


Case Study

Physical and chemical properties of soils from different geological formations affecting *Tuber melanosporum* plantations: a case study in Acqualagna area (central Italy)

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Abstract

Soil properties play a pivotal role in the fructification of the Périgord black truffle (*Tuber melanosporum* Vittad.). However, the precise manner in which soil composition influences truffle production remains unclear. This study considered three plantation sites located in Acqualagna (central Italy), an important area for the cultivation of black truffles. The sites differed in terms of productivity and geological terrains. Site 1, which produced 4 kg yr⁻¹, was on Schlier sediments (marls and clayey marls; Miocene age), site 2, which yielded 12 kg yr⁻¹, was on alluvial deposits (heterogeneous, mainly sandy gravels and gravels; Holocene age), and site 3 produced 40 kg yr⁻¹ was on Scaglia variegata (alternating marly limestones, calcareous marls, and marls; Eocene age). A total of 62 soil samples were collected and analyzed for their physical, chemical, organic, and mineralogical composition. Among the samples a control area without the presence of truffle was included. The soils composition differed for the content in phyllosilicates and calcite which ranged from 124 to 398 g kg⁻¹ and 98 to 450 g kg⁻¹, respectively. Principal component analysis extracted three factors, accounting for 82% of the total variance. Factor 1 (46.2% of the variance) was strongly and positively correlated with sheet silicates, smectite, clay and negatively with skeleton (fraction > 2 mm). The soils from Scaglia variegata and Schlier clustered around the active carbonate content which was 266 g kg⁻¹ in Scaglia and 250 g kg⁻¹ in Schlier. These soils clustered also for the clay fraction (389 g kg⁻¹ and 428 g kg⁻¹, respectively) as well as for the smectite (268 g kg⁻¹ and 307 g kg⁻¹, respectively). In spite, the soils from alluvial deposits clustered per skeleton (638 g kg⁻¹). In terms of soil productivity, it can be posited that the contribution of the skeleton was always dominant. The carbonate contents allow for the differentiation between intermediate soils (alluvial deposits) and low-productivity soils (Schlier). Additionally, the clay component was found to be high in both low (428 g kg⁻¹) and high productivity soils (389 g kg⁻¹). Conversely, it was low in medium productivity soils (125 g kg⁻¹). The automatic linear modelling (ALM) indicated that among all of the variables considered in this study, six of them were included in the equation and explained 94.3% of the variation in soil classified as suitable for black truffle production. In particular, calcite was identified as the most important predictor variable, followed by available P and K. Regarding the influence of functional groups of humic substances, amide I (1641 cm⁻¹) exerted a positive effect while lignin residues (1510 cm⁻¹) had a negative one. The findings of this study may assist in the selection of optimal soils for black truffle cultivation.

Keywords Black truffle · Calcite · Diffractometric analyses · FT-IR · Humic substances · Phosphorus · Skeleton · Smectite

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1 Introduction

Tuber melanosporum Vittad., commonly named Périgord black truffle, is a hypogeous ascomycete endemic to Mediterranean areas where it forms ectomycorrhizal associations with roots of oak (*Quercus* spp.) and hazel (*Corylus avellana* L.) especially. It is native to the calcareous regions of Mediterranean area as well as France, Spain, and Italy, where it thrives on well-drained open forests or farmlands characterized by high soil pH, warm and dry summers with strong water soil deficit, and cold winter temperatures [1]. Owing to its great culinary and commercial value, in the last decades its cultivation has spread throughout Europe, New Zealand, Australia, United States, and other countries [2]. Also, productive truffle orchards presently provide rural landowners with an alternative to agricultural subsidies, as they promote the restoration of abandoned cereal lands and require relatively low agricultural inputs [3]. Truffle fresh weight annual production varies widely from a few to even over 100 kg ha⁻¹ [4] with average retail prices ranging from 700 to 1500 euros kg⁻¹ fresh weight depending on the season and weather conditions [5]. In Italy, Périgord black truffles mainly occur in the central-northern and southern Apennines, where *T. melanosporum* and *T. magnatum* occur in large quantities, with Norcia and Spoleto represent the major trade market centers for this truffle.

The cultivation of the Périgord black truffle is a complex process that requires careful consideration of interrelated biological, ecological, and management issues. Understanding and optimizing these different elements is the key to successful truffle farming.

In this context, the chemical and physical parameters of soils are considered key elements. The most preferred parent materials are those from reddish marly limestones, at least in central Italy, although alluvial deposits from the Quaternary period are similarly recognized as excellent [5]. Suitable soils for black truffle production must be well-structured, porous, and aerated [6, 7]. Different studies have reported that the best soils are developed from stony or gravelly substrates or highly fractured hard rock, which ensure proper drainage of excess water from the soil profile [6, 8–12]. Soil texture is a good way of distinguishing between soils that produce black truffles and those that do not. Soils with low water-holding capacity and high compaction do not meet the requirements of black truffles [13]. Moreover the clay content, the soil aeration, the organic matter, and the biological activity are crucial factors for truffle cultivation.

Previous studies have focused on the role of soil calcium carbonate for the growth and development of black truffles. It is generally accepted that the soil must be calcareous, with a pH between 7.5 and 8.5 [14].

Some authors have described considerable variation in soils carbonate levels (1–70%) as a potential promoter of truffle production. However, the authors did not provided data to demonstrate the relationship between soil carbonate and black truffle production [15]. A high content of active carbonate is correlated with variability in the size and production of fruiting bodies [16–21]. In contrast, other authors have demonstrated that the most productive soils for truffles are those that have undergone leaching or have lost carbonate [8, 10–12].

The intricacies of physical soil parameters and their impact on the truffle growth remain poorly understood, despite their acknowledged significance. Soil organic matter plays an important role in sustaining the complex microbial communities [22] and in particular that associated with truffle habitats. It is noteworthy that ectomycorrhizal fungi and truffles release extracellular enzymes (i.e. cellulase and polyphenol oxidase) which are essential for the degradation of complex organic compounds [23, 24]. These enzymes contribute to the cycling of essential nutrients in the soil. A mutually beneficial relationship exists between fungi and host plants, whereby nutrients such as nitrogen are released and can be taken up by the plants. The chemical composition of organic matter exerts a significant influence on the growth of fungi, particularly during the mycelium development stage. Gryndler et al. [25] observed that certain organic matter components, including gallic acid, cellulose and calcium formate, exhibited a positive effect on the stimulation of hyphal growth. In contrast, inorganic compounds did not produce any stimulation. Conversely, Callot [10] observed that polyphenols (i.e. tannins) had a positive effect on the *in vitro* growth of *Tuber melanosporum*. Phenolic compounds are generally accepted as the main 'building blocks' of humic substances (HS) [26]. Phenolic acids account for a significant portion of HS, up to 35%. They are distinguished by their reducing or electron-donation capacity, a key attribute that distinguishes them from other chemical compounds.

Humic substances exert a significant influence on soil structure, by enhancing soil aggregation, fostering a diverse microbial community [27] and ensuring a consistent supply of nutrients. In general, HS play a pivotal role in maintaining the overall health and productivity of soil ecosystems [28, 29]. To date, research has focused on the HS biological activities, and their impact on plant growth, soil fertility, and nutrient cycling. Nevertheless, there has been no particular attention paid to the impact of HS on black truffle production. An evaluation of HS content in soils that are highly productive for black truffles could help to elucidate their role in a complex system such as soil.

The focus of this study is to clarify the existing knowledge on the pedological conditions that promote the growth of *T. melanosporum* in Acqualagna, an area situated in the central-northern Apennines (Marche Region, Italy) renowned for the cultivation and production of Périgord black truffles. The Apennines are a mountain range that originated as a result of extension and compression regimes caused by the subduction of the African lithosphere to the west and southwest. They represent the backbone of the Italian peninsula. Marche region landscape is characterized by marine sedimentary rocks that have been shaped by modern drainage. The alternation of stratified sediments with different grain sizes and compositions is a common feature, with lithological thicknesses varying from centimetric to decametric scales. The admixture of different lithologies serves to distinguish these materials from other soil types with homogeneous substrates.

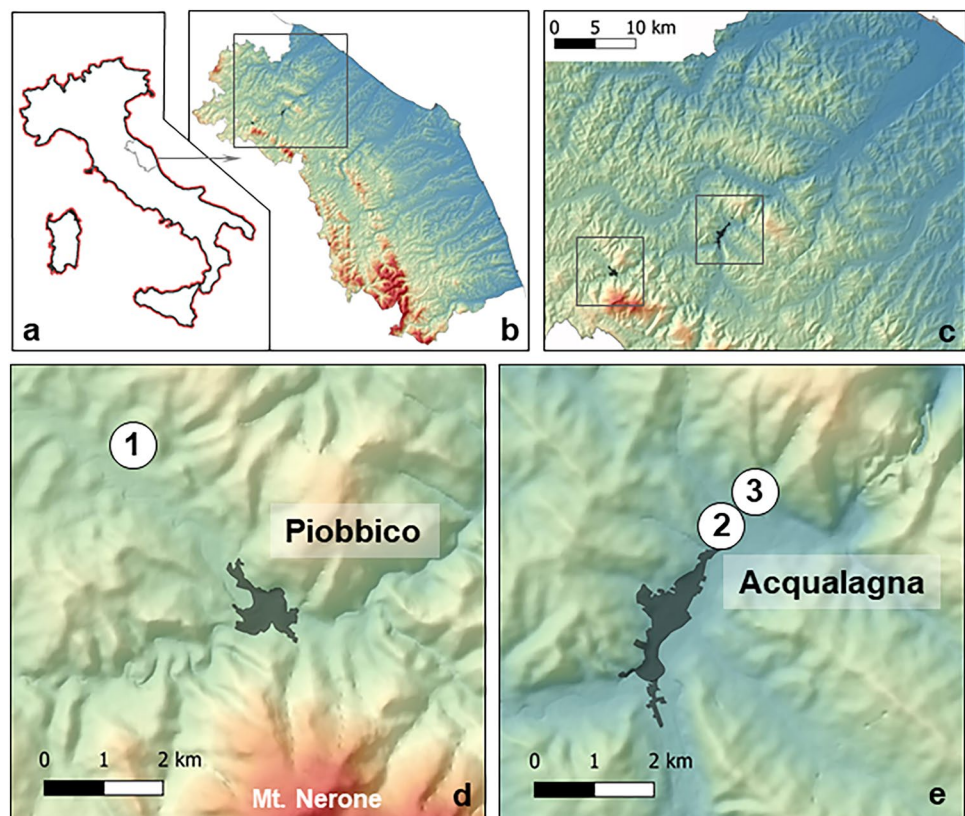
The objective of this study was to examine and identify the physico-chemical parameters of soils derived from different parent rocks and their humic substances. The interaction between these organic compounds and various soil properties can influence the availability of nutrients, which are essential for truffle farming. Consequently, the management of soil properties can lead to an improvement in truffle yield and quality.

2 Materials and methods

2.1 Study area and production techniques

The study sites are located within the Metauro river basin, near the municipalities of Acqualagna and Piobbico (Pesaro-Urbino) in the central-northern Apennines (Marche Region, Italy) (Fig. 1a–c), an area renowned for the Périgord black truffle cultivation and production [30]. The local climate is classified as continental and sub-humid, with an annual rainfall of about 1100 mm predominantly concentrated during the summer. The temperature rises from a minimum of -5°C in January to a maximum of 25°C in July. The area's geologic stratigraphy is predominantly characterised by evaporitic sediments (Trias), followed by a very thick marine succession from the Giurassic to the Tertiary (approximately 200-million-year sedimentation). The sediments exhibit a variety of compositions, including carbonates, pelagic clays, emipelagites and clastic materials in the upper part of the succession (middle Miocene), whereas the uppermost Miocene

Fig. 1 Location map: **a** Italy with the outline of the Marche Region; **b** orography of the Marche Region; **c** engagement of section b showing the location of Piobbico and Acqualagna towns; **d**, **e** locations of the sites 1 (Piantata), 2 (Bacciaglia) and 3 (Pelingo)



and Pliocene are characterised by shallow marine to continental sedimentation. The compressional regime affecting the sedimentary basin since the Miocene is responsible for clastic sedimentation and generation of the Apennines Range, which is still under formation [31].

In the present study, three plantation stands named Piantata, Bacciaglia and Pelingo with similar extension area (about 1 ha) but different productivity were selected. The stands were planted in 2005 and consist mainly of Downy oaks (*Quercus pubescens* Willd.), with a minor presence of Hop-hornbeams (*Ostrya carpinifolia* Scop.) and Holms (*Quercus ilex* L.), with a 5 × 5 m planting layout. In these plantations, pruning is performed mainly during the vegetative rest, whereas green pruning is sporadic. The soil is worked up to 20 cm through an inter-row cultivator equipped with pivoting blades (ET Aratruf®, Rinieri, Forlì, Italy), and the inoculum with *T. melanosporum* is performed once a year. Irrigation is carried out manually when necessary until complete absorption of the hob is achieved, which typically requires the addition of approximately 70–80 L of water per plant. Since 2009, the majority of the trees have been producing black truffles and display the characteristic brùlés. The average of fresh ascomata collected in the last three years was of 4 ± 1 , 12 ± 3 and 40 ± 5 kg yr⁻¹ for Piantata, Bacciaglia and Pelingo, respectively.

The three stands are located on different geologic terrains. The first, designated Piantata (site 1, Fig. 1d) is on Schlier sediments (marls and clayey marls, Miocene), at about 450 m above sea level. The site exhibits an average slope of 26% toward South-West. Bacciaglia (site 2, Fig. 1e) is on young alluvial deposits (heterogeneous deposits, mainly gravels and sandy-gravels, Olocene), at about 200 m above sea level. It is located on a relatively flat surface along the side of a local valley bottom. Pelingo (site 3, Fig. 1e) is on the Scaglia variegata sediments (alternations of marly limestones, calcareous marls and marls, Eocene), at about 270 m above sea level, with an average slope of 22% toward South. Additionally, samples (controls) were collected from the Bisciario sediments (marls and calcareous marls, with additional cherts, diatomaceous and volcanoclastic materials), where no mycorrhized plants were observed.

2.2 Soil morphology and sampling

In March 2019, three soil profiles were randomly selected and excavated from each stand. The soils were described [32] for their morphological features (Table 1). In both Piantata and Pelingo, the soils were formed by an Ah horizon rich in clay-size fraction, followed by a clayey Bt and BC horizon reaching the depth of approximately 50 cm. These soils were classified as Calcari Eudeptic Cambisols following the WRB classification [33] and as Vertic Eutrudept following USDA classification. In Bacciaglia, the soil horizons followed the sequence Ah-Bw-BCk, characterised by a notable abundance of skeleton occurring on the surface and along all the profiles. In this case, the soil was classified as a Skeletic-Calcari Regosol [33] and as a Lithic Eutrudept (USDA). A sample of approximately 1 kg was taken from each horizon in each profile. Within each stand, fifteen soil samples (ca. 500 g) were collected from the 0–20 cm layer following a random sampling design. Additionally, control samples (n=8) were collected in non-production areas where there are no mycorrhized plants. After sorting out litter and large plant root fragments, the samples were placed in labeled plastic bags, sealed, and transported to the laboratory. Samples were air dried inside a controlled room at 20 °C, crushed by a rolling pin to break up clods, passed through a 2-mm sieve, and stored. Particles greater than 2 mm in diameter are considered as the proportion of soil skeleton. In total, sixty-two samples of soil were collected and analyzed.

There were a number of constraints that limited the number of plots that could be sampled. The first is that the truffle in question is highly prized, of very high quality and its production area is very limited. Another factor is that truffle cultivation in central Italy is fragmented, based on small and very small plots of land, and several landowners often ask for minimal soil disturbance during sampling so as not to compromise production.

2.3 Soil analyses

Soil pH was measured potentiometrically in 1:2.5 soil water extracts. Calcium carbonate (CC) equivalent was determined by the calcimeter method and gravimetric loss of CO₂. The hydrometer method was used to conduct particle size analysis, and sodium hexametaphosphate was used as a dispersant [34]. Organic carbon (OC) and total nitrogen (TN) were determined by dry combustion with a CNS Vario Macro elemental analyzer (Elementar, Hanau, Germany). For OC, the data were corrected for inorganic C. Exchangeable bases, including calcium (Ex Ca), magnesium (Ex Mg), potassium (Ex K) and sodium (Ex Na), were extracted using barium chloride [35], and the cation exchange capacity (CEC) was calculated as sum of the bases. Olsen extractable-P (available P) was obtained by shaking 1.0 g of soil with 20 mL of 0.5 mol L⁻¹ sodium bicarbonate solution (pH 8.5) for 30 min [36]. The ion concentrations were determined by inductively coupled plasma-optical emission spectroscopy (ICP-OES) on a SPECTRO CIROS (Spectro Analytical Instruments, Kleve, Germany).

Table 1 Morphological description of the representative profiles

	Depth, cm	Skeleton ^a	Texture ^b	Structure ^c	Consistency ^d	Plasticity ^e	Reaction ^f	Porosity ^g	Boundary ^h
Site 1 Piantata									
Ah	0–10	f, a	c	3, f sbk	fi, s	p	sa	2vf	cs
Bt	10–22	c, a	c	3, f sbk	fi, s	p	sa	2vf	cs
BC	22–40	c, a	c	3, f sbk	fi, s	p	sa	2vf	cs
Site 2 Bacciaglia									
Ah	0–7	a, fg-mg, sr-wa	cl	2, c sbk	fr	l	sa	4c	cs
Bw	7–20	a, fg-mg, sr-wa	cl	2, c sbk	fr	l	sa	4c	cs
BCK	20–50	a, fg-mg, sr-wa	cl	2, c sbk	fr	l	sa	4c	cs
Site 3 Pelingo									
Ah	0–5	vf, fg	c	3, f sbk	fi, s	p	sa	2vf	cs
Bt	5–16	vf, fg	c	3, f sbk	fi, s	p	sa	2vf	cs
BCK	16–50		c	3, f sbk	fi, s	p	sa	2vf	cs

^aa=abundant, c=common, f=few, vf=very few; fg=fine gravel, mg=medium gravel; s=subrounded, sr=spherical rounded, wr=well-rounded

^bc=clay, cl=clay loam

^c1=weak, 2=moderate, 3=strong; th=thin, f=fine, m=medium, c=coarse; cr=crumb, abk=angular blocky, sbk=subangular blocky, pl=platy

^dm=moist, fr=friable, fi=firm; w=wet, ss=slightly sticky, s=sticky

^el=low, w=wet, ps=slightly plastic, p=plastic

^fsa=slightly alkaline

^g1=very low, 2=low, 3=medium, 4=high; c=coarse, vf=very fine

^ha=abrupt, c=clear; b=broken, w=wavy, s=smooth

Humic substances were extracted by air-dried soils with 0.1 M NaOH, as previously described [37]. Humic substances were freeze dry before spectroscopic characterization.

Each soil analysis was performed in duplicate. The data were averaged and used to calculate the mean for each stands soil sample.

2.4 Diffractometric analyses

The soil mineralogy was identified by diffractometric analyses. To carry out this analysis, three g of sieved soil (< 2 mm) were ground by hand in a mortar and pestle before passing through a 250- μ m sieve. Each sample was loaded by lightly filling the sample holders with finely ground powders, and the surface was then flattened with the sharp edge of a razor blade. The samples were analyzed by a Philips X'Pert Pro diffractometer (Co tube and secondary monochromator). Powders were back loaded in order to minimize the chances of preferred orientations. The mineral constituents were computed on a semi-quantitative basis according to the so-called full-pattern approach [38, 39] and using a mineral database [40]. Smectite components in the mineral assemblage are representative of true smectite and also of smectitic mixed-layers.

2.5 FT-IR analyses of humic substances

FT-IR spectra of lyophilized HS were performed by using a Bruker Tensor 27 FT-IR Spectrophotometer (Bruker, Ettlingen, Germany) equipped with an attenuated total reflection (ATR) diamond crystal sampling device. The spectra were collected from 4000 to 400 cm^{-1} and averaged over 100 scans with a resolution of 4 cm^{-1} . Background spectrum was made before recording each sample's spectrum using the diamond crystal. All spectra were processed using baseline correction. The region between 1800 and 1240 cm^{-1} , corresponding to the typical functional groups of humic substances, was examined by curve-fitting analysis with Grams/386 spectroscopic software (version 6.00, Galactic Industries Corporation, Salem, NH, USA).

Spectra were fitted with the Gaussian function and the best fitting parameters were determined by minimization of the reduced Chi-square (χ^2). A good agreement between experimental and calculated profiles was reached, with coefficients of determination (R²) from 0.999 to 0.964 and the standard error (SE) from 0.0001 to 0.0012 [41].

The following deconvoluted bands at 1646 cm⁻¹ (C=O stretching of amide I, quinone, in conjugated ketones and water), 1579 cm⁻¹ (-COO⁻ asymmetric stretching and aromatic C=C stretching), 1535 cm⁻¹ (amide II, aromatic C=C stretching), 1510 cm⁻¹ (aromatic skeletal vibrations in lignin), 1450 cm⁻¹ (CH₂ and CH₃ deformation), 1415–1395 cm⁻¹ (aliphatic CH deformation, -COO⁻ symmetric stretching), and 1330–1245 cm⁻¹ (C–O stretching of phenols and ethers, C–OH deformation) were identified [42].

Integrations were performed on the identified peaks to calculate peak area and subsequently converted into percentage peak area. It was used as a relative quantity of different functional groups. Three replicates were performed for spectra analysis.

2.6 Statistical analyses

Bartlett's test was applied to the data set to assess the homogeneity of variance. Where necessary, the angular transformation was employed to normalise the data. The analysis of variance (ANOVA) was performed to evaluate the effect of geology on soil physical and chemical properties including skeleton, clay-size fraction, sand, silt, pH, calcium carbonates, active carbonates, organic carbon, total nitrogen, C:N ratio, available phosphorus, exchangeable Ca, exchangeable Na, exchangeable Mg, exchangeable K, cation exchange capacity, sheet silicates, smectite, feldspars, quartz and calcite as well as the percentage peak area of FT-IR spectra. The Student–Newman–Keuls test was applied to compare the differences between the group means, and the mean weighted on fine earth content. The correlations between the variables were determined using Pearson's coefficient.

To identify the structure of the interdependence between the main soil parameters, a joint principal components analysis (PCA) was performed on the studied variables. The standardized variables were submitted to PCA; rotated orthogonal components (varimax rotation method) were extracted. The relative scores were then determined. Only PCs with eigenvalue > 1 were considered for the discussion. Automatic Linear Modelling (ALM) was used to determine the soil parameters that best-influenced truffle production. The independent variables (predictors) were processed via forward stepwise regression to calculate their importance on the target variable (production). ALM was performed at a confidence level of 95%. All statistics were executed with IBM SPSS for Windows version 28.0.1.1.

3 Results

3.1 Main chemical and physical properties

The mineralogy of the soils in all the areas studied was predominantly inherited from the parent material, with sheet silicates, particularly smectite, representing the most abundant mineral phase. This was observed in Schlier (398 and 307 g kg⁻¹, respectively) and in Scaglia variegata (330, 268, 450 g kg⁻¹, respectively), where it was accompanied by the presence of calcite (Table 2). The observed mineralogy was statistically significant ($p \leq 0.05$).

A relatively high quartz content was identified in Bisciario and Schlier (152 and 146 g kg⁻¹) in comparison to Scaglia variegata and alluvial deposits ($p < 0.05$) (Table 2). Additionally, the feldspar content differed significantly between Schlier (50 g kg⁻¹) and Bisciario, Scaglia variegata, and alluvial deposits (35, 33, and 26 g kg⁻¹, respectively) ($p < 0.05$) (Table 2).

The main chemical and physical soil characteristics of the studied plantations are shown in Table 2.

The soils were moderately alkaline, ranging from pH 7.9 in Scaglia variegata to pH 7.7 ($p < 0.05$) in Bisciario. Likewise, the carbonate content was between 536 g kg⁻¹ in Scaglia variegata and 130 g kg⁻¹ in Bisciario for CC and between 266 and 133 g kg⁻¹ for AC.

The results showed a significant statistical difference ($p < 0.05$) in the amounts of larger particles (greater than 2 mm in diameter) or skeleton between the mean values of alluvial deposits (638 g kg⁻¹) and those of Scaglia variegata (122 g kg⁻¹), Schlier (145 g kg⁻¹) and Bisciario (446 g kg⁻¹). The clay-size fraction was significantly ($p < 0.05$) higher in the Schlier and Scaglia variegata (428 and 389 g kg⁻¹) compared to the Bisciario and alluvial deposits (250 and 125 g kg⁻¹).

The cation exchange capacity was higher in Schlier (22.43 cmol kg⁻¹), Bisciario (18.14 cmol kg⁻¹) and Scaglia variegata (18.15 cmol kg⁻¹) compared to alluvial deposits (9.29 cmol kg⁻¹) ($p < 0.05$). This was primarily due to the presence of clay.

Table 2 Mean properties of the soils weighted as a function of fine earth content corresponding to ANOVA on different parent material and recommended ranges (n = 62)

Variable	Parent material								$p \leq$	Recommended range ^a
	Scaglia variegata ^b		Alluvial deposits ^b		Schlier ^b		Bisciaro ^b			
	Mean	SE	Mean	SE	Mean	SE	Mean	SE		
Skeleton (g kg ⁻¹)	122c*	12	638a	31	145c	23	446b	28	0.001	20–900
Sand (g kg ⁻¹)	226a	4	148b	8	227a	7	200a	15	0.005	280
Silt (g kg ⁻¹)	262a	3	87c	9	199b	4	102c	11	0.001	514
Clay (g kg ⁻¹)	389a	10	125c	14	428a	13	250b	15	0.001	<460
pH	7.9a	0.1	7.8ab	0.1	7.8ab	0.1	7.7b	0.1	0.001	7.8–8.5
Calcium carbonates (g kg ⁻¹)	536a	6	198c	17	327b	7	130d	44	0.005	10–837
Active carbonates (g kg ⁻¹)	266a	6	97b	10	250a	7	133b	11	0.001	2–81
OC (g kg ⁻¹)	11.2ab	0.8	8.9b	0.7	13.9a	0.8	15.7a	1.1	0.005	8.7–46
TN (g kg ⁻¹)	1.07ab	0.2	0.89b	0.1	1.45a	0.1	1.46a	0.1	0.010	1–3
C:N	10.3	0.2	10.1	0.2	9.6	0.2	10.8	0.2	0.040	8–15
Available P (mg kg ⁻¹)	17.5a	2.1	7.9b	0.8	7.6b	0.6	7.9b	0.3	0.001	0.1–0.3%(total)
Exchangeable Ca (cmol kg ⁻¹)	17.16a	0.3	8.69b	0.7	21.12a	0.9	17.08a	1.4	0.001	5.23–46.16
Exchangeable K (cmol kg ⁻¹)	0.36ab	0.02	0.24b	0.02	0.56a	0.04	0.43ab	0.02	0.001	0.07–1.47
Exchangeable Mg (cmol kg ⁻¹)	0.51ab	0.04	0.31b	0.02	0.65a	0.05	0.55ab	0.09	0.001	0.41–6.34
Exchangeable Na (cmol kg ⁻¹)	0.10a	0.01	0.03c	0.01	0.08ab	0.01	0.06bc	0.01	0.001	n.d
CEC (cmol kg ⁻¹)	18.15a	0.36	9.29b	0.71	22.43a	0.99	18.14a	1.59	0.001	11.66–50.04
Sheet silicates (g kg ⁻¹)	330b	7	124d	11	398a	15	247c	9	0.001	n.d
Smectite (g kg ⁻¹)	268a	4	100c	8	307a	12	201b	3	0.001	n.d
Feldspars (g kg ⁻¹)	33b	2	26b	2	50a	3	35b	5	0.001	n.d
Quartz (g kg ⁻¹)	64b	2	38c	3	146a	12	152a	11	0.001	n.d
Calcite (g kg ⁻¹)	450a	4	172c	15	260b	6	98d	4	0.001	n.d

n number of soils, SE standard error

*In the same row differences among parent material were at $p \leq 0.05$

^bLulli et al. [9], Raglione and Owczarek [7], García-Montero et al. [20, 21]

^cControl soil samples were collected outside the studied stands in the neighboring and non-productive areas; additional control samples were collected on Bisciaro sediments

The organic carbon and total nitrogen (TN) contents were higher in Bisciaro (15.7 and 1.46 g kg⁻¹, respectively) and Schlier (13.9 and 1.45 g kg⁻¹, respectively), and lower in alluvial deposits (8.9 and 0.89 g kg⁻¹, respectively) ($p < 0.05$). Conversely, the available P is consistently higher in Scaglia variegata (17.5 g kg⁻¹) than in the alluvial deposits, Schlier and Bisciaro (7.9, 7.6 and 7.9 g kg⁻¹, $p < 0.05$, respectively).

3.2 Humic substances

To give deeper details on the semi-quantitative distribution of HS functional groups for each soil (Fig. 2), deconvoluted band integrals (% of total area) in the region between 1800 and 1240 cm⁻¹ were processed by ANOVA test (Table 3). The carbonyl group in amide I (1646 cm⁻¹) was statistically greater in Scaglia variegata (42.41%) and alluvial deposit (34.30%), but no statistical difference was found between Schlier and Bisciaro. The carboxylate group percentage (1579 cm⁻¹) was highest in Bisciaro (34.4%), though it did not differ significantly from Scaglia variegata and alluvial deposits. In contrast, Schlier was statistically different from the others by exhibiting the lowest value (20.52%). As for the other bands coupled with the bending of carboxylate groups at 1415 cm⁻¹, a statistically significant difference was observed between Schlier (13.12%) and Bisciaro (18.22%). In the case of Scaglia variegata and alluvial deposits this band shifted at 1381 cm⁻¹. In the alluvial deposits, it accounted for 19.96% and it was statistically diverse from the Scaglia variegata (12.91%) and Schlier (8.62%). For the aromatic rings (1535 cm⁻¹), the highest value was found in Schlier (17.86%), while the lowest was seen in Bisciaro (4.50%). No statistically significant difference was identified between Scaglia variegata and alluvial deposits. The aromatic skeletal vibration of

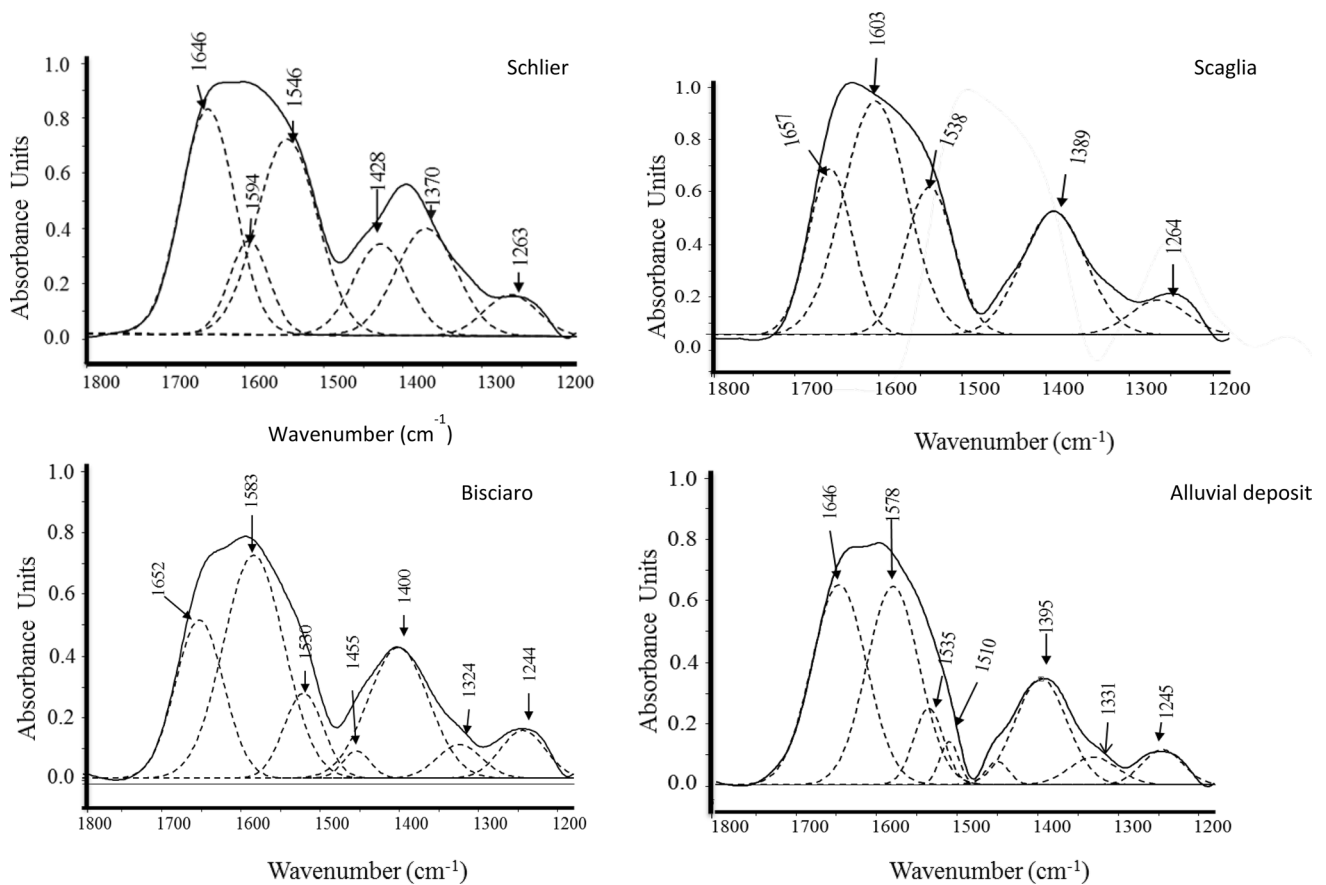


Fig. 2 Original spectra (solid line) from 1800 to 1200 cm^{-1} of humic substances and their deconvoluted spectra by using the curve-fitting process (dotted line)

Table 3 Mean values and ANOVA results of the percentage area of deconvoluted peaks in the region between 1800 cm^{-1} and 1240 cm^{-1} of the humic substances extracted from soils on different parent material ($n=62$)

Spectra region	Parent material								$p \leq$
	Scaglia variegata		Alluvial deposits		Schlier		Bisciario		
Frequency (cm^{-1})	Mean	SE	Mean	SE	Mean	SE	Mean	SE	
1646	42.41a*	1.3	34.30b	0.7	25.88c	0.9	21.38c	0.8	0.001
1579	31.51a	1.1	30.23a	0.8	20.52b	2.9	34.34a	2.3	0.050
1535	8.57b	0.4	8.10b	0.5	17.86a	1.3	4.50c	0.4	0.001
1510	n.d	n.d	2.19	0.3	n.d	n.d	n.d	n.d	0.001
1450	n.d	n.d	1.01b	0.1	1.05b	0.1	2.52a	0.3	0.002
1415	n.d	n.d	n.d	n.d	13.12b	1.4	18.22a	1.5	0.001
1381	12.91b	1.2	19.96a	0.9	8.62b	1.3	n.d	n.d	0.001
1331	n.d	n.d	0.77	0.21	2.77	0.7	2.00	0.2	0.094
1245	5.33a	0.3	2.49b	0.31	4.56a	0.2	5.19a	0.6	0.001

Deconvoluted band at ca. 1646 cm^{-1} was C=O stretching of amide I, quinone and water, 1579 cm^{-1} was $-\text{COO}^-$ asymmetric stretching and aromatic C=C stretching, 1535 cm^{-1} was amide II, aromatic C=C stretching, 1510 cm^{-1} was aromatic skeletal vibrations in lignin, 1450 cm^{-1} was CH_2 and CH_3 deformation), 1415–1381 cm^{-1} was aliphatic CH deformation, $-\text{COO}^-$ symmetric stretching, and 1330–1245 cm^{-1} was C–O stretching of phenols and ethers, C–OH deformation [42]

SE standard error

*In the same row differences among parent material were at $p \leq 0.05$

lignin (1510 cm^{-1}) was detected only in the alluvial deposit. In regard to C–O vibration in phenols and ethers (1245 cm^{-1}), the alluvial deposits (2.49%) exhibited statistically significant differences from others sites.

3.3 Relationships among variables

Soil parameters correlated each other (Table 4). Skeleton had a strong and negative correlation with sand ($r = -0.93, p \leq 0.01$), clay ($r = -0.98, p \leq 0.01$) and AC ($r = -0.97, p \leq 0.01$). Active carbonates positively correlated with CC ($r = 0.80, p \leq 0.01$) and Ex Ca ($r = 0.82, p \leq 0.01$). Cation exchange capacity strictly correlated with Ex Ca ($r = 0.99, p \leq 0.01$), clay ($r = 0.93, p \leq 0.01$), and OC ($r = 0.71, p \leq 0.01$), while OC sturdily correlated with TN ($r = 0.95, p \leq 0.01$) (not shown). With respect to minerals, smectite (Sme) finely correlated with sheet silicates ($r = 0.99, p \leq 0.01$), feldspars with quartz ($r = 0.79, p \leq 0.01$), and sheet silicates with clay ($r = 0.98, p \leq 0.01$) and CEC ($r = 0.97, p \leq 0.01$).

In general, correlations between functional groups were not robust, albeit they were significant at two levels of significance. For instance (Table 4), the C=O in amide I (1646 cm^{-1}) was correlated with quartz ($r = -0.67, p \leq 0.01$), mica ($r = -0.66, p \leq 0.01$) and calcite ($r = 0.58, p \leq 0.01$). No correlation was found between carboxylate group (1579 cm^{-1}) and mineral and chemical parameters. In contrast, the bending of carboxylate groups (1415 cm^{-1}) showed the highest correlations with CEC ($r = 0.73, p \leq 0.01$) and quartz ($r = 0.93, p \leq 0.01$). The aromatic ring (1535 cm^{-1}) was correlated ($r = -0.65, p \leq 0.01$) with aromatic skeletal vibration of lignin (1510 cm^{-1}). The latter group (1510 cm^{-1}) correlated with mineral parameters (e.g., with smectite C), while low correlations were found for CC and OC.

Furthermore, the calcite content had a positive impact on ascomata production (prod), as shown in Table 4. Notably, the calcite concentration accounted for 80% of the variance in ascomata production ($r = 0.80, p \leq 0.01$). Other correlations were with C=O in amide I at 1641 cm^{-1} ($r = 0.84, p \leq 0.01$) and carboxylate at 1415 cm^{-1} ($r = -0.71, p \leq 0.01$). To a less extent, also CC and AvP showed a positive relationship with the ascomata production ($r = 0.76, p \leq 0.01$ and $r = 0.68, p \leq 0.01$, respectively).

PCA extracted three factors, accounting for 82% of the total variance (Table 5). Factor 1 explained 46.2% of the variance and was correlated with sheet silicates, smectite, clay, Ex Na, CEC, Ex Ca, AC, silt, C–O in phenols (1242 cm^{-1}), quartz, skeleton and aromatic skeletal vibration of lignin (1510 cm^{-1}). Factor 2 was found to account for 22.7% of the variance and was correlated with OC, Ex K, Ex Mg, feldspars, and with C=O in amide I (1641 cm^{-1}). The remaining of the variance (13.1%) was explained by Factor 3, which was mostly correlated with available P, calcite and CC. Plotting data according to PC1 and PC2 has allowed identifying two main clusters: alluvial deposits (in the left) from Schlier, Scaglia variegata and Bisciario (in the right) (Fig. 3a). In particular (Fig. 3b), alluvial deposits were highly endowed in the skeleton and aromatic skeletal vibration of lignin (1510 cm^{-1}), while Schlier and Scaglia variegata were characterized by high values of AC, clay and smectite. Furthermore, Schlier was higher in OC, Ex K, Ex Mg, feldspars than Scaglia variegata. PC1 and PC3 instead well separated alluvial deposits (on the left) from Scaglia variegata (upper right) and from Schlier and Bisciario (center-low of the graph) (Fig. 4a). Scaglia variegata was more endowed in CC, calcite and available P, while Schlier and Bisciario were characterized by a relatively high content in quartz and feldspars (Fig. 4b).

Truffle production was also estimated on the variables by ALM. The model, tested on our plantations, showed that out of the 21 initial variables, 6 were incorporated into the equation while the remaining variables were excluded. The predictor variables accounted for 94.3% of the variance of the dependent variable. The relative importance of each variable is reported (Fig. 5). Since the coefficients are relative, the sum of these coefficients was equal to 1. The importance of the predictors (Fig. 5a) decreased following the order: calcite ($0.445, p \leq 0.001$), C=O in amide I (1641 cm^{-1}) ($0.223, p \leq 0.001$), available P ($0.157, p \leq 0.004$), Ex K ($0.084, p \leq 0.024$), feldspars ($0.066, p \leq 0.049$) and aromatic skeletal vibration of lignin (1510 cm^{-1}) ($0.016, p \leq 0.077$). From the coefficients (Fig. 5b), calcite, C=O in amide I (1641 cm^{-1}) and available P had a positive effect while Ex K, feldspars and aromatic skeletal vibration of lignin (1510 cm^{-1}) had a negative one.

4 Discussion

4.1 Properties of soils related to lithology

In the studied area, the lithology has given rise to soils characterized by a high limestone content and extremely variable granulometry. The combination of clay and skeleton created an environment optimal for the growth of *T. melanosporum*, specifically in soil that is not too asphyctic and drained well [43]. In terms of composition, the content of sheet silicates and smectites, which due to their expansion and compression process, could create problems for *T. melanosporum* growth, was mitigated by the skeleton. The presence of skeletons made the soil looser and mulching.

Table 4 Pearson's correlation (r) among soil variables and truffle production (n = 62)

Sk	Sand	Clay	CC	AC	OC	AVP	ExCa	ExK	ExMg	ExNa	CEC	Sme	Sheet/Sil	Fd	Qz	Cal	Mica	1641	1510	1535	1510	1450	1415	1381	1331	1242	
Sand	-0.93**	-																									
Clay	-0.98**	0.90**																									
CC	-0.76**	0.63**	-																								
AC	-0.97**	0.88**	0.80**	-																							
OC	-0.53**	0.67**	0.57**	0.47**	-																						
AVP	-0.35*	0.34*	n.s.	0.62**	0.30*	-																					
ExCa	-0.89**	0.88**	0.93**	0.43**	0.69**	n.s.	-																				
ExK	-0.61**	0.68**	0.66**	n.s.	0.57**	0.86**	n.s.	0.74**	-																		
ExMg	-0.65**	0.69**	0.68**	n.s.	0.62**	0.75**	0.31*	0.73**	0.85**	-																	
ExNa	-0.73**	0.60**	0.71**	0.63**	0.73**	n.s.	0.29*	0.66**	0.39**	0.55**	-																
CEC	-0.89**	0.88**	0.93**	0.42**	0.82**	0.71**	n.s.	0.99**	0.77**	0.76**	0.67**	-															
Sme	-0.96**	0.91**	0.98**	0.57**	0.91**	0.60**	n.s.	0.96**	0.68**	0.70**	0.69**	0.96**	-														
Sheet	-0.95**	0.90**	0.98**	0.53**	0.90**	0.62**	n.s.	0.97**	0.71**	0.72**	0.68**	0.97**	0.99**	-													
Sil																											
Fd	-0.70**	0.74**	0.77**	n.s.	0.64**	0.69**	n.s.	0.84**	0.76**	0.72**	0.42**	0.85**	0.82**	-													
Qz	-0.62**	0.66**	0.73**	n.s.	0.55**	0.68**	n.s.	0.84**	0.75**	0.68**	0.37*	0.84**	0.80**	0.85**	-												
Cal	-0.71**	0.58**	0.59**	0.99**	0.75**	n.s.	0.64**	0.36*	n.s.	0.59**	0.36*	0.51**	0.47**	n.s.	n.s.	-											
Mica	-0.31*	0.29*	0.42**	n.s.	0.37*	-0.38**	0.53**	0.50**	0.42**	n.s.	0.53**	0.47**	0.51**	0.62**	0.70**	n.s.	-										
1641	n.s.	n.s.	0.52**	n.s.	-0.30*	0.48**	n.s.	-0.33*	n.s.	n.s.	-0.29*	n.s.	n.s.	-0.39**	-0.67**	0.58**	-0.66**	-									
1579	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	-							
1535	-0.34*	n.s.	0.42**	n.s.	0.33*	n.s.	0.39**	0.44**	0.36*	n.s.	0.40**	0.39**	0.42**	0.54**	0.46**	n.s.	0.47**	-0.34*	-0.65**	-							
1510	0.68**	-0.63**	-0.69**	-0.46**	-0.64**	-0.39**	n.s.	-0.62**	-0.46**	-0.47**	-0.62**	-0.69**	-0.68**	-0.42**	-0.54**	n.s.	n.s.	n.s.	n.s.	n.s.	-						
1450	0.35*	-0.29*	-0.31*	-0.45**	-0.34*	n.s.	n.s.	n.s.	n.s.	-0.33*	n.s.	-0.30*	n.s.	n.s.	-0.46**	n.s.	-0.54**	0.31*	n.s.	n.s.	-						
1415	-0.41**	0.48**	0.54**	n.s.	0.32*	0.61**	-0.36*	0.73**	0.65**	0.56**	n.s.	0.73**	0.65**	0.74**	0.93**	-0.31*	0.69**	-0.72**	n.s.	0.41**	-0.41**	n.s.	-				
1381	0.42**	-0.46**	-0.46**	n.s.	-0.34*	-0.43**	n.s.	-0.58**	-0.43**	-0.36*	-0.40**	-0.58**	-0.52**	-0.36*	-0.60**	n.s.	-0.41**	0.33*	n.s.	n.s.	0.57**	n.s.	-0.61**	-			
1331	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.40**	-0.51**	n.s.	n.s.	0.72**	n.s.	-0.34*	-			
1245	-0.61**	0.49**	0.60**	0.56**	0.58**	0.29*	0.31*	0.48**	0.31*	0.31*	0.53**	0.48**	0.55**	0.53**	0.32*	0.53**	n.s.	n.s.	0.30*	-0.55**	n.s.	n.s.	n.s.	n.s.	-		
Prod	n.s.	n.s.	n.s.	0.76**	n.s.	n.s.	0.68**	n.s.	-0.32*	n.s.	0.30*	n.s.	n.s.	-0.40**	-0.58**	0.80**	-0.64**	0.84**	n.s.	-0.33*	n.s.	-0.42**	-0.71**	n.s.	-0.29*	0.31*	

Deconvoluted bands at ca. 1646 cm⁻¹ was C=O stretching of amide I, quinone and water, 1579 cm⁻¹ was -COO⁻ asymmetric stretching and aromatic C=C stretching, 1535 cm⁻¹ was amide II, aromatic C=C stretching, 1510 cm⁻¹ was aromatic skeletal vibrations in lignin, 1450 cm⁻¹ was CH₂ and CH₃ deformation, 1415-1395 cm⁻¹ was aliphatic CH deformation, -COO⁻ symmetric stretching, and 1330-1245 cm⁻¹ was C-O stretching of phenols and ethers, C-OH deformation

Sk skeleton, TOTC total carbonates, AC actives carbonates, OC organic carbon, AVP available phosphorus, ExCa exchangeable calcium, ExK exchangeable potassium, ExMg exchangeable magnesium, ExNa exchangeable sodium, CEC cation exchange capacity, Sme smectite, Sheet sil sheet silicates, Fd feldspars, Qz quartz, Cal calcite, Prod carophore productivity

*≤0.05; **≤0.01; n.s., not significant

Table 5 Loadings values of some physical and chemical variables on the axes identified by principal components analysis for the studied soils

Variable	PC1	PC2	PC3
Skeleton	-0.905	-0.347	-0.162
Sheet silicates	0.903	0.390	0.115
Smectite	0.901	0.392	0.125
Clay	0.900	0.398	0.123
Exchangeable Na	0.868	0.263	0.138
Cation exchange capacity	0.864	0.460	0.032
Exchangeable Ca	0.863	0.447	0.035
Active carbonates	0.853	0.353	0.257
Silt	0.798	0.077	0.554
1510 cm ⁻¹	-0.781	-0.200	0.009
1242 cm ⁻¹	0.743	0.234	0.084
Quartz	0.732	0.519	-0.365
1537 cm ⁻¹	-0.442	0.282	0.202
Organic C	0.183	0.875	0.103
Exchangeable K	0.355	0.861	-0.001
Exchangeable Mg	0.426	0.798	0.129
Feldspars	0.487	0.761	-0.173
1641 cm ⁻¹	-0.135	-0.696	0.123
Available P	0.106	0.275	0.886
Calcite	0.549	-0.210	0.784
Calcium carbonates	0.630	-0.153	0.728
1575 cm ⁻¹	-0.325	-0.142	0.496

Deconvoluted band at ca. 1646 cm⁻¹ was C=O stretching of amide I, quinone and water, 1579 cm⁻¹ was -COO- asymmetric stretching and aromatic C=C stretching, 1535 cm⁻¹ was amide II, aromatic C=C stretching, 1510 cm⁻¹ was aromatic skeletal vibrations in lignin, 1450 cm⁻¹ was CH₂ and CH₃ deformation, 1415-1395 cm⁻¹ was aliphatic CH deformation, -COO- symmetric stretching, and 1245 cm⁻¹ was C-O stretching of phenols and ethers, C-OH deformation

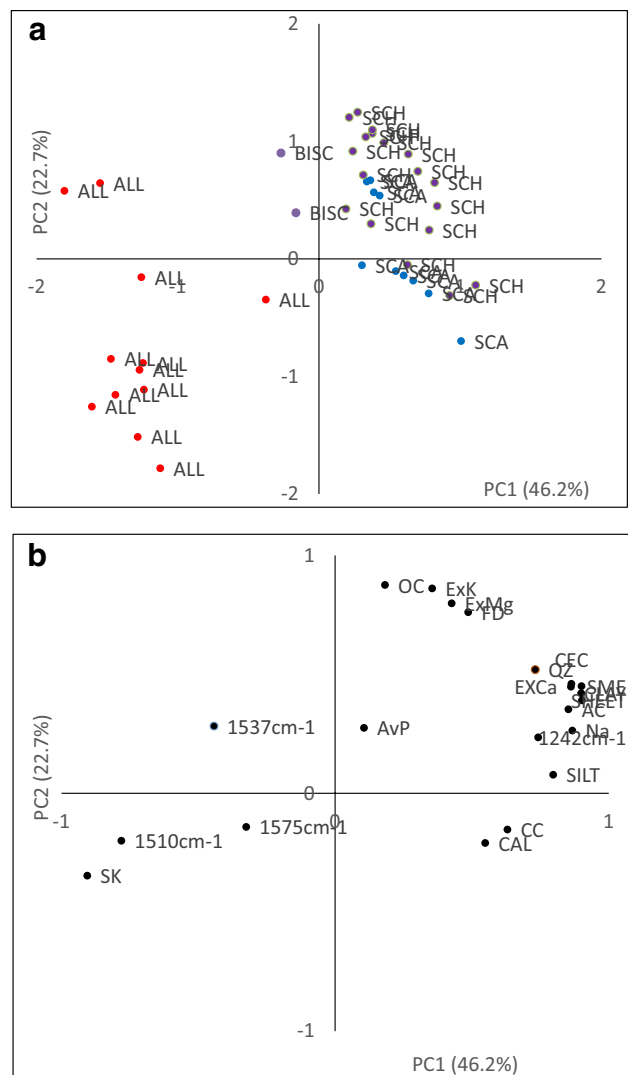
The typical reaction of buffering power from carbonates was the optimal one for the growth of black truffle [17, 18]. Black truffle prefers soils with low organic matter in A horizon [10]; this is also due to the formation of the brûlés which acts as a deterrent to vegetable forms. The low endowment of organic carbon, and the C:N ratio values between 9 and 10 in our soils are considered optimal [44], indicating good mineralization of the organic matter. Adequate availability of active carbonate (generally 4–16%) may play an important role in truffle fructification [20].

The correlation matrix indicates that soil physical and chemical properties and processes are the predominant variables explaining the differences among the studied areas. The strong inverse relationship between skeleton and clay-size fraction and sand reflected differences in the parent material, which can be further elucidated by considering the effects of weathering.

The scattered points in the PCA demonstrate the soil heterogeneity. Soils from alluvial deposits were well separated from those from Scaglia variegata and Schlier (Figs. 3a and 4a). Additionally, the close relationship between exchangeable calcium and cationic exchange capacity and clay (Table 3, Fig. 3b) indicated the importance of calcium in these soils. Constitutional alkalinity was also evidenced by the pH values and elevated presence of active carbonates as found elsewhere [45]. Nevertheless, the physical and chemical composition of the soils ruled out a major role for some variables in determining significant differences existing among the geologic substrates in Acqualagna. It is therefore likely that the silicate minerals occurring in our soils represent the primary detrital material originating from the geological terrains. In contrast, the pedogenic effects are poorly detectable on the bulk soil materials. Therefore, a primary mineralogical signature is expected in soils developed in geologic young areas, such as the central Apennines.

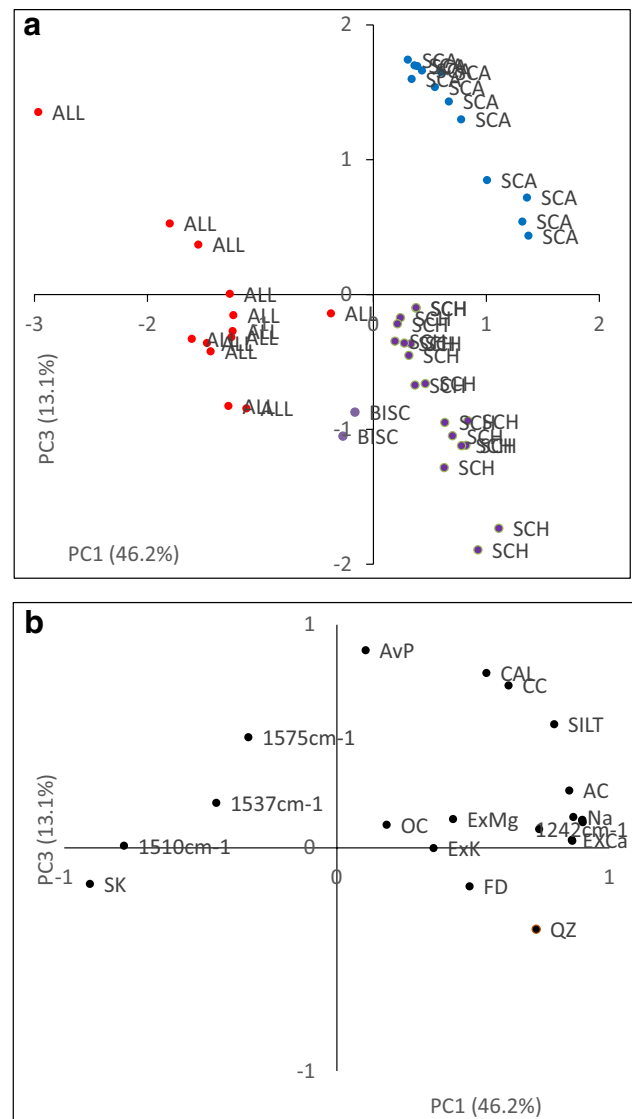
Clay-size fraction and skeleton were significant variables in discriminating soils from Scaglia variegata and Schlier from those originating from alluvial deposits. The Schlier sediments, which consist of marls and clayey marls, and the Scaglia variegata sediments, which are composed of marly limestones, calcareous marls, and marls, are known to be parent rocks from which soils with high clay and expansive clay mineral contents originate.

Fig. 3 Position of the soil samples distinct by geology in the reduced space of axes 1–2 (a) determined by principal components analysis (red, Alluvial deposits; grey, Schlier; blue, Scaglia variegata; purple, Bisciaro) and position of variables (b) (AC active carbonates, CC carbonates, CEC cation exchange capacity, ExCa exchangeable calcium, ExK exchangeable potassium, ExMg exchangeable magnesium, ExNa exchangeable sodium, Fd feldspars, OC organic carbon, Qz quartz, Sk skeleton, Sheet sheet silicates; 1510 cm^{-1} , lignin; 1575 cm^{-1} , aromatic C; 1537 cm^{-1} , amide II)



On the other hand, alluvial deposits formed by heterogeneous deposits, mainly gravels and sandy-gravels, give origin to soils rich in skeleton. Furthermore, PCA positively correlated with active carbonates, sheet silicates and smectite, whose presence was relevant in soils from Scaglia variegata and Schlier, while scarce in soils from alluvial deposits. However, there were notable differences between Schlier and Scaglia variegata, as the former was rich in quartz, and the latter in calcite. The high calcite content in soils from Scaglia variegata was due to depositional conditions. These observations suggest that lithology is one of the main driving factors determining the soils' chemical, physical, and mineralogical properties in the studied areas. Yet, because the areas were immediately adjacent, there were no significant differences in climatic conditions and topography. Like our observations, Sierra et al. and Cardelli et al. [46, 47] also have found strict correlations between the nature of the parent material and some physical and chemical properties of soils of other areas. In the present study, the chemical composition of humic substances was observed to undergo alteration in soils exhibiting differences in their parent material and mineral composition. In particular, in Schlier and Scaglia variegata, AC deposits, clay and smectite were identified as the dominant components, whereas in alluvial deposits, the presence of lignin residues (1510 cm^{-1}) indicated that wood or leaf debris deposition may be contributing factors to the composition of HS. Overall, there was considerable variation in the composition of the humic substances observed at the different sites. This evidence suggests that the interaction between organic matter and the changing lithology was diverse [48].

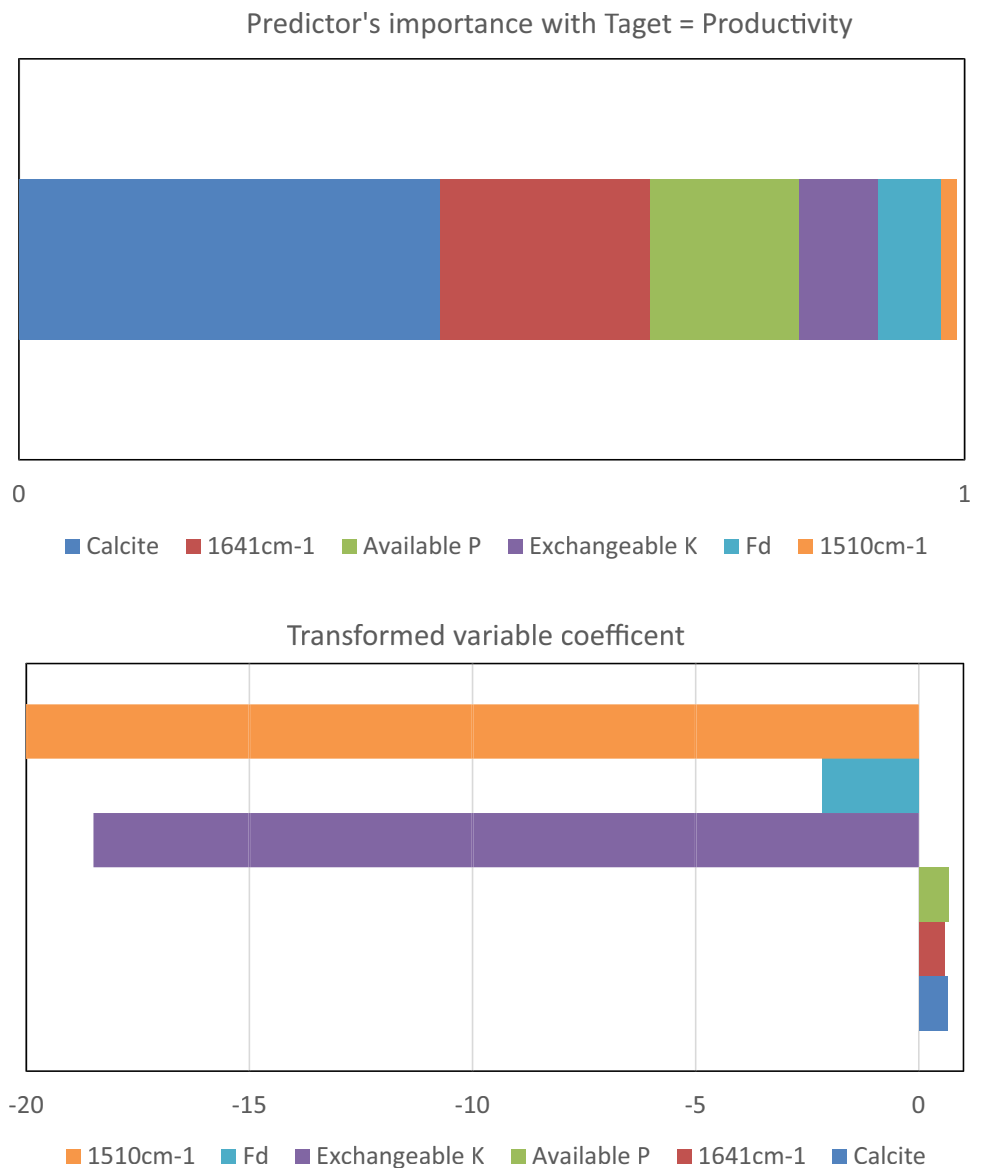
Fig. 4 Position of the soil samples distinct by geology in the reduced space of axes 1–3 (a) determined by PCA (red, alluvial deposits; grey, Schlier; blue, Scaglia variegata; purple, Bisciaro) and variables projected in the same plane (b) (AC active carbonates, AP available phosphorus, CEC cation exchange capacity, ExCa exchangeable calcium, ExK exchangeable potassium, ExMg exchangeable magnesium, ExNa exchangeable sodium, TC total carbonates, Qz quartz, Sk skeleton; 1510 cm^{-1} , lignin; 1575 cm^{-1} , aromatic C; 1537 cm^{-1} , amide II; 1242 cm^{-1} , phenols and ethers)



4.2 Relationships among soil properties and black truffle production

The Acqualagna area is recognized as suitable for the cultivation of *T. melanosporum*, based on both practical experience and historical traditional cultivation. Our soil analyses indicate that the majority of values fall within the recommended ranges [7, 9, 44, 49–51]. In particular, a link between soil physical and chemical properties and the black truffle production was found. Our findings indicate a strictly positive correlation between the ascospores production and the following soil properties: calcite, total carbonates, and available P (Table 3 and Fig. 4). Yet, the most calcareous soils in terms of total carbonates, calcite and active carbonates bear the most productive truffle beds (site 3, Scaglia variegata). Carbonates are considered a well-known factor driving black truffle production [9, 14]. Active carbonates, defined as a fine fraction of calcareous rock and thus of total carbonates, are distinguished by their high chemical activity and represent an important reserve of exchangeable Ca^{2+} . Active carbonates play a crucial role in regulating soil pH and maintaining the soil structure. It has been established that calcium is a key element for the growth of the mycelium of *T. melanosporum* [52]. This is likely due to their ability to acidify their environment and dissolve calcium carbonate providing carbonates, bicarbonates and other salts [53]. Our findings align with the results reported by García-Montero et al. [18], who observed the growth of *T. melanosporum* mycelia directly from the carbonated soil fraction. [53]. Additionally, a higher abundance of active carbonate inside brûlés than outside was also found in soils from Jurassic and Cretaceous limestones and dolomites [20, 21], and this well accounted for up to 43% of ascospores production [16–18]. A recent study conducted in Spain

Fig. 5 ALM graphical results. **a** Predictive importance of soil chemical variables to car-pophore production: calcite, 1641 cm^{-1} (amide I), available P, exchangeable K, feldspars, 1510 cm^{-1} (lignin). **b** Trans-formed variable coefficients: 1510 cm^{-1} (lignin), feldspars, exchangeable K (negative), and available P, 1641 cm^{-1} (amide I), calcite (positive)



[54] demonstrated a negative correlation between carbonate content and black truffle productivity. The study proposed that watering may act as a significant interfering factor on truffle production, thereby providing a plausible explanation for this result. Furthermore, the authors highlighted the need for additional research into the physico-chemical characteristics of the topsoil and their impact on truffle fruiting.

Likewise, Jaillard et al. [55] found that very calcareous soils may not be optimal for truffle production, particularly in comparison to dolomitic soils with an active carbonate content of $4 \pm 7\text{ g kg}^{-1}$.

In addition to carbonates, the most productive soils were found to be strictly correlated with the content in clay-size fraction (Tables 2 and 3). Moreover, skeleton and clay, which were inversely correlated, displayed the same trend (low skeleton and high clay) in low (site 1, Schlier) and high productive soils (site 3, Scaglia variegata) (Fig. 4a, b). Conversely, the opposite trend (high skeleton and low clay) was observed in intermediate productive soils (site 2, alluvial deposits) (Fig. 4a, b). It is challenging to provide an explanation that is directly applicable in this context. Although soil aeration and water availability were not directly determined, the aforementioned results suggest that the balancing of these two factors is not the sole prerequisite for black truffle fructification in the Acqualagna area. This is corroborated by the observation that the same results were observed in both high and low productive soils. Soil stoniness regarded as a favourable condition for the cultivation of *T. melanosporum* due to its beneficial effect on drainage and aeration [9, 17, 18]. Conversely, surface stoniness reduces summer evapotranspiration and provides protection against soil compaction and erosion induced by rainfall. On the other hand, although an excess of stones might reduce water-holding capacity,

higher clay contents can offset this drawback. In agreement with our results, high clay content as well as bulk density and water-holding capacity, are favourable for the cultivation of black truffles [54].

Overall, the results suggest that the soil properties can be interrelated, yet collinearity between variables might be present [56]. To overcome this issue and determine the main factors influencing ascomata formation in our plantations, ALM was applied on the independent variables with ascomata production as the target variable. The resulting model showed that 6 variables entered the equation, accounting for 94.3% of the variations in the production; calcite was the most important predictive variable, followed by available P, exchangeable K and feldspars. In this regard, it is known that for a good ascomata production the percentage in calcite should increase with the clay content, in particular if the expandable type (smectite) is dominant [57]. Indeed, the high-productive soils (site 3, Scaglia variegata) had a twofold higher calcite: smectite ratio than the low-productive soils (0.85) (site 1, Schlier). Calcite is also considered important because controls the activities of $\text{Ca}^{2+}(\text{aq})$ in soils containing this mineral. Our results agree with others previously reported [7, 58], which showed that pH, carbonates, and organic matter content were the most discriminant variables in the Carpathian and Apennine Mountains. In terms of soil-available phosphorus, our findings demonstrate that there is a positive relationship between truffle production and phosphorus. This is supported by the evidence that the hyphae have highly efficient absorption systems which enable them to obtain phosphorus and other nutrients from the surrounding soil [59].

The characterisation of humic substances (HS) has yielded valuable insights into the potential influence of specific functional groups on black truffle production. ALM also found that the Amide I (1641 cm^{-1}) had a positive effect whilst the lignin residues (1510 cm^{-1}) had a negative one. Amide I has been demonstrated to possess biostimulant activity [60]. Conversely, the negative impact of lignin residues may be attributed to their complex formation with HS, which renders them highly resistant to microbial degradation [61]. Consequently, the high level of lignin compounds may contribute to the stabilization of soil organic carbon [62]. It has been demonstrated that HS has the capacity to enhance soil fertility and improve soil structure, regulating pH in the manner that creates an environment optimal for the growth not only of the host plants but also of the truffles themselves. Thus, the result is an improved yield and quality in truffle cultivation.

5 Conclusions

The aim of this study is to find out how differences in the parent material can affect the soil properties of Périgord black truffle plantations under constant cultivation techniques, and how these differences affect the productivity of black truffles in the Acqualagna area. The high carbonate contents found in high-productivity soils allow for the differentiation between intermediate soils and low-productivity soils. The balance between the skeleton and clay fraction is a key factor. Furthermore, humic substances containing amide I and quinonic groups and low lignin residues have been shown to have a positive impact on truffle production. The results of this study may contribute to the improvement of the black truffle cultivation, which represents an alternative for the sustainable development of regions affected by depopulation, such as those of central Italy. A considerable number of studies on black truffles have been conducted on soils developed from marine limestone (e.g. Spanish studies). These soils are distinguished by a high carbonate content. However, soils derived from sediments, such as those found in the central Apennines, which exhibit a lower carbonate content, have also demonstrated remarkable suitability for truffle cultivation. Although the study area was not particularly extensive, the results achieved are promising in light of the potential for further research in larger study areas. Truffles play a significant socio-economic and ecological role in the environment. In this context, truffle plantations are subject to a number of critical challenges associated with climate change, including irregular rainfall and poor soil conditions. Consequently, there is a need to enhance understanding of the diverse habitats in which truffles naturally grow in order to provide effective support to truffle farms and growers.

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Data availability The raw data used for statistical processing are available on request.

Declarations

Competing interests The authors declare no competing interests.

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