nonetheless, we surmise that other factors promoting soil water availability must be involved in truffle fructification, particularly under Mediterranean climates where summer drought is severe. Our results suggest that, on the one hand, a high proportion of stones in the 20-cm topsoil is an outstanding way of preventing soil water from evaporating (Duchaufour 1984). On the other hand, although an excess of stones reduces water-holding capacity dramatically, higher clay contents can offset this drawback. Clay, along with organic matter, boosts the formation of micropores and aggregates, which in turn increases water-holding capacity. This statement is supported by our results, as WHC showed a positive and significant relationship with PR, while our multivariate analysis identified clay content as a significant factor but not organic matter.

Table 7 Logistic models for predicting production success, in watering-independent plots (top) and nonwatered plots (bottom)

Water(N=70)						
Term		Coef.	Std. err.	OR	Wald-χ^2	p
Intercept		5.85166	1.586		13.608	0.000
FE		−0.14977	0.069	0.861	2.845	0.030
WHC		0.10079	0.070	1.106	4.711	0.151
SILT		−0.05915	0.035	0.943	2.063	0.092
AIC	Kappa	Gwet	K-thres	G-thres	AUC	Rdev
86.9	0.605	0.600	0.658	0.658	0.794	78.9
No watering (N=50)						
Term		Coef.	Std. err.	OR	Wald-χ^2	p
Intercept		2.43478	2.174		1.254	0.263
FE		−0.06350	0.026	0.938	5.965	0.015
SILT		−0.15776	0.069	0.854	5.228	0.022
CLAY		0.10734	0.056	1.113	3.674	0.055
TCARB		0.10205	0.044	1.107	5.379	0.020
AIC	Kappa	Gwet	K-thres	G-thres	AUC	Rdev
60.0	0.718	0.723	0.574	0.574	0.859	50.0

Stepwise method and the Akaike's information criterion (AIC) was used for variable selection, which are presented in the order they entered the model. *OR* odds ratios. Kappa and Gwet are the statistics for the goodness of fit; K-thres and G-thres are the thresholds (according to Kappa or Gwet statistics, respectively) for an optimum area under the curve (AUC) of the receiver operating characteristic. *Rdev* is the residual deviance of each model, in both cases not significantly different from 0 (p>0.10). See Table 1 for variable acronyms and units

Table 8 Confusion matrix for the variable truffle production (PR) in both logistic models, derived for optimum probability thresholds (see Table 7)

		Predicted Yes	No
Watering-independent model			
Observed	Yes	29	11
	No	3	27
Nonwatered plots model			
Observed	Yes	19	5
	No	2	24

Several studies note the crucial role of both factors (water availability and aeration) in influencing truffle production (Lulli et al. 1999, Olivera et al. 2011). It is well known that sporocarp formation is typically induced by high humidity along with other factors such as a reduction in temperature, neutral or slightly acidic pH, and pulses of low energy light, although a universal set of conditions that lead to fructification in all fungi cannot be defined (Murat et al. 2008). Moreover, Hacskaylo (1973) asserted that oxygen absorption of mycorrhizal roots in the soil is greater than that of nonmycorrhizal roots, thus affecting the growth and the life cycle of both plant and fungus and their mycorrhizal relationship. Overall, our results suggest that a key issue for the triggering of sporocarp formation is the balance between good aeration and a sufficient water supply. Indeed, all the soil physical properties we found significantly related to black truffle fructification in plantations, i.e., silt and clay content, fine earth fraction, soil water-holding capacity, and bulk density, are related with the quantity of oxygen and water that the soil can retain in its voids. In any case, to envisage which could be the cause-effect relationships between the abovementioned soil attributes and the fructification process of *T. melanosporum*, we have to bear in mind that *T. melanosporum* ectomycorrhizae belong to the short distance exploration type (Agerer 2006). Their hydrophilic cystidia (Agerer 2001) only extend a few millimeters from the mantle, acquiring nutrients in the immediate vicinity of the ectomycorrhizal root tips.

Other well-known factors driving truffle production, like total soil carbonates (García-Montero et al. 2012), had an outwardly contradictory behavior in our samples: A high

Table 9 Mean values for every selected edaphic variable, split by the two levels of PR

PR	FE	SILT	CLAY	WHC	OM	TCARB
Yes	58.37	29.27	27.42	66.37	1.83	32.61
No	71.61	35.12	26.14	53.84	1.96	38.72

See Table 1 for variable acronyms and units content of carbonates in the soil could be self-defeating for truffle fruiting when considering the whole set of plots. Nevertheless, the opposite has been found in nonwatered plots, which hints that an overload of carbonates in watered plots, under a Mediterranean regime characterized by a harsh summer drought, can lead to an underlying secondary carbonate enrichment (Callot et al. 1996) and hence to a drainage depletion. Moreover, this carbonate enrichment must be at the expense of the depletion of carbonates in the topsoil; hence, watered plots would tend to have lower values of carbonates in surface horizons (though not statistically significant). Given that watering has a positive and significant effect on truffle fructification, the negative relationship between $CaCO_3$ content and PR can be derived from the PR-W interaction, which is actually significant for our data (p=0.011 for TCARB). These multiple interactions cannot be interpreted with confidence from our results, but Ourzik (1999) and Ricard (2003) highlight the importance of maintaining a constant content of fine limestone in the surface horizons but also emphasize that there is insufficient knowledge regarding how physical-chemical topsoil properties influence truffle cultivation.

Our results support the positive effect of watering on truffle production previously reported by Bonet et al. (2006) and Olivera et al. (2011). Conversely, our results do not support previous findings on cultivation practices. Shallow ploughing has traditionally been considered advantageous for black truffle production by reducing weed competition and enhancing soil structure. In fact, recent methods of truffle cultivation recommend deep soil cultivation (Chevalier and Sourzat 2012) to regenerate root systems and improve water economy. Nevertheless, our results do not affirm that ploughing satisfactorily corrects unfavorable physical conditions of the soil. A detailed study of the structure characteristics of the sampled soils is needed to explain this lack of relationship.

The third objective of the present work has been satisfactorily achieved, as we provide orchard owners with a straightforward, reliable, and operationally valid model for reducing the risk of failure in future plantations. The method used for model selection (*best model* approach) typically produces equations that function properly for predicting the dependent variable but may not explain the relationships between explanatory and predicted variables. Thus, in the first model, WHC shows a nonsignificant coefficient, though when included in the model improves its prediction ability. The high and significant correlation coefficient between FE and WHC is probably hampering the explanatory power of the model. A similar finding occurs with FE and SILT, which show a weaker but also significant positive correlation coefficient.

The predictive performance of the model works best for nonproductive plots (less than 10 % of plots were misclassified); hence, land owners interested in truffle culture can reduce the probability of an investment disaster by using the model. Moreover, our models use variables that can be easily acquired from standard soil analysis. Thus, we recommend that soil inspection includes analyses of the physical properties to provide a consistent basis for distinguishing the suitability of a plot. As for any model, it is important to note that our model should be tested with independent data and recalibrated when used in other regions with contrasting climatic attributes.

We have shown that soil physical properties influence the success or failure in producing black truffles. The balance between good aeration and sufficient water content in the soil is one of the key factors controlling fructification of black truffles in plantations. Further work is needed to address the multiple interactions among soil properties and other factors like tree age, mycorrhizal seedling quality, inoculum origin, or stand density. The research on this subject will help to improve black truffle cultivation, which represents presently a real alternative for the sustainable development of regions seriously affected by depopulation all over Spain and other Mediterranean countries.

Acknowledgments This work was carried out in the currently closed Centro de Investigación Forestal de Valonsadero (Junta de Castilla y León, Spain). The projects PET2007-013-C07 of the INIA (Ministerio de Ciencia e Innovación, Gobierno de España) and Mycosilva+ (European Union) supported the research. We would like to thank the owners of the truffle orchards in Teruel for their kind access of their plots for this study, and particularly Manuel Redón Iserte for his help in the fieldwork.

References

Agerer R (1991) Characterization of ectomycorrhiza. In: Norris JR, Read DJ, Varma A (eds) Techniques for the study of mycorrhiza, vol 23, Method Microbiol, vol., pp 25–73

Agerer R (2001) Exploration types or ectomycorrhizae. a proposal to classify ectomycorrhizal mycelial systems according to their patterns of differentiation and putative ecological importance. Mycorrhiza 11:107–114

Agerer R (2006) Fungal relationships and structural identity of their ectomycorrhizae. Mycol Prog 5:67–107

Águeda B, Ágreda T, Alonso Ponce R, Hernández A (2011) Truficultura en Castilla y León. In: Martínez-Peña F, Oria De Rueda JA, Ágreda T (eds) Manual para la gestión del recurso micológico forestal en Castilla y León. Somacyl-Junta de Castilla y León, Valladolid, pp 271–291

Alonso Ponce R, Águeda B, Ágreda T, Modrego MP, Aldea J, Martínez-Peña F (2010) Un modelo de potencialidad climática para la trufa negra (*Tuber melanosporum*) en Teruel (España). For Syst 19:208–220

ASTM (1958) Procedures for testing soils. American Society for Testing and Materials, Philadelphia

Barry-Etienne D, Ricard JM, Diette S, Moundy PJ, Chandioux O, Fioresse D, Jaillard B, Serre F, Jourdan C (2008) Distribution of Tuber melanosporum mycorrhizas on rootstocks of holm-oaks (Quercus ilex) in production. In: 3º Congresso Internazionale di Spoleto sul tartufo, Spoleto, Italia, 2008

Bencivenga M, Granetti B (1989) Indagine preliminare sul contenuto in macro e microelementi del terreno e dei carpofori di *Tuber melanosporum* Vitt. Micol Ital 3:25–30

Bencivenga M, Calandra R, Granetti B (1988) Ricerche sui terreni e sulla flora delle tartufaie naturali dei Tuber melanosporum Vitt. dell'Italia centrale. Atti del Secondo Congresso Internazionale sul Tartufo. Spoleto.

Bonet JA, Fischer CR, Colinas C (2006) Cultivation of black truffle to promote reforestation and land-use stability. Agron Sustain Dev 26: 69–76

Bonneau M, Souchier B (1987) Edafología. 2: Constituyentes y propiedades del suelo. Masson, Barcelona

Bragato G, Vignozzi N, Pellegrini S, Sladonja B (2010) Physical characteristics of the soil environment suitable for *Tuber magnatum* production in fluvial landscapes. Plant Soil 329:51–63

Bruhn JN, Mihail JD, Pruett GE (2013) Truffle seedling production method has long-term consequences for tree growth and root colonization. Agroforest Syst 87:DOI 10.1007/s10457-10012-19588-10453

Büntgen U, Egli S, Camarero JJ, Fischer EM, Stobbe U, Kauserud H, Tegel W, Sproll L, Stenseth NC (2012) Drought-induced Périgord black truffle decline. Nat Clim Chang 2:827–829

Callot G, Jaillard B, Braye JP, Jalade M (1996) Incidence des caractéristiques structurales du sous-sol sur l'entrée en production de *Tuber melanosporum* et d'autres champignons mycorhiziens. Agronomie 16:405–419

Chevalier G, Sourzat P (2012) Soils and techniques for cultivating *Tuber melanosporum* and *Tuber aestivum* in Europe. In: Zambonelli A, Bonito GM (eds) Edible ectomycorrhizal mushrooms, current knowledge and future prospects. Springer, London, pp 163–189

Colinas C, Capdevila JM, Oliach D, Fischer C, Bonet JA (2007) Mapa de aptitud para el cultivo de la trufa negra (Tuber melanosporum Vitt.) en Cataluña. Centre Tecnològic Forestal de Catalunya, Solsona

Davis J, Freitas F (1970) Physical and chemical methods of soil and water analysis. Soils Bulletin no.10. FAO, Rome

Domingo JM, Fernández de Villarán R, Corral E, Rapp Í (2006) Estimación de la capacidad de retención de agua en el suelo: revisión del parámetro CRA. Investig Agrar Sist Recur For 15(1): 14–23

Domínguez JA, López Leiva C, Rodríguez Barreal JA, Sáiz de Omeñaca JA (2003) Caracterización de rodales truferos en la comunidad valenciana. Ecología 17:181–190

Duchaufour P (1984) Edafología. 1: Edafogénesis y clasificación. Masson, Barcelona

FAO (1998) World Reference Base for Soil Resources. World Soil Resources Reports, 84. FAO, Roma

García Pérez A (2005a) Métodos avanzados de estadística aplicada. Métodos robustos y de remuestreo. UNED, Madrid

García Pérez A (2005b) Métodos avanzados de estadística aplicada. Técnicas avanzadas. UNED, Madrid

García-Barreda S, Reyna S (2013) Response of *Tuber melanosporum* fruiting to canopy opening in a *Pinus-Quercus* forest. Ecol Eng 53: 54–60

García-Montero LG, Quintana A, Valverde-Asenjo I, Díaz P (2009) Calcareus amendments in truffle culture: a soil nutrition hypothesis. Soil Biol Biochem 41:1227–1232

García-Montero LG, Valverde-Asenjo I, Moreno D, Díaz P, Hernando I, Menta C, Tarasconi K (2012) Influence of edaphic factors on edible ectomycorrhizal mushrooms: new hypotheses on soil nutrition and C sinks associated to ectomycorrhizae and soil fauna using the *Tuber* brûlé model. In: Zambonelli A, Bonito GM (eds) Edible ectomycorrhizal mushrooms, current knowledge and future prospects. Springer, London, pp 83–104

Giraud M (1990) Mycorhizes: prélèvement et analyse. In: Verlhac A (ed) La truffe guide practique. CTIFL, Reims, pp 77–88

Gwet K (2002) Kappa statistic is not satisfactory for assessing the extent of agreement between raters. Stat Methods Inter-Rater Reliab Assess 1:1–5

Hacskaylo E (1973) Carbohydrate physiology of ectomycorrhizae. In: Marks GC, Kozlowski TT (eds) Ectomycorrhizae: their ecology and physiology. Academic Press, London, pp 207–231

Hall I, Zambonelli A (2012) Laying the foundations. In: Zambonelli A, Bonito GM (eds) Edible ectomycorrhizal mushrooms. Current knowledge and future prospects. Springer, London, pp 3–16

Hosmer DW, Lemeshow S (2000) Applied Logistic Regression. Wiley, New York

Kües U, Martin F (2011) On the road to understanding truffles in the underground. Fun Gen Biol 48:555–560

Lulli L, Bragato G, Gardin L (1999) Occurrence of *Tuber melanosporum* in relation to soil surface layer properties and soil differenciation. Plant Soil 214:85–92

MAPA (1994) Métodos oficiales de análisis. Tomo III. Ministerio de Agricultura, Pesca y Alimentación, Madrid

Martin F, Kohler A, Murat C, Balestrini R, Coutinho PM, Jaillon O, Montanini B, Morin E, Noel B, Percudani R, Porcel B, Rubini A, Amicucci A, Amselem J, Anthouard V, Arcioni S, Artiguenave F, Aury JM, Ballario P, Bolchi A, Brenna A, Brun A, Buee M, Cantarel B, Chevalier G, Couloux A, Da Silva C, Denoeud F, Duplessis S, Ghignone S, Hilselberger B, Iotti M, Marcais B, Mello A, Miranda M, Pacioni G, Quesneville H, Riccioni C, Ruotolo R, Splivallo R, Stocchi V, Tisserant E, Viscomi AR, Zambonelli A, Zampieri E, Henrissat B, Lebrun MH, Paolocci F, Bonfante P, Ottonello S, Wincker P (2010) Perigord black truffle genome uncovers evolutionary origins and mechanisms of symbiosis. Nature 464:1033–1038

Murat C, Mello A, Abbà A, Vizzini A, Bonfante P, Varma A (2008) Edible mycorrhizal fungi: identification, life cycle and morphogenesis. In: Varma A (ed) Mycorrhiza: genetics and molecular biology, eco-function, biotechnology, eco-physiology, structure and systematics. Springer-Verlag, Berlin, pp 707–732

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

Ourzik A (1999) Les sols argilocalcaires à potentiel truffier de la Vienne. In: Corvoisier M, Olivier JM, Chevalier G (eds) V Congres International: Science et Culture de la Truffe, 1999. pp 273–274

R Development Core Team (2009) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna

Rauscher T, Agerer R, Chevalier G (1995) Ektomykorrhizen von *Tuber melanosporum*, *Tuber mesentericum* und *Tuber rufum* (Tuberales) an *Corylus avellana*. Nova Hedwigia 61(3–4):281–322

Reyna S (ed) (2012) Truficultura. Fundamentos y técnicas. Mundi-Prensa, Madrid

Ricard JM (2003) La truffe. Guide technique de trufficulture. Centre Technique Interprofessionnel des Fruits et Légummes (CTIFL), Paris

Rubini A, Belfiori B, Riccioni C, Paolocci F (2012) Genomics of *Tuber melanosporum*: new knowledge concerning reproductive biology, symbiosis, and aroma production. In: Zambonelli A, Bonito GM (eds) Edible ectomycorrhizal mushrooms. Current knowledge and future prospects. Soil Biology 34. Springer, London, pp 55–72

Rubio A, Elena R, Sánchez Palomares O, Blanco A, Sánchez F, Gómez V (2002) Soil evaluation for *Castanea sativa* afforestation in northeastern Spain. New For 23:131–141

Samils N, Olivera A, Danell E, Alexander SJ, Fischer C, Colinas C (2008) The socioeconomic impact of truffle cultivation in rural Spain. Econ Bot 62:331–340

Sánchez Palomares O, Sánchez Serrano F, Carretero MP (1999) Modelos y cartografía de estimaciones climáticas termopluviométricas para la España peninsular. INIA, Madrid

Soil Conservation Service (1972) Soil survey laboratory methods and procedures for collecting soil samples. US Department of Agriculture. Soil Survey Investigations. Rep. no. 1. Government Printing Office, Washington D.C

Valverde-Asenjo I, García-Montero LG, Quintana A, Velázquez J (2009) Calcareus amendments to soil to erradicate *Tuber brumale* from *T. melanosporum* cultivations : a multivariate statistical approach. Mycorrhiza 19:159–165

Zampieri E, Rizzello R, Bonfante P, Mello A (2012) The detection of mating type genes of *Tuber melanosporum* in productive and non productive soils. Appl Soil Ecol 57:9–15