FORESTRY IDEAS, 2022, vol. 28, No 2 (64): 393–404

Soil properties of the most productive *Tuber aestivum* habitats from midpart of Western Bulgaria

Teodor Nedelin*, Kameliya Petrova, and Slavcho Savev

Forestry University, Sofia, Bulgaria. E-mail*: t.nedelin@ltu.bg, teodor_nedelin@hotmail.com

Received: 14 July 2022 / Accepted: 15 November 2022 / Available online: 22 November 2022

Abstract

Only recently, truffle hunting in Bulgaria has established itself as an important source of income and due to the specific socio-economic aspects, there is a tendency for continuous increase of the truffle hunters' number. *Tuber aestivum* is the most important commercial truffle in Bulgaria. Because truffles are ectomycorrhizal fungi, at least part of their life cycle is related to plant partner, which in most cases is a tree species. Among those factors that affect growth and distribution of vegetation and of *Tuber* host plants, are pedoclimatic conditions. They are determined from a large extent on geographical and orographic characteristics, creating specific microclimatic conditions suitable for *Tuber* fruitbodies development. In this study we focus on various soil properties of the most productive *T. aestivum* habitats from midpart of Western Bulgaria. We have used Principal component and classification analysis (PCA) to determine the most important soil factors for fruitbody formation. The common feature of the most productive *T. aestivum* habitats from midpart of Western Bulgaria are high cation exchange capacity values (CEC) and low $CaCO_3$ content. Moreover, our research confirms that Ca^{2+}, total organic carbon and total nitrogen are among the most important factors for *T. aestivum* production. The results extend our knowledge on *T. aestivum* ecology and can be used to select the best areas for establishing truffle plantations in Bulgaria.

Key words: PCA, soil environment, summer truffle.

Introduction

Although it was found back in 1965 (Hinkova 1965, Dimitrova and Gyosheva 2008), it became clear that Burgundy truffle – *Tuber aestivum* Vittad. (syn. *Tuber uncinatum* Chatin) occurs frequently in a variety of habitats in Bulgaria. The annual truffle production for all commercial true truffle species (*Tuber* sp.) from natural sites during the last few years in Bulgaria according to unofficial and official sources exceed 500 t in average year but in good one can reach over double. For instance, according to the National Statistical Institute, the 2020-year export is 299 294 kg total of all *Tuber* sp., 210 720 kg of them are exported to Italy. More than two thirds of these truffles are represented by *T. aestivum* and this makes it the most economically important in Bulgaria. Unlike other commercial truffle species like *T. magna-*

tum Picco, which occurrence is restricted to small areas characterised by particularly suitable environmental parameters (Mello et al. 2004), *T. aestivum* occupies a wider ecological niche.

There is sufficient evidence, that most of the truffles have very wide range of plant species as ectomycorrhizal partners and in the past, this was subject to various studies (Bonito et al., 2010; Stobbe et al. 2013b, García-Montero et al. 2014).

Because truffles grow underground or are partially buried, the soil is their environment. For this reason, its physical, granulometric, hydrological and chemical properties separately and as a combination as well and the soil biota, i.e., soil complex environment are of utmost importance (Gryndler et al. 2017).

The moisture content in soils and particularly hydrological regime affects to the physiological activity of vegetation, which in turn affects the truffle harvest. Increase and inhibition of moisture occurs in plane relief and along rivers due to the rising groundwater levels. In thermophilic vegetation zone of Western Bulgaria, under the influence of continental climate with warm summers and rather cold winters, the soil moisture is mostly result of precipitation and therefore these soils are usually very dry in the second half of the vegetation period.

During the last two decades numerous studies of pedological conditions were done for *T. aestivum* productive sites in Europe, which spread from Spain and Sweden to Transylvanian Subcarpathian Hill in Romania (Wedén et al. 2004, García-Montero et al. 2009, Păcurar et al. 2019). However, there is no available information for Bulgaria until now.

The aim of this study is to fill this gap – to describe and analyse the soil properties of the most productive *T. aestivum* habitats from midpart of Western Bulgaria. Present data can be used to select the best areas for establishing truffle plantations in Bulgaria.

Material and Methods

Study area

Ten representative productive *T. aestivum* habitats were chosen from more than a hundred known to the authors in a maximum distance of 120 km from Sofia as a mean point of Central part of Western Bulgaria. The criteria were the higher ratio – productivity/area (kg·m^{-2}) of truffle habitats, the duration of fruitbody formation and the heterogeneity in soil type and bedrock/vegetation/climate patterns (altitude) compared to the other appropriative sites within the region. Most of the habitats are visited on a weekly or biweekly bases from the end of May till the end of November or till the end of fruitbodies formation. The main topographical characteristics of studied truffle area and productivity are listed in the Table 1, but exact geographical coordinates are not provided due to the lack of legal framework regarding the collection of truffles.

The climate is moderate continental with average annual precipitation for the study region from 550 to 860 mm, with a maximum from May to June. The average annual temperature is from 11.2 ºC (S5 at 290 m a.s.l.) to 7.8 ºC (S4 at 1020 m a.s.l.), average maximum – from 28.4 ºC to 22.6 ºC (August and July) and average minimum from –5.3 ºC to -6.5 ºC (January) (Kyutchukova 1979, Koleva and Peneva 1990).

Table 1. Topographic, vegetation and productivity characteristics of studied area.

Sample	Elevation, m a.s.l.	Exposition	Slope, %	Relief	Microrelief	Host trees	Truffle productivity habitats area*	Annual productivity**
S1	550	NW	2	plane	plane	Qc	M	2
S2	580	E	4	plane	mound	Qc	S	3
S3	570	SW	2	plane	plane	Qc, Tt	M	2
S4	1020	NE	17	slope lower part	plane	Tt, Cs	M	1
S5	290	SW	2	plane	depressed	Cb, Qc, Qd	S	2
S6	720	NE	2	plane	depressed	Tt	M	1
S7	530	S	3	plane	plane	Tt	L	1
S8	890	NE	24	slope lower part	excavation	Cb, Fs	M	2
S9	640	W	3	plane	plane	Tt	S	1
S10	810	SW	14	slope upper part	plane	Ps, Pn	L	1

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; Qc – *Quercus cerris* L., Qd – *Quercus dalechampii* Ten., Tt – *Tilia tomentosa* Moench., Cs – *Castanea sativa* Mill., Cb – *Carpinus betulus* L., Fs – *Fagus sylvatica* L., Ps – *Pinus sylvestris* L., Pn – *Pinus nigra* Arn.

Soil sampling

A total 40 soil samples (30 plus 10) are collected at approximately 7 cm below the surface after removing litter and accompanying vegetation. Three soil cylinder cores are taken up to 10 cm from three *T. aestivum* fruitbodies found at each truffle habitat. Additionally, approximately 1 kg of soil is sampled for each place from not more than 15 cm distance of *T. aestivum* fruitbody. All soils analyses were performed in specialised laboratory at Forestry University, Sofia.

Soil analyses

To determine soil pH H_2O ISO 10390:2005 was used. Bulk density was measured according to ISO 11272:1998 and particle density by pycnometer method (ISO 787-10:1993). Soil porosity was determined by formula (1).

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

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

Soil organic matter was determined by modified Turin's method – 120 ºC, 45 min and $CuSO_4$ as a catalyst (Kononova 1963, Filcheva and Tsadilas 2002). Total nitrogen – Kjeldal's 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). For exchangeable cations (Ca^{2+}, Mg^{2+}, K^+, Na^+) determination was used ISO 11260:2018. Content of calcium carbonates (percent) – Scheibler's method – ISO 10693:1995. Saturated water content, field capacity, wilting point and hygroscopic moisture content – Kachinskii method (Kachinskii 1963).

Statistical analysis

Collected data of all productive truffle habitats were analysed using R 4.1.2 (R Core Team, 2022) and main descriptive statistics of soil parameters were presented. Soil texture triangle diagram was built using R package soil texture 1.1.5 (Moeys 2018). For C:N ratio, ANOVA was performed, followed of a Tukey's HSD post hoc test, preceded by Shapiro-Wilk normality test and Levene's test of homogeneity of variance. Principal component and classification analysis (PCA) was done with the aid of package FactoMineR 2.4 (Husson et al. 2017) for 18 selected soil parameters. For graphic visualisation package ggplot2 3.3.5 (Wickham 2016) was used.

Results and Discussion

Geographical characteristics

Studied soils of most productive *T. aestivum* habitats from midpart of Western Bulgaria are located from 300 to 1000 m a.s.l. with all expositions included (Table 1). According to Stobbe et al. (2013a) southern habitats with a warm climate reach higher altitudes (Morocco, ~1,600 m a.s.l.), whereas northern habitats are often located near sea level (Sweden, <50 m a.s.l.), but it seems that even in Bulgaria, Burgundy truffle can reach very high altitudes close to the upper limit in southern parts of a country. The highest known elevation on which fruitbodies of *T. aestivum* are found in Bulgaria is ~1430 m a.s.l. and the lowest 15–30 m a.s.l. near Danube and the Black Sea (T. Nedelin unpublished data). The slopes are from gently to moderately steep. At higher altitudes the Burgundy truffle fruitbodies prefers growing from strongly sloping to steep terrains. One of the reasons might be the greater solar energy coming perpendicular to the surface when the crop began at the end of summer and/or the beginning of autumn at these places combined to the favorable soil moisture. The relief in most cases is plane or lower part of a slope, where the water drains away. The microrelief is plane to more or less depressed, but it can be also mound and is related to the particular climate conditions. Both last characteristics are very important in the respective season and host ability to form *T. aestivum* ECM. For instance, plane relief is preferred for artificial plantations in Hungary at low elevation (Gógán et al. 2012).

Host plants

Host plants of the studied areas are presented in Table 1. The most common (S1-S5, S8) are Fagaceae and with Pinaceae (S10), they play an important ecological role as ectomycorrhizal partners (Bonito and Smith 2016). Lindens are also one of the main Burgundy truffle hosts and it was found in the most productive sites (S3, S4, S6, S7, S9) An interesting observation is that *Corylus avellana*, although is widespread is not among the most productive *T. aestivum* host plants in the region. The possible reason is that it occurs mostly on acidic soils near Sofia.

Our results show that not all plant species have the same significance. They differ from the seasonal dynamics in the formation of fruitbodies and their quantity. For instance, in Bulgaria, *Pinus sylvestris* and *Pinus nigra*, are most productive from the mid-summer, while *Tilia* sp. from the end of the spring (data not shown). However, it is difficult to separate the exact influence of pedoclimatic conditions and those of a host species.

Physical and granulometric soil properties

The soil granulometric diagram (Fig. 1) reveals broad range in soil particle size distribution. The most appropriative soils of productive areas are clay, silty clay loam, clay loam, silt loam, loam, sandy clay loam and sandy loam.

Unlike Carpathian Basin, where more than two-thirds of Burgundy truffle habitats have heavy clay soils (Bratek et al. 2010) only few in our studied area are with higher content of clay, but not too heavy (for example S5). In such, truffles are usually developing on the surface or up to 5–7 cm depth and can be seen with naked eyes. The crop in these cases sometimes can be remarkable (more than 1 kg per once visitation at ≈10 m^2 or less area. The fruitbodies are often larger and from our observation a single truffle can reach 200 g and more but the fruiting period is relatively short and production is very dependable from climate conditions. Compared to the other studies (Wedén et al. 2004, Raglione and Owczarek 2005, Bruhn and Hall 2011, Gógán et al. 2012, Robin et al. 2013, Stobbe et al. 2013a, Jamali 2017, Hilszczańska et al. 2019), truffle representative productive habitats from midpart of Western Bulgaria resembles the granulometric structure of most *T. aestivum* productive soils from Poland, Sweden, Italy, France, Belgium, Romania, Hungary and Iran as well as those on which artificial plantations have been established in Canada and in Israel.

Studied soils are Vertisols, Cambisols, Luvisols, Fluvisols and Rendzinas. The last one is from the

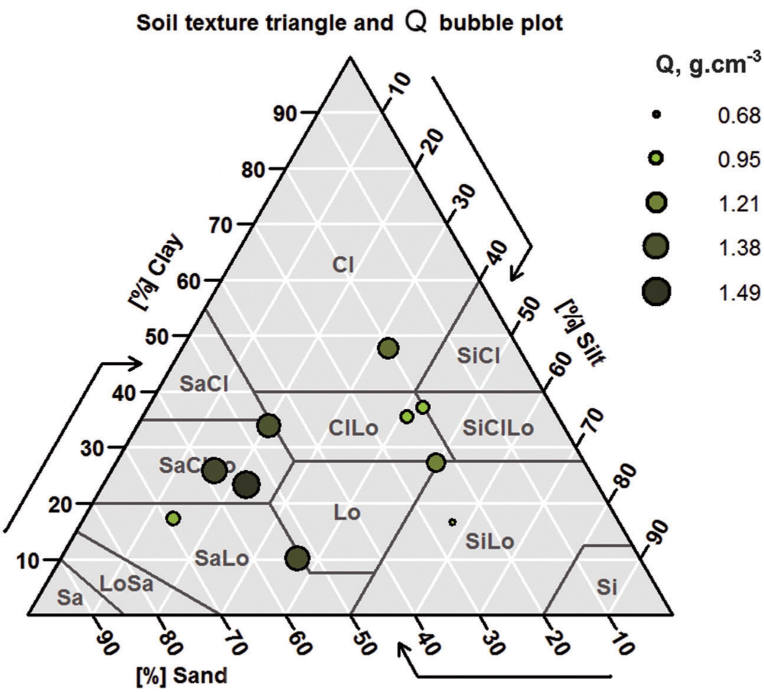

Fig. 1. Soil texture triangle and bulk density plot of studied truffle habitats.

most favourable type in Western and Central Europe (Chevalier and Sourzat 2012, Stobbe et al. 2013a).

The bedrock is represented by sedimentary rocks, andesite, limestone, marlstone and conglomerate (Nishava KiT 2019; Silva 2003 2019). To our knowledge, andesite is not preferred bedrock for Burgundy truffle habitats and is still not reported in the literature.

In recent studies, bulk density (*BD*) of *T. aestivum* productive soils is reported around 1 (Bragato et al. 2021a, b). Our results show that *BD* varies from 0.68 to 1.49. The lowest value is observed in *P. nigra* and *P. sylvestris* plantation developed on Rendzinas with limestone bedrock (S10).

In Table 2 are presented main descriptive statistics parameters of studied soils.

Hydrological and chemical soil properties

Soil parameters such as water availability could influence truffle plantation success (Todesco et al. 2019). Three (S7, S9 and S10) of most productive habitats have similar hydrological properties. In them, soils are well drained, with higher val-

Table 2. Descriptive statistics of some of the most important properties of soil of studied truffle habitats.

Parameters	Mean	St.Dev.	min	max
HMC, Hygroscopic moisture content, %	10.73	±3.51	5.35	17.29
SWC, Saturated water content, %	46.89	±17.45	30.48	85.70
FC, Field capacity, %	31.76	±14.31	20.14	64.47
PWP, Permanent wilting point, %	14.38	±4.70	7.16	23.17
PD, Particle density, $g \cdot cm^{-3}$	2.53	±0.06	2.46	2.67
P, Porosity, %	53.90	±10.92	40.00	73.00
Q, Bulk density, $g \cdot cm^{-3}$	1.16	±0.27	0.68	1.49
pH, H_2O	7.41	±0.56	6.80	8.10
Sand, %	39.28	±18.62	20.16	68.89
Silt, %	33.27	±14.87	13.87	57.50
Clay, %	27.45	±11.30	10.04	47.66
SOM, Soil organic matter, %	5.58	±2.17	1.28	8.18
TOC, Total organic carbon, %	3.24	±1.26	0.75	4.74
TN, Total nitrogen, %	0.30	±0.12	0.09	0.52
C:N	10.74	±1.58	8.29	13.06
P_2O_5, $mg \cdot 100\ g^{-1}$	12.20	±7.64	2.24	24.72
K_2O $mg \cdot 100\ g^{-1}$	45.84	±17.31	22.61	76.69
$CaCO_3$, %	2.32	±3.40	0.60	11.90
$Ca^{2+,}$ Exchangeable calcium, $cmol(+) \cdot kg^{-1}$	44.36	±28.55	17.14	92.29
K^+, Exchangeable potassium, $cmol(+) \cdot kg^{-1}$	0.91	±0.44	0.40	1.95
$Mg^{2+,}$ Exchangeable magnesium, $cmol(+) \cdot kg^{-1}$	3.83	±1.80	2.17	7.53
Na^+, Exchangeable sodium, $cmol(+) \cdot kg^{-1}$	0.10	±0.12	0.03	0.40
EA, Exchangeable acidity, $cmol(+) \cdot kg^{-1}$	0.02	±0.00	0.01	0.02
CEC, Cation exchange capacity, $cmol(+) \cdot kg^{-1}$	49.13	±29.26	20.33	100.00

ues of HMC and both SWC and FC. The higher values of FC suggest that even at higher summer temperatures and southern expositions these soils have enough moisture to ensure fruitbodies development.

The mean value (7.4) and the range of pH (H_2O) – 6.8–8.1 of studied soils (Table 2) corresponds to the value pointed out to Burgundy truffle in Europe (Stobbe et al. 2013a, Păcurar et al. 2019). It refers also to the most soils in Bulgaria, spreading up to 1000–1200 m a.s.l.

SOM, TOC and TN vary in a wide range. For example, S7 soil is very poorly stocked according to the parameters above, but is one of the most productive truffle sites near Sofia with a lot of large and long-distance spreading truffle patches. This can be explained due to the suitable plant host (*T. tomentosa*), its age and plantation structure, available herbaceous plants (*Geum urbanum* L., *Ranunculus ficaria* L. and *Anemone nemorosa* L.), some of them are also known to support ECM (Gryndler et al. 2014) and can stored a plenty of carbohydrates respectively. It should be noted that the area is surrounded from two sites by water.

In most studied soils, C:N ratio is around 10, which corresponds to a number of studies, not only for *T. aestivum* but for some other truffles with commerce values (Hilszczańska et al. 2008, Chevalier and Sourzat 2012). This ratio indicates a preference of *T. aestivum* for soils that are poor in the readily degradable nitrogen. Compared to some observations from Italy (Piacenza), Sweden and France (Weden et al. 2004, Innangi et al. 2020), studied soils near Sofia shows slightly lower values resembling those from the region of Parma with a mean value of 10.8 (Gregori 2010). Our results show also, that in soils with loose and optimal structure, according to their bulk density, C:N ratio is ~10, while in those with compact and very compact structure, C:N ratio is ~12 (p = 0.0393, Tukey HSD = -1.974).

Calcium (Ca^{2+}) is essential element for plant growth, but also numerous of studies point out that it is a key factor for *Tuber* sp. occurrence and fruitbody formation (García-Montero et al. 2009, Gryndler et al. 2017, Hilszczańska et al. 2019). It is provided mostly from soil solids. Through ion exchange, elements such as Ca^{2+} and K^+ are released and escape into the soil solution where they can be readily absorbed by ECM. Its concentration in soil has a seasonal fluctuation, especially in broadleaves forests, and after its leaves fall to the ground, they decompose rapidly and release large amounts of Ca^{2+} ions that become adsorbed as exchangeable Ca^{2+} on humus and clay. Compared to the other studies (Hilszczańska et al. 2019), exchangeable calcium in most productive truffle habitats in midpart of Western Bulgaria varies in broader range – from 17.14 to 92.29 cmol(+)·kg^{-1}.

According to the total $CaCO_3$ content, with the exception of S10, studied soils are poor or little stocked. The cation exchange capacity (CEC) is an important variable, dependent on the amount of humus in the soil, and the amount and type of clays (Weil and Brady 2017). Low CEC soils need quick but frequent watering, while high CEC soils need slow water applied less often. In most natural or artificial forest sites, where *T. aestivum* occurs, CEC ranges from 5 to 45 cmol(+)·kg^{-1} (García-Montero et al. 2008, Robin et al. 2016). However, our result shows a range from 20 to 100 cmol(+)·kg^{-1} and mean of our study productive truffle habitats is around 50 (Table 2).

PCA – BIPLOT (Fig. 2) are built to present soils variables interaction and

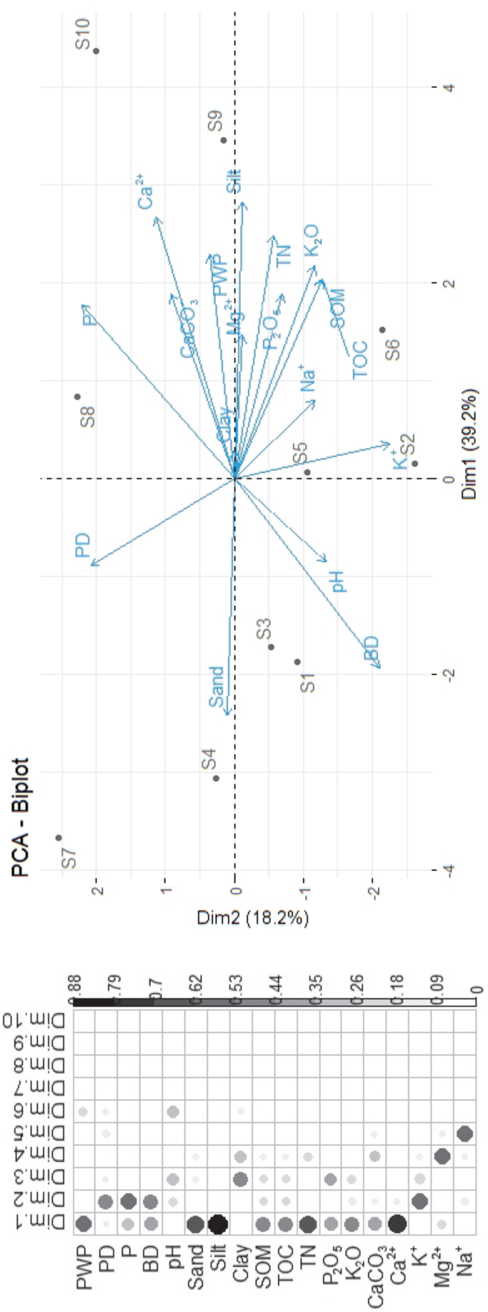

Fig. 2. PCA – BIPLOT and bubble plot (factor coordinates of dimensions), according to the most productive *T. aestivum* habitats in midpart of Western Bulgaria.

each soil sample impact (individual contribution) on the most productive *T. aestivum* habitats in midpart of Western Bulgaria.

Principal component analysis (PCA)

First two principal components (dimensions) express 57.4 % of the total inertia (Fig. 3), and first 5 of them fit more than 91 % of it.

The first principal component summarizes 39.2 % of the overall variance. The main structure elements of Dim 1 are presented from the variables (factor coordinates of PCA variables are presented in S-1): Silt (0.939), Ca^{2+} (0.887), TN (0.825), Sand (-0.807), i.e. its presence have negative effect on fruitbody formation of *T. aestivum* if we assume that first three components are favourable and PWP (0.759). Another possible hypothesis for the above, can be the fact that the Burgundy truffle ECM is of short distance exploration type (Zambonelli et. al. 1995) and in a higher sand content, the resistance in the soil is less, and would be conducive to the development of competitive long distances type ECM fungi.

The second principal component summarizes 18.2 % of the overall variance. The main structure elements of Dim 2 are constructed from the variables: K^+ (-0.751), P (0.735) and *BD* (-0.700). According to PCA – BIPLOT diagram, bulk soil density is negatively correlated with porosity and in other hand, K+ are negatively correlated to particle density. The third principal component focuses mainly on Clay and pH (15.1 %).

Individual contribution to PCA is presented in Figure 4 according to their bulk density. The variation of physical, granulometric, hydrological and chemical soil properties in optimal-lose soils from midpart of Western Bulgaria is much great-

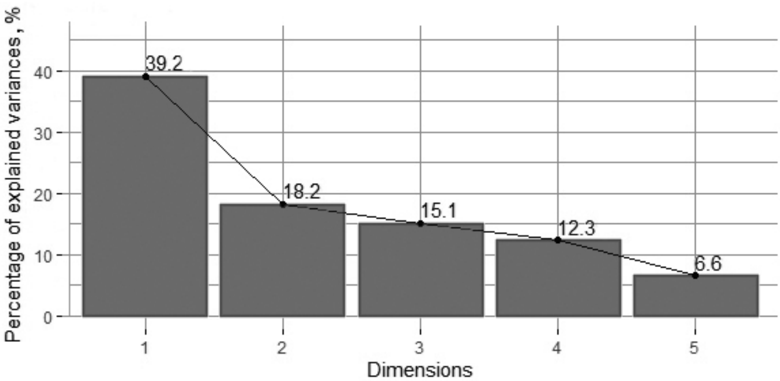

Fig. 3. Scree plot of % of total variance according to principal components (dimensions).

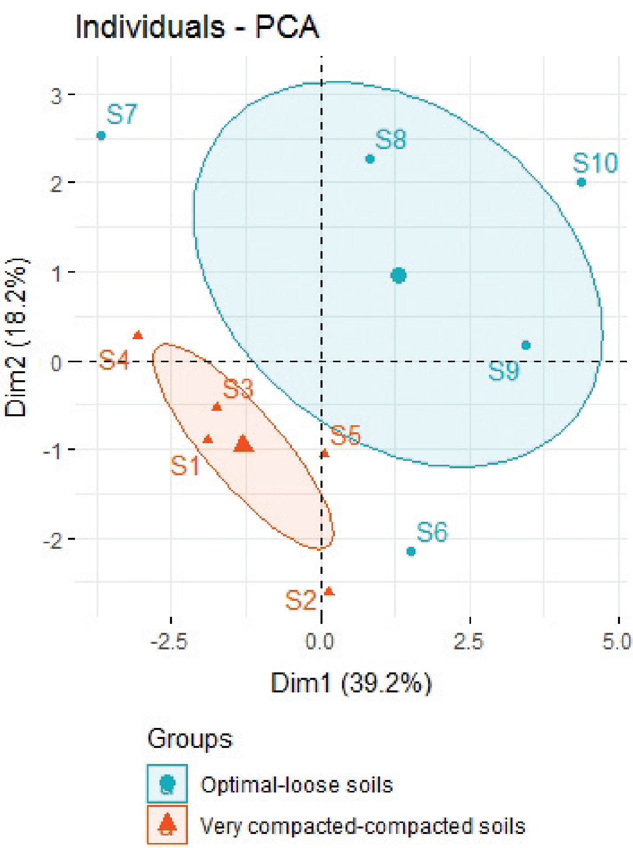

Fig. 4. Productive truffle habitats, according to their bulk density.

er than the very compacted-compacted soil group. According to our observations, in first, *T. aestivum* crop is more dependable on external (out of soil) parameters such as climate and especially precipitation. According to our observations, Rendzinas are among the most suitable soils and we suggest that *T. aestivum* ectomycorrhiza is not only element but also function of the forest ecosystems spreading in such soils. This is because most plants do not have simple, plant-only roots – they have mycorrhizae (Begon and Townsend 2021).

Conclusions

It has been found that productivity of *T. aestivum* habitats from midpart of Western Bulgaria varies between years in extremely wide borders and even all pedological conditions are perfect, there is no guaranty that the formation of fruitbodies will occur. The reason for this is complex, but the temperature and precipitation are of the most importance in combination with the soil mechanical and chemical properties as well as the hydrological regime in the soil, which determines the spatial distribution of the ECM roots. Most productive *T. aestivum* habitats from midpart of Western Bulgaria are characterising with great diversity of physical, hydrological and chemical soil parameters. Their common feature is very high Cation exchange capacity values (CEC) and very low and low $CaCO_3$ content.

Compared to other *T. aestivum* PCA studies (and to other commercial truffles also like *T. melanosporum* Vittad.) our research confirms that Ca^{2+}, TOC and TN are among the most important factors for productive sites. As this is already well known, future efforts should focus on the factors affecting to the formation of ECM and fruitbodies in the context of interaction between the competing ectomycorrhizal fungal species.

Acknowledgments

The present study was performed in the framework of Project No 1214/27.04.22 'Study of soil parameters influencing the distribution and development of the common black summer truffle (*Tuber aestivum* Vittad.) in Western Bulgaria' funded by Scientific Research Sector at University of Forestry, Sofia, Bulgaria.

References

Agerer R., Rambold G. 2004–2011. DEEMY – an information system for characterization and determination of ectomycorrhizae. Ludwig-Maximilians-Universität München and SNSB – Botanische Staatssammlung München, Germany. http://www.deemy.de (Accessed March 13, 2022).

Begon M., Townsend R. 2021. Ecology: From Individuals to Ecosystems. Fifth Edition. Wiley. 864 p.

Bonito G., Gryganskyi A., Trappe J., 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. https://doi.org/10.1111/j.1365-294X.2010.04855.x

Bonito G., Smith M. 2016. General Systematic Position of the Truffles: Evolutionary theories. In True Truffle (*Tuber* spp.) in the World: Soil Ecology, Systematics and Biochemistry; Zambonelli A., Iotti. M., Murat. C. (Eds). Springer: Berlin/Heidelberg, Germany: 3–18.

Bragato G., Fornasier F., Bagi I., Egli S., Marjanović Ž. 2021a. Soil parameters explain short-distance variation in production of *Tuber aestivum* Vittad. in an oak plantation in the central-northern part of the Great Hungarian Plain (Jaszsag region, Hungary). Forest Ecology and Management 479, 118578. https://doi.org/10.1016/j.foreco.2020.118578

Bragato G., Mosetti D., Turpaud P., Benucci G., Donnini D. 2021b. Presence and Production of *Tuber aestivum* in relation with the soil environment of a semi-natural experimental truffle orchard in Spoleto, Central Italy. http://dx.doi.org/10.2139/ssrn.3996982

Bratek Z., Merényi Z., Illyés Z., László P., Anton A., Papp L., Merkl O., Garay J., Vikor J., Brandt S. 2010. Studies on the ecophysiology of *Tuber aestivum* populations in the Carpatho-Pannonian region. *Österreichische Zeitschrift für Pilzkunde* 19: 221–226.

Bruhn J., Hall M. 2011. Burgundy Black Truffle Cultivation in an Agroforestry Practice. Agroforestry in Action, University of Missouri, Center for Agroforestry: 1–20.

Chevalier G., Sourzat P. 2012. Soils and techniques for cultivating *Tuber melanosporum* and *Tuber aestivum* in Europe. In Edible ectomycorrhizal mushrooms. Springer, Berlin, Heidelberg: 163–189. https://doi.org/10.1007/978-3-642-33823-6_10

Dimitrova E., Gyosheva M. 2008. Hypogeous ascomycetes in Bulgaria. Phytologia Balcanica 14(3): 309–314.

Filcheva E., Tsadila C. 2002. Influence of clinoptilolite and compost on soil properties. Communications in Soil Science and

Plant Analysis 33(3–4): 595–607. https://doi.org/10.1081/CSS-120002766
García-Montero L.G, Moreno D., Monleon V., Arredondo-Ruiz F. 2014. Natural production of *Tuber aestivum* in central Spain: *Pinus* spp. *versus Quercus* spp. brûles. Forest Systems 23(2): 394–399. http://dx.doi.org/10.5424/fs/2014232-05112
García-Montero L., Díaz P., Martín-Fernández S., Casermeiro, M. 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. https://doi.org/10.1080/09064710701743286
García-Montero L., Quintana A., Valverde-Asenjo I., Díaz P. 2009. Calcareous amendments in truffle culture: a soil nutrition hypothesis. Soil Biology and Biochemistry 41(6): 1227–1232. https://doi.org/10.1016/j.soilbio.2009.03.003
Gógán A., 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. doi: 10.5586/am.2012.015
Gregori G. 2010. L‘espèce *Tuber uncinatum* en Italie: écologie, commercialisation et valorisation. Österreichische Zeitschrift für Pilzkunde 19: 265–271.
Gryndler M., Beskid O., Hujslová M., Konvalinková T., Bukovská P., Zemková L., Hršelová H., Jansa J. 2017. Soil receptivity for ectomycorrhizal fungi: *Tuber aestivum* is specifically stimulated by calcium carbonate and certain organic compounds, but not mycorrhizospheric bacteria. Applied Soil Ecology 117–118: 38–45. https://doi.org/10.1016/j.apsoil.2017.05.007
Gryndler M., Černá L., Bukovská P., Hršelová H., Jansa J. 2014. *Tuber aestivum* association with non-host roots. Mycorrhiza 24(8): 603–610. https://doi.org/10.1007/s00572-014-0580-9
Hilszczańska D., Sierota Z., Palenzona M. 2008. New Tuber species found in Poland. Mycorrhiza 18(4): 223–226. https://doi.org/10.1007/s00572-008-0175-4
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(3): 1713–1718. https://doi.org/10.15244/pjoes/89588
Hinkova T. 1965. Materials on the fungus flora of Bulgaria. Yearbook of Sofia University, Faculty of Biology, book 2 Botanica 58: 95–105 (in Bulgarian).
Husson F., Lê S., Pagès J. 2017. Exploratory multivariate analysis by example using R. CRC press. 243 p.
Innangi M., Fioretto A., Fondón C., García-Montero L., Marzaioli R., Pinto S., Rutigliano F., 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 80, 150648. doi: 10.1016/j.pedobi.2020.150648
ISO 10390:2005. Soil quality. Determination of pH: 1–7.
ISO 10693:1995. Soil quality. Determination of carbonate content – Volumetric method: 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 test 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).
Jamali S. 2017. First report of identification and molecular characterization of *Tuber aestivum* in Iran. Agroforestry Systems 91(2): 335–343. https://doi.org/10.1007/s10457-016-9932-0
Kachinskii A. 1963. Soil physics. Part 2, Mos-

cow 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. (Ed.). 1979. Climate handbook of People's Republic of Bulgaria. Vol. 3. Air temperature, soil temperature, frost. Nauka i Izkustvo Publishing House. 811 p.

Mello A., Murat C., Vizzini A., Gavazza V., Bonfante P. 2004. *Tuber magnatum* Pico, a species of limited geographical distribution: its genetic diversity inside and outside a truffle ground. Environ Microbiol 7(1): 55–65. https://doi.org/10.1111/j.1462-2920.2004.00678.x

Moeys J. 2018. The soil texture wizard: R functions for plotting, classifying, transforming and exploring soil texture data. CRAN. R-Project. 104 p. https://cran.r-project.org/web/packages/soiltexture/vignettes/soiltexture_vignette.pdf (Accessed March 15, 2022).

Nahm M., Tegel W., Gold C. 2019. A search for naturally grown Burgundy truffles (*Tuber aestivum*) in hazelnut plantations in Germany: Results of a survey. Agriculture and Natural Resources 53(1): 84–86. doi: 10.34044/j.anres.2019.53.1.14

Nishava KiT 2019. Forest management plan of the Slivnitsa Forest Service. (in Bulgarian).

Păcurar H., Dirja M., Mihai M., Păcurar I., Roșca S., Bilașco Ș. 2019. Identification of soils factors influence in the distributions of *Tuber aestivum* in Transylvanian Subcarpathian hill, Romania. Notulae Botanicae Horti Agrobotanici Cluj-Napoca 47(2): 478–486. https://doi.org/10.15835/nbha47111378

R Core Team 2022. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/ (Accessed March 15, 2022).

Raglione M., Owczarek M. 2005. The soils of natural environments for growth of truffles in Italy. Mycologia Balcanica 2: 209–216.

Robin C., Goutal-Pousse N., Le Tacon F. 2016. Soil characteristics for *Tuber aestivum* (syn. *T. uncinatum*). In True Truffle (*Tuber* spp.) in the World: Soil Ecology, Systematics and Biochemistry. Zambonelli A., Iotti M., Murat C. (Eds), Springer International Publishing: 211–231.

Silva 2003. 2019. Forest management plan of the Sofia Forest Service. (in Bulgarian).

Stobbe U., Egli S., Tegel W., Peter M., Sproll L., Büntgen U. 2013a. Potential and limitations of Burgundy truffle cultivation. Applied microbiology and biotechnology 97(12): 5215–5224. https://doi.org/10.1007/s00253-013-4956-0

Stobbe U., Stobbe A., Sproll L., Tegel W., Peter M., Büntgen U., Egli S. 2013b. New evidence for the symbiosis between *Tuber aestivum* and *Picea abies*. Mycorrhiza 23(8): 669–673. doi: 10.1007/s00572-013-0508-9

Todesco F., Belmondo S., Guignet Y., Laurent L., Fizzala S., Le Tacon F., Murat C. 2009. Soil temperature and hydric potential influences the monthly variations of soil *Tuber aestivum* DNA in a highly productive orchard. Scientific Reports 9, 12964. https://doi.org/10.1038/s41598-019-49602-2

Wedén C., Chevalier G., Danell E. 2004. *Tuber aestivum* (syn. *T. uncinatum*) biotopes and their history on Gotland, Sweden. Mycological Research 108(3): 304–310. https://doi.org/10.1007/s00572-013-0508-9

Weil R., Brady N. 2017. The nature and properties of soils. Fifteenth edition. Pearson Education Limited. 1104 p.

Wickham H. 2016. ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag New York. 267 p. https://ggplot2.tidyverse.org (Accessed March 15, 2022).