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Assessment of basic soil parameters related to the spread and development of Tuber aestivum fruiting bodies in Western Bulgaria

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Abstract. *Tuber aestivum* is one of the most widespread truffle species which is found on the territory of Bulgaria. Its natural habitats include a wide range of soil units and a wide spectrum of ectomycorrhizal host tree species. In our study we have obtained valuable data of basic soil parameters in natural habitats of the summer truffle in Western Bulgaria. The soil reaction of the studied soils varies significantly. The pH (H_2O) of soils was assessed as slightly alkaline to neutral, and in fewer cases it was slightly acidic. In most productive truffle sample plots the average pH value was 6.9, the soils had moderately fine texture and were rich in SOC. The average Org.C/N ratio was 10, therefore the transformation of organic matter is high, and the soil is well stocked with total nitrogen. All the studied soils were rich in exchangeable calcium and highly saturated with bases.

Key words: summer truffle, Burgundy truffle, soil texture, soil chemical properties.

Introduction

The family Tuberaceae is one of the most diverse lineages of fungi that form truffles (Læssøe & Hansen, 2007) although it is not composed strictly of truffle fungi (Bonito et al., 2013). Truffles are fruiting bodies of ascomycetous fungi belonging to the genus *Tuber* (Ascomycota, Pezizales). The number of species in this genus remains unclear but it was assumed that it comprises at least 180 species worldwide (Bonito et al., 2010). In 2019 two new species (*T. pulchrosporum* and *T. thracicum*) which belong to this genus were found and described in Bulgaria (Polemis et al., 2019). It should be noted that species of *Tuber* can form not only ectomycorrhizas but also mycorrhizas (Lancellotti et al., 2014) and endomycorrhizas with orchids (Selosse et al., 2004).

One of the commercial truffle species is *Tuber aestivum* (Vittad.) commonly known as summer or Burgundy truffle. Some authors suggested that T. *aestivum* and *T. uncinatum* Chatin are synonyms (Paolocci et al., 2004; Wedén et al., 2004). In 2013 it has been proven that *Tuber aestivum* and *Tuber uncinatum* Chatin are conspecific (Molinier et al., 2013). It is considered to be the third most widespread truffle in Europe (Hall et al., 2007; Chevalier, 2010, 2012) with broad ecological amplitude (Robin et al., 2016). It is found in both urban and natural habitats at a wide range of elevations (Nedelin et al., 2017). The summer truffle may form ectomycorrhizas with a wide range of host plants of different genera such as *Pinus*, *Picea*, *Carya*, *Castanea*, *Cistus*, *Corylus*, *Fagus*, *Ostrya*, *Tilia*, and *Quercus* (Wang et al., 2008; Chevalier, 2009; Benucci et al., 2012; Hilszczańska et al., 2019). According to Zambonelli et al. (2015), it has an immense potential for cultivation because of its adaption to a wide range of soils, climate, and host plants. Innagi et al. (2020) pointed out that in the temperate habitats the fruiting season has two maxima (July and November). Fruiting of truffles cannot take place even when climatic conditions are favorable if certain soil characteristics are inappropriate. The soil conditions which are favorable to truffle fruiting can vary significantly by species (Chevalier & Sourzat, 2012).

In recent decades black truffles are more valued because of their unique taste and aroma (Berch, 2013; Berch & Bonito, 2014) and hence the high price on the market. For the past two decades, truffle hunting in Bulgaria has attracted ever growing attention (Nedelin et al., 2022). Although several authors have focused their research worldwide on soil characteristics (Chevalier & Sourzat, 2012; Gógán et al., 2012; Păcurar et al., 2019), which are a major factor in the development of fruiting bodies of *T. aestivum*, in Bulgaria there is still a lack of data on this topic. Our study was conducted to fulfil this gap of information. The main aim was to obtain data for soil parameters in natural distribution areas of *T. aestivum*. It was also intended to gain knowledge which soil characteristics play a key role to determine the favourable conditions for the development of a larger amount of truffle ascomata in the studied areas. A comparison of soil characteristic has been made between the areas with the largest amount of fruiting bodies and those with the least ones.

Materials and methods

Study area

To highlight and better understand the favourable soil conditions for *T. aestivum* ascomata, 22 sample plots were selected from over 50 investigated truffle areas in Western Bulgaria. The criteria for the selection were the higher ratio – productivity/area (kg·m^{-2}) of truffle sample plots, tree vegetation and altitude.

The field surveys were conducted two times per month from the end of May to the end of November 2023. Table 1 shows the main topographical characteristics of studied truffle sample plots and their momentary productivity. The specific geographical coordinates are not provided in the table due to the lack of legal framework regarding the truffle hunting in Republic of Bulgaria and to protect the truffle habitats from poachers. All the studied areas are located within 120 km from Sofia city.

The climate in the studied areas is moderate continental with average annual precipitation of 550 mm up to 860 mm. The maximum precipitation occurs from May to June (Koleva & Peneva, 1990).

Soil sampling

All soil samples were taken from the natural distribution truffle areas. They were collected according to FAO recommendations (FAO, 2006). From each sample plot, soil samples were taken in three repetitions of the soil layer (0-10 cm), where the *T. aestivum* ascomata occurs. The soil was collected after removing litter layers and the accompanying vegetation on the soil surface. To obtain data on bulk density of the soils, soil samples were taken using a metal cylinder in three repetitions from each sample plot. Approximately 2 kg of soil were collected from each sample plot in no more than 15 cm distance of *T. aestivum* fruiting bodies. Soil analyses were carried out in Central Laboratory of University of Forestry, Sofia.

Soil analyses and methods

Each soil sample was tested to determine the following parameters:

- Active soil reaction - pH(H_2O) (ISO 10390:2005);
- Bulk density (ISO 11272:1998);
- Particle density by pycnometer method (ISO 78710:1993).
- Soil porosity was determined by formula (1):

$$SP = \left(1 - \frac{BD}{PD}\right) \times 100 \quad (1),$$

where: SP is soil porosity in %, BD is bulk density in g cm^{-3} and PD is particle density.

- Soil organic matter was determined by modified Turin's method – 120°C, 45 min and $CuSO_4$ as a catalyst (Kononova, 1963, Filcheva & Tsadilas, 2002);
- Total nitrogen – Kjeldahl method (Kjeltec Auto 1030 Analyser);
- Available phosphorus (P_2O_5) and available potassium (K_2O) – acetate-lactate method (Ivanov, 1984);
- Soil texture was measured as clay – <2 µm; silt – 2–63 µm and sand – 63–2000 µm (ISO 11277:2009);
- Exchangeable cations (Ca^{2+}, Mg^{2+}, K^+, Na^+) after ISO 11260:2018;
- Soil water holding capacity and field capacity – Kachinskii method (Kachinskii, 1963).

Table 1. Topography, tree vegetation (hosts) and momentary productivity of the studied truffle sample plots.

Sample plot	Altitude, m	Exposition	Slope, %	Soil unit WRB, (2006, 2007)	Relief	Micro relief	Host trees	Truffle productivity	Annual productivity
1	580	SE	3	Vertisols	plane	plane	Qc, Ca, Cb	L	1
2	515	NE	2	Fluvisols	plane	plane	Tc	M	2
3	670	SW	10	Rendzinas	slope middle part	plane	Qp	L	1
4	815	NW	15	Rendzinas	slope middle part	plane	Tt	S	3
5	810	SW	12	Rendzinas	slope middle part	plane	Pn	L	1
6	670	NE	15	Rendzinas	slope upper part	plane	Pn, Ps	M	3
7	670	NE	15	Rendzinas	slope upper part	plane	Pn, Ps	M	3
8	770	SE	16	Rendzinas	slope lower part	plane	Co, Qc, Qp	L	1
9	880	SE	18	Rendzinas	slope lower part	plane	Qc, Qp, Tp, Ca	M	3
10	765	SW	14	Rendzinas	slope middle part	depres sed	Pn	S	2
11	765	SW	14	Rendzinas	slope middle part	depres sed	Pn	S	2
12	715	S	8	Rendzinas	slope lower part	plane	Pn	M	3
13	640	SW	10	Rendzinas	slope upper part	plane	Pn	L	1
14	820	NE	16	Luvisols	slope lower part	depres sed	Fs, Cb, Qs, Co, Ps	M	3
15	330	NE	6	Luvisols	depression	excava tion	Tt, Qf, Qd, Qf	S	2
16	325	NW	3	Luvisols	depression	plane	Tp, Qc, Cb, Qr, Co	S	1
17	330	NW	13	Luvisols	slope lower part	depres sed	Tt, Qd, Qc	M	2
18	320	NW	10	Luvisols	slope lower part	depres sed	Tt, Cb, Qc,Qf	M	1
19	300	NE	3	Luvisols	depression	plane	P I-214, Cb, Tt, Qd	L	1
20	295	NE	2	Vertisols	plane	plane	Qc, Cb, Ca,	S	3
21	325	NE	2	Vertisols	plane	plane	Tt, Qc, Cb, Qd	M	3
22	300	NE	2	Vertisols	plane	plane	Pb	S	3

Note: * S ≤ 25 m^2, 25 m^2 < M ≤ 50 m^2, L > 50 m^2; 1 > 5 kg, 2 = 2.5÷5 kg, 3 < 2.5 kg;

Ca- *Celtis australis* L., Cb – *Carpinus betulus* L., Cs – *Castanea sativa* Mill., Co - *Carpinus orientalis* Mill., Fs – *Fagus sylvatica* L., Pb - *Populus bachelieri*; P I-214 – *Populus* I-214, Pn – *Pinus nigra* Arn., Ps – *Pinus sylvestris* L., Qc – *Quercus cerris* L., Qd – *Quercus dalechampii* Ten., Qf- *Quercus frainetto* Ten., Qp- *Quercus petraea* (Matt.) Liebl., Tc- *Tilia cordata* Mill., Tp- *Tilia platyphyllos* Scop., Tt – *Tilia tomentosa* Moench.

Results and Discussion

Host plants

The results in Table 1 showed that in the studied sample plots the black summer truffle forms an ectomycorrhiza with a wide range of broad-leaved species (Betulaceae, Fagaceae, Malvaceae and Salicaceae) and some conifers (Pinaceae). The most common tree hosts belong to Fagaceae and Pinaceae. According to Fischer et al. (2017) most of black truffles occur and are collected from oak (*Quercus* spp.) forests. The highest truffle productivity was observed in stands composed of species mainly belonging to genus *Quercus*. García-Montero et al. (2014) suggests that *Pinus* spp. could also be considered as good hosts for *T. aestivum* mycorrhizae and significant amounts of truffles can be produced in pine forest areas. Our study confirms that suggestion. In two of the sample plots (SP5 and SP13) with highest collected amounts of fruiting bodies tree host was *Pinus nigra* Arn.

Soil structure

Soil structure of the soils in which *T. aestivum* fruiting bodies occur in the studied areas was assessed as good to excellent (Guimarães et al., 2011). Robin et al. (2016) mentions that soil structure is being highly loose and stable. The good soil structure provides a good water, air and temperature regime in soil which has a favorable effect on the growth of truffle fruiting bodies.

Bulk density, particle density and soil porosity

The obtained data on soil bulk density (BD) and porosity pointed out in Table 2 showed that soils in all studied sample plots are characterized by favorable soil physical properties. According to Mukhopadhyay et al. (2019) a BD of less than or equal to 1.3 $g{\cdot}cm^{-3}$ is considered as good. In our study, bulk density of soils in truffle sample plots is assessed as loose (0.90 $g{\cdot}cm^{-3}$) to good (1.33 $g{\cdot}cm^{-3}$). The average bulk density value for the surface horizon (0-10 cm) in the most productive truffle soils was 1.09 $g{\cdot}cm^{-3}$, and in soils with low amounts of fruiting bodies it was 1.20 $g{\cdot}cm^{-3}$. Although the difference between the two values is not significant, this suggests that as the bulk density values increase, the conditions for the growth of truffle ascomata deteriorate. Hakkou et al. (2023) obtained similar results to ours about the bulk density and defined soils as loose to slightly dense. Soil porosity varied in the narrow range between good (45%) and very good (63%) (Dilkova, 2014). These results showed that the physical properties of the studied soils create favorable conditions for the easier growth of the underground fruiting bodies of the summer truffle.

Table 2. Physical properties of the soil units.

Sample plot	Bulk density, $g{\cdot}cm^{-3}$	Particle density	Porosity, %
1	1.18	2.35	50
2	1.35	2.65	49
3	0.96	2.39	60
4	1.32	2.65	50
5	1.22	2.28	46
6	1.11	2.26	51
7	1.37	2.54	46
8	0.90	2.46	63
9	0.99	2.17	54
10	1.04	2.44	57
11	1.01	2.38	58
12	1.17	2.37	51
13	1.04	2.56	59
14	1.06	2.56	59
15	1.24	2.44	49
16	1.32	2.42	45
17	1.08	2.51	57
18	0.93	2.35	61
19	1.20	2.60	54
20	1.33	2.43	45
21	1.18	2.31	49
22	1.23	2.33	47

Soil texture

Soil texture is an important factor affecting the growth of truffle ascomata (Gezer et al., 2014). It is well documented in the literature that *T. aestivum* occurs on a great variety of soil texture (García-Montero et al., 2012; Ponce et al., 2014; Jaillard et al., 2014; Hilszczańska et al., 2019; Hakkou et al., 2023). According to Robin et al. (2016) fruiting bodies of black truffles occur in soils with textures that vary considerably – from silty clayey to loamy sandy. The results obtained for soil texture in our study are shown on Fig. 1.

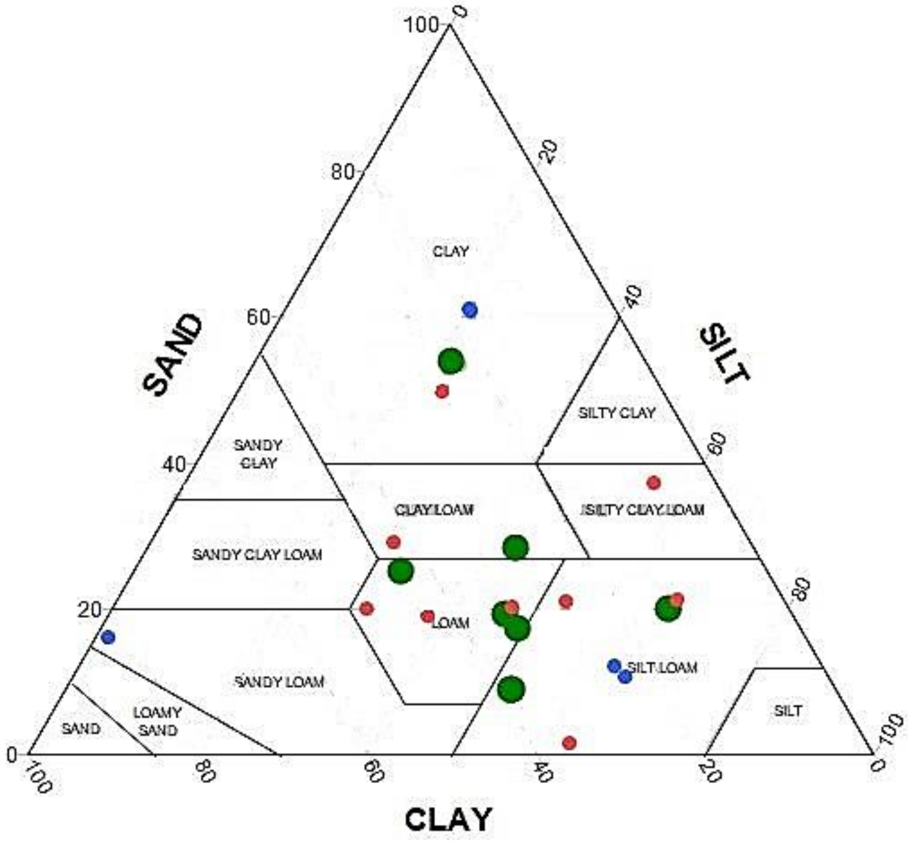

Fig. 1. Soil texture of the soils in the sample plots.
*Note: In green colour – – soil texture of the soils with highest amounts of truffle fruiting bodies, in blue – with moderate amounts and in red - with low amounts.

Fig. 1 show the soil texture of the soils from which the highest amounts of summer truffle fruiting bodies were found and collected (> 5 kg per a sample plot). Although loam texture is considered to offer the most favorable conditions for growth, the most common soil texture in the areas with the highest amounts of summer truffle fruiting bodies was silty clay loam. In contrast to the study of Abourouh (2020), which indicated that the sand fraction predominated (80-90%), in our study we found out that the dominant fraction in the most productive truffle soils was silt, followed by clay. In general, the soils in which the largest amounts of fruiting bodies were found, are characterized by a moderately fine texture.

Blue colored circles show the soil texture of soils from which moderate amounts (2.5÷5 kg) of fruiting bodies were collected. These soils are characterized by heterogeneous soil texture, varying from sandy loam, silt loam to clay. Soil texture of soils with low amounts (< 2.5 kg) of ascomata are presented on Fig. 1 in red. These soils also vary significantly in their soil texture and there is no clear trend in the distribution of individual fractions.

Soil reaction

Soil reaction is one of the most important soil characteristics (Thomas, 2012; Nedelin et al., 2022), which determines the direction and rate of biochemical processes in the soil and the availability of nutrients for the ectomycorrhizal fungi such as *T. aestivum*. Some authors claim that ideal pH value for the fruiting bodies of Burgundy truffles is 7.5 (Thomas, 2012), while others argue that pH is generally weakly alkaline to neutral, or even mildly acidic (6.0 –7.6) (Hilszczańska et al., 2019). According to Bragato et al. (2022), soil pH may vary from slightly acid to slightly alkaline, and that variation may significantly affect the habitat of ectomycorrhizal fungi. Our research also confirmed that pH varies between slightly acidic to slightly alkaline (Table 3). We established that the average pH value between the most productive plots was 6.9.

Soil organic matter

The obtained results indicate that the soil characteristics influencing *T. aestivum* production in Western Bulgaria are very specific and depend on soil unit. One of the main factors, which is linked to the high truffle productivity, is the accumulation of soil organic carbon (SOC) in the soil surface layer. Levels of SOC in high productivity areas varied in wide range from low (18.45 g/kg^{-1}) to high (58.32 g/kg^{-1}) (Table 3).

Table 3. Chemical characteristics of the studied soils.

Sample plot number	pH (H_2O)	SOC	TNC	C/N	P_2O_5	K_2O	Exch. Ca	Exch. Mg	Exch. K	Exch. Na	CEC	BS
		g/kg^{-1}			mg/100 g		cmol(+)·kg^{-1}					%
1	6.3	51.00	4.292	12	6.36	55.1	19.05	26.13	1.17	0.04	26.13	99
2	7.3	16.13	1.859	9	23.86	40.1	27.41	31.33	0.72	0.15	31.33	100
3	6.6	58.32	6.742	9	2.12	41.3	40.71	44.27	0.59	0.03	44.27	100
4	6.8	25.19	2.578	10	5.23	39.8	14.68	17.90	0.90	0.01	17.90	100
5	7.2	35.19	3.710	9	25.00	67.3	35.83	38.93	1.43	0.03	38.93	100
6	7.4	41.06	4.040	10	1.52	33.7	38.86	41.88	0.65	0.02	41.88	100
7	6.5	18.00	1.918	9	1.36	58.0	16.86	22.29	1.61	0.02	22.29	95
8	6.2	40.05	4.267	9	2.58	33.3	37.06	40.39	0.65	0.06	40.39	97
9	7.2	66.94	7.572	9	5.68	81.7	39.35	47.46	2.66	0.02	47.46	100
10	5.8	20.44	1.549	13	1.67	15.4	7.09	13.86	0.41	0.02	13.86	84
11	7.1	42.25	5.366	8	23.44	76.3	39.60	44.21	2.50	0.02	44.21	100
12	7.6	48.73	4.740	10	3.90	19.6	39.85	47.41	0.55	0.02	47.41	100
13	7.3	18.45	1.802	10	1.80	14.5	19.95	21.67	0.43	0.02	21.67	100
14	7.4	28.06	3.295	9	1.95	25.3	29.51	36.52	0.73	0.02	36.52	100
15	7.3	34.97	3.304	11	21.10	56.4	32.28	36.03	1.58	0.02	36.03	100
16	7.1	29.55	3.237	9	4.06	35.3	26.87	31.91	0.83	0.09	31.91	100
17	7	18.69	2.009	9	1.40	25.6	15.08	17.99	0.63	0.06	17.99	100
18	7.4	43.15	3.542	12	14.84	36.4	29.76	33.63	0.90	0.25	33.63	100
19	7.3	24.84	3.111	8	5.00	43.4	23.44	25.97	1.06	0.01	25.97	100
20	6.2	30.00	2.910	10	5.63	31.3	23.63	27.96	0.74	0.04	27.96	97
21	6.3	21.12	2.388	9	2.66	22.6	24.14	29.01	0.43	0.03	29.01	97
22	6.8	29.55	2.760	11	3.67	20.3	23.76	28.51	0.43	0.34	28.51	99

Note: * SOC – Soil Organic Carbon, TNC – Total Nitrogen Content, CEC – Cation Exchange Capacity and BS – Base Saturation.

The average value of SOC in these soils was 37.57 g/kg^{-1}, which is assessed as moderate (Vanmechelen et al. 1997). The same applies to soils with moderate and low productivity. Several studies (Hilszczańska et al., 2019; Bragato et al., 2022) reported similar results for the SOC in soils of the summer truffle areas.

The obtained data for C/N ratio in the studied soils was low (around 10) and corresponds to other studies on this topic (García-Montero et al., 2008; Hilszczanska & Sierota, 2010). In high productivity areas, soil had an average C/N ratio of 10, which is assessed as very low (Vanmechelen et al., 1997) and indicates a very fast decomposition of soil organic matter and release of nitrogen in the soil. It should be noted that the total nitrogen content (TNC) was assessed as high (3.11 g/kg^{-1}) to very high (6.74 g/kg^{-1}). An exception was the soil in sample plot 13, where TNC was assessed as moderate (Vanmechelen et al., 1997).

In the study of Hilszczanska & Sierota (2010) it was found that low phosphorus concentration and a high Ca/Mg ratio were favourable for the development of *T. aestivum* mycorrhizae. We have obtained similar results. In the most productive truffle sample plots, the average values of Ca/Mg ratio were 14. With the decrease of the amount of *Tuber aestivum* fruiting bodies, Ca/Mg ratio also decreases. It reaches average values of 10 in soils with average truffle productivity and in soils with low productivity it was 8. These results indicate that summer truffle prefers soils, in which exchangeable calcium predominates over exchangeable magnesium in the soil.

In all sample plots the K/Mg ratio in the soils was below one, which indicates that the uptake of magnesium was not negatively affected. No relationship was found between momentary fruiting body productivity and the amount of available phosphorus and potassium in the soil.

The obtained results in the most productive truffle areas suggested that black truffles preferred soils with average values of CEC above 30 cmol (+).kg^{-1}. All the soil samples were saturated with bases. Base saturation of the soil in most productive areas varied in narrow range between 97% and 100%, which indicates that summer truffle fruiting bodies highly depend on saturation of soil.

Soil moisture

Soil moisture is one of the most important factors for developing ascomata of summer truffles and it has had a significant impact on their productivity. It is well known fact that soil moisture is mostly influenced by season, soil organic matter and soil texture. We have obtained data for the main hydrological properties of the soil in the studied truffle areas, which are presented in Table 4.

In the most productive truffle areas it was established that the average value of total water capacity of the soils is very high – 79% and the field capacity is 46%. The results obtained correspond to the research of Ponce et al. (2014), which pointed out that fructification of black truffles highly depend on soil water content. It should be noted that the average value of water holding capacity of the soil in most productive truffle areas was 31%, and in low productive areas it was 23%.

Table 4. Soil hydrological properties.

Sample plot number	Total water capacity	Field capacity	Soil moisture	Wilting point	Water holding capacity
	%				
1	69	39	10	13	26
2	53	33	10	14	20
3	92	59	15	21	38
4	61	34	6	8	26
5	74	46	13	17	29
6	83	44	14	19	26
7	57	29	11	15	14
8	91	56	14	19	37
9	98	54	17	22	32
10	80	40	4	5	35
11	82	44	16	22	22
12	76	36	10	14	22
13	92	50	7	9	41
14	73	37	11	15	22
15	62	34	12	16	18
16	60	35	11	14	20
17	81	49	6	9	40
18	89	45	11	14	30
19	68	36	9	12	24
20	69	35	12	15	19
21	83	40	12	17	23
22	69	30	11	15	14

Conclusions

In conclusion the most productive summer truffle areas have several soil characteristics in common – they are with good to excellent structure, soil reaction is slightly acidic to slightly alkaline, with moderately fine texture, rich in SOC and well stocked with total nitrogen. In our study, we established that summer truffles prefer soils with very low C/N ratio, which are highly saturated with bases. The most productive truffle areas were characterized by the very high values of CEC and an average value of water holding capacity of 31%.

References

Abourouh M., (2020). *Terfess et truffes de la Maâmora: importances biologique, écologiques et socioéconomique*. Consultation nationale "Restauration de la forêt de chêne-liège pour le développement et la valorisation des truffes dans la forêt de la Maâmora". FAO, 78 p. [in French]

Benucci, G.M.N., Bonito, G., Falini, L.B., & Bencivenga, M. (2012). Mycorrhization of Pecan trees (*Carya illinoinensis*) with commercial truffle species: *Tuber aestivum* Vittad. and *Tuber borchii* Vittad. *Mycorrhiza,* 22, 383–392. doi: 10.1007/s00572-011-0413-z

Berch, S.M. (2013). Truffle cultivation and comercially harvested native truffles. In: Proceeding "*International symposium on Forest Mushroom*". Hosted by Korea Forest Research Institute and Korean Forest Mushroom Society. August 6, 2013, Seoul, Republic of Korea, 85-97.

Berch, S.M., & Bonito, G. (2014). Cultivation of Mediterranean species of *Tuber* (Tuberaceae) in British Columbia, Canada. *Mycorrhiza,* 24, 473–479. doi: 10.1007/s00572-014-0562-y

Bonito, G.M., Gryganskyi, A.P., Trappe, J.M., & Vilgalys, R. (2010). A global meta-analysis of *Tuber* ITS rDNA sequences: species diversity, host associations and long-distance dispersal. *Molecular Ecology,* 19, 4994–5008. doi: 10.1111/j.1365-294x.2010.04855.x

Bonito, G., Smith, M.E., Nowak, M., Healy, R.A., Guevara, G., Cázares, E., Kinoshita, A., Nouhra, E.R., Domínguez, L.S., Tedersoo, L., Murat , C., Wang, Y., Arroyo Moreno, M., Pfister, D.H., Nara, K., Zambonelli, A., Trappe, J.M., & Vilgalys, R. (2013). Historical biogeography and diversification of truffles in the tuberaceae and their newly identified southern hemisphere sister lineage. *PLoS ONE*, 8, e52765. doi: 10.1371/journal.pone.0052765

Bragato, G., Mosetti, D., Turpaud, P., Benucci, G.M.N., & Donnini, D. (2022). Presence and production of *tuber aestivum* in relation with the soil environment of a semi-natural experimental truffle orchard in Spoleto, Central Italy. *Applied Soil Ecology,* 178, 104565. doi: 10.1016/j.apsoil.2022.104565.

Chevalier, G. (2009). The truffle of Europe (*Tuber aestivum* Vittad.): ecology and possibility of cultivation. In: Abstracts: first conference on the "European" Truffle *Tuber aestivum/uncinatum*, 6–8.11.2009, Faculty Centre of Biodiversity, University of Vienna, Vienna, 1–2.

Chevalier, G. (2010). The truffle of Europe (*Tuber aestivum*): geographic limits, ecology and possibility of cultivation. In: Proceedings of the First Conference on the "European" Truffle, Greilhuber A., Hausknecht I., Urban A., Eds., Wien, Austria: Österreichische Mykologische Gesellschaft, 249 p.

Chevalier, G. (2012). Europe, a continent with high potential for the cultivation of the Burgundy truffle (*Tuber aestivum/uncinatum*). *Acta Mycologica,* 47 (2), 127–132.

Chevalier, G., & Sourzat, P. (2012). Soils and Techniques for Cultivating *Tuber melanosporum* and *Tuber aestivum* in Europe. In: Zambonelli, A., & Bonito, G.M. (Eds.), *Edible Ectomycorrhizal Mushrooms*. Springer-Verlag Berlin Heidelberg, 163–190. ISBN 978-3-642-33822-9.

Dilkova, R. (2014). *Structure, physical properties and aeration of soils in Bulgaria*. PSSE, Sofia, 300 p.

FAO. (2006). *Guidelines for Soil Description. Fourth Edition*. Fao, Rome, 97 p.

Filcheva, E., & Tsadilas, C. (2002). Influence of clinoptilolite and compost on soil properties. *Communications in Soil Science and Plant Analysis*, 33(3–4), 595–607. doi: 10.1081/CSS-120002766

Fischer, C.R., Oliach, D., Bonet, J.A., & Colinas, C. (2017). *Best Practices for Cultivation of Truffles.* Forest Sciences Centre of Catalonia, Solsona, Spain; Yaşama Dair Vakıf, Antalaya, Turkey, 68 p. ISBN 978-84-697-8163-0.

García-Montero, L.G., Díaz, P., Martín-Fernández, S., & Casermeiro, M.A. (2008). Soil factors that favour the production of *Tuber melanosporum* carpophores over other truffle species: a multivariate statistical approach. *Acta Agriculturae Scandinavica, Section B – Soil and Plant Science*, 58(4), 322-329. doi: 10.1080/09064710701743286

García-Montero, L.G., 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, G.M. (Eds.), *Edible Ectomycorrhizal Mushrooms: Current Knowledge and Future Prospects.* Springer Berlin Heidelberg, Germany, 83 p.

García-Montero, L.G., Moreno, D., Monleon, V.J., & Arredondo-Ruiz, F. (2014). Natural production of *Tuber aestivum* in central Spain: *Pinus* spp. versus *Quercus* spp brûlés. *Forest Systems*, 23, 394-399. doi: 10.5424/fs/2014232-05112

Gezer, K., Kaygusuz, O., Çelik, A., & Işiloğlu, M. (2014). Ecological characteristics of truffles growing in Denizli Province, Turkey. *Journal of Food, Agriculture & Environment*, 12(2), 1105-1109.

Gógán, A.C., Nagy, Z., Dégi, Z., Bagi, I., & Dimény, J. (2012). Ecological characteristics of a Hungarian summer truffle (*Tuber aestivum* Vittad.) producing area. *Acta Mycologica*, 47(2), 133–138.

Guimarães, R.M.L., Ball, B.C., & Tormena, C.A. (2011). Improvements in the visual evaluation of soil structure. *Soil Use and Management*, 27(3), 395–403. doi: 10.1111/j.1475-2743.2011.00354.x

Hakkou, S., Sabir, M., & Machouri, N. (2023). Contribution to the study of the production factors of Moroccan truffles (Soil, Climate, Vegetation). *Journal of Materials and Environmental Science*, 14(1), 13-30.

Hall, I.R., Brown, G.T., & Zambonelli, A. (2007). *Taming the truffle: the history, lore and science of the ultimate mushroom*. Timber Press, Portland, Australia, 304 p.

Hilszczańska, D., & Sierota, Z. (2010). First attempt towards cultivation of *Tuber aestivum* in Poland. *Österreichische Zeitschrift für Pilzkunde*, 19, 209 - 212.

Hilszczańska, D., Szmidla, H., Sikora, K., & Rosa-Gruszecka, A. (2019). Soil Properties Conducive to the Formation of *Tuber aestivum* Vitt. Fruiting Bodies. *Polish Journal of Environmental Studies*, 28, 1713–1718. doi: 10.15244/pjoes/89588

Innangi, M, Fioretto, A., Fondón, C. L., García-Montero, L.G., Marzaiolia, R., Pintoc, S., Rutiglianoa, F. A., & Menta, C. (2020). *Tuber aestivum* is associated with changes in soil chemistry and reduced biological quality in a *Quercus pubescens* stand in Northern Italy. *Pedobiologia (Jena)*, 80, 150648. doi: 10.1016/j.pedobi.2020.150648

ISO 10390:(2005). Soil quality. Determination of pH: 1–7.

ISO 11260:(2018). Soil quality. Determination of effective cation exchange capacity and base saturation level using barium chloride solution: 1–12.

ISO 11272:(1998). Soil quality. Determination of dry bulk density: 1–19.

ISO 11277:(2009). Soil quality. Determination of particle size distribution in mineral soil material – Method by sieving and sedimentation: 1–34.

ISO 787-10:(1993). General methods of tests for pigments and extenders – Part 10: Determination of density – Pycnometer method: 1–5.

Ivanov, P. (1984). A new acetate lactate method for determining the availability of phosphorus and potassium plants in the soil. *Soil Science and Agrochemistry*, 19(4), 88–98. [in Bulgarian]

Jaillard, B., Barry-Etienne, D., Colinas, C., De Miguel, A.M., Genola, L., Libre, A., Nevue, P., Oliach, D., Saenz, W., Sáez, M., Salducci, X., Souche, G., Sourzate, P., & Villeneuve, M. (2014). Alkalinity and structure of soils determine the truffle production in the Pyrenean Regions. *Forest Systems*, 23(2), 364-377. doi: 10.5424/fs/2014232-04933

Kachinskii, A. (1963). *Soil physics. Part 2,* Moscow High School Publishers, 7–11. [in Russian]

Koleva, E., & Peneva, R. (1990). *Climate handbook of Bulgaria. Vol. 5. The precipitation in Bulgaria.* Publishing House of the Bulgarian Academy of Sciences, 196 p.

Kononova, M. (1963). *Soil Organic Matter. Its Nature and Properties. Second edition.* Pergamon press, 544 p. [in Russian]

Kyutchukova, M. (1979). *Climate handbook of People's Republic of Bulgaria. Vol. 3. Air temperature, soil temperature, frost*. Science and Art Publishing House, 811 p. [in Bulgarian]

Lancellotti, E., Iotti, M., Zambonelli, A., & Franceschini, A. (2014). Characterization of *Tuber borchii* and *Arbutus unedo* mycorrhizas. *Mycorrhiza*, 24, 481-486. doi: 10.1007/s00572-014-0564-9

Læssøe, T., & Hansen, K. (2007). Truffle trouble: what happened to the Tuberales? *Mycological Research,* 111(9), 1075–1099. doi: 10.1016/j.mycres.2007.08.004

Molinier, V., Bouffaud, M.L, Castel, T., Mounier, A., Colombet, A., Recorbet, G., Frochot, H., & Wipf, D. (2013). Monitoring the fate of a 30-year-old truffle orchard in Burgundy: from *Tuber melanosporum* to *Tuber aestivum*. *Agroforest Systems*, 87, 1439-1449. doi: 10.1007/s10457-013-9649-2

Mukhopadhyay, S., Masto, R.E., Tripathi, R.C., & Srivastava, N.K. (2019). Chapter 14 - Application of soil quality indicators for the phytorestoration of mine spoil dumps. In: Pandey, V.C., & Bauddh, K. (Eds.), *Phytomanagement of Polluted Sites: Market Opportunities In Sustainable Phytoremediation.* Elsevier, 361-388. doi: 10.1016/B978-0-12-813912-7.00014-4

Nedelin, T., Gyosheva, M., & Lacheva M. (2017). Hypogeus macrofungi on the territory of the Sofia and Plovdiv city parks, Bulgaria. *Annual of Sofia University"St. Kliment Ohridski", Book 2-Botany,* 101, 32-38.

Nedelin, T., Petrova, K., & Savev, S. (2022). Soil properties of the most productive Tuber aestivum habitats from midpart of Western Bulgaria. *Forestry Ideas*, 28(2), 393-404.

Păcurar, H., Dîrja, M., But, M., Păcurar, I., Roșca, S., & Bilașco, Ș. (2018). Identification of Soils Factors Influence in the Distributions of *Tuber aestivum* in Transylvanian Subcarpathian Hills, Romania. *Notulae Botanicae Horti Agrobotanici,* 47, 478–486. doi: 10.15835/nbha47111378.

Paolocci, F., Rubini, A., Riccioni, C., Topini, F., & Arcioni, S. (2004). *Tuber aestivum* and *Tuber uncinatum*: two morphotypes or two species? *FEMS Microbiology Letters*, 235(1), 109–115. doi: 10.1016/j. femsle.2004.04.029

Polemis, E., Konstantinidis, G., Fryssouli, V., Slavova, M., Tsampazis, T., Nakkas, V., Assyov, B., Kaounas, V., & Zervakis, G.I. (2019). *Tuber pulchrosporum* sp. nov., a black truffle of the *Aestivum* clade (Tuberaceae, Pezizales) from the Balkan peninsula. *MycoKeys*, 47, 35–51. doi: 10.3897/mycokeys.47.32085

Ponce, A.R., Ágreda, T., Águeda, B., Aldea, J., Martínez-Peña, F., & Modrego, M.P. (2014). Soil physical properties influence "black truffle" fructification in plantations. *Mycorrhiza,* 24 (S1), 55.

Selosse, M.A., Faccio, A., Scappaticci, G., & Bonfante, P. (2004). Chlorophyllous and achlorophyllous specimens of *Epipactis microphylla* (Neottieae, Orchidaceae) are associated with ectomycorrhizal septomycetes, including truffles. *Microbial Ecology,* 47, 416–426. doi: 10.1007/s00248-003-2034-3

Robin, C., Goutal-Pousse, N., & Le Tacon, F. (2016). Soil Characteristics for *Tuber aestivum* (Syn. *T. uncinatum*). In: Zambonelli, A., Iotti, M., & Murat, C. (Eds.), *True Truffle (Tuber spp.) in the World: Soil Ecology, Systematics and Biochemistry.* Springer International Publishing, 211-231. doi: 10.1007/978-3-319-31436-5_13)

Thomas, P.W. (2012). The role of pH in *Tuber aestivum* syn. *uncinatum* mycorrhiza development within commercial orchards. *Acta Mycologica,* 47, 161−167. doi: 10.5586/am.2012.019

Vanmechelen, L., Groenemans, R., & van Ranst, E. (1997). *Forest Soil Condition in Europe. Results of Large-Scale Soil Survey*. Technical Report. EC, UN/ECE, Ministry of Flemish Community; Brussels, Geneva. Forest Soil Co-ordinating Centre. International Cooperative Programme on Assessment and Monitoring of Air Pollution on Forest, UNECE, 261 p.

Wang, G., Li, Y., Li, D., & Tang, Y. (2008). Determination of 5 alpha-androst-16-en-3alpha-ol in truffle fermentation broth by solid-phase extraction coupled with gas chromatography flame ionization detector/electron impact mass spectrometry. *Journal of Chromatography B-Analytical Technologies in the Biomedical and Life Sciences,* 870(2), 209-215. doi: 10.1016/j.jchromb.2008.06.014

Wedén, C., Danell, E., Camacho, F.J., & Backlund, A. (2004). The population of the hypogeous fungus *Tuber aestivum* syn. *T. uncinatum* on the island of Gotland. *Mycorrhiza*, 14, 19-23.

World Reference Base for Soil Resources. (2006, first update 2007). World Soil Resources Report № 103. FAO, Rome, 110 p.

Zambonelli, A., Iotti, M., & Hall, I. (2015). Current status of truffle cultivation: recent results and future perspectives. *Italian Journal of Mycology,* 44(1), 31-40. doi: 10.6092/issn.2465-311X/5593

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