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Original Research

Soil Properties Conducive to the Formation of *Tuber aestivum* Vitt. Fruiting Bodies

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Abstract

Summer truffle (*Tuber aestivum*), also known as Burgundy truffle, is getting interest in Poland in terms of cultivation as a promising incentive for rural areas. Yet the occurrence of the fungus in wider scale in our country has been confirmed in the last decade. Ecological factors that determine the occurrence of *T. aestivum* are rather well known in the Mediterranean region, whereas such knowledge is limited in northern Europe. The aim of this work was to find the correlations between essential nutrients in surface horizons of soils typical of truffle occurrence. The study area is situated in the Nida Basin in southern Poland. Principal component analysis (PCA) showed that active carbonate content is the variable that accounts for the greatest percentage of occupancy in the *T. aestivum* habitat. In this paper we propose that active carbonate is a major factor in the fruiting of summer truffle. The obtained results could have applications in natural harvesting and truffle culture.

Keywords: summer truffle, habitats, soil composition, stands

Introduction

Burgundy truffle *Tuber aestivum* Vittad., one of the species among black truffles, is a prized fungus due to its taste and aroma. This hypogeous species is found throughout Europe [1, 2] and belongs to the Pezizales, a large group of ectomycorrhizal fungi that form mutualistic symbioses with the roots of angiosperms and gymnosperms [3]. *T. aestivum* forms ectomycorrhizal symbioses with many different species, including *Corylus avellana*, *Quercus robur*, *Fagus sylvatica*, *Tilia cordata*, and *Pinus nigra*. This truffle species prefers calcareous soils with pH levels near or above 7-8, although it occurs in beech woods on lime-deficient soils in the United Kingdom [4].

Both historical [5] and contemporary data show that *T. aestivum* and other truffle species have been reported from Poland [6-8] and the situation is similar for neighbouring countries the Czech Republic [9] and Germany [10]. So far, known localities of the fungus have been confirmed in southern Poland on soils that represent rendzinas type of soil formed on bedrock that belongs to marlstone, marly limestone, and gypsum [7]. It seems that the best conditions for fructification of *T. aestivum* are in mixed broadleaved forests which are no older than 30 years [6].

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The importance of soil chemical and physical characteristics for truffle (e.g. *T. aestivum*) ectomycorrhiza development and fruiting bodies (ascocarps) formation in plantation and natural stands is well documented [11]. However, Granetti and co-authors [3] claim that there is a lack of knowledge as to how the physical-chemical properties of soil shape natural truffle development. In addition, the microbiology, site ecology, and host specificity of *T. aestivum* is poorly understood [10, 12]. Gryndler and co-authors [13] suggest that non-host (not known to form ectomycorrhiza with a given fungal symbiont) plants may also be important for truffle production since the association of *T. aestivum* mycelium with the non-host plants plays a functional role. The presence of the mycelium can induce substantial changes in the vegetation layer of *T. aestivum* inhabited stands and result in important consequences for carbon and nutrient cycling in the ecosystem. Furthermore, the suggestion that truffle-related soil microorganisms may be less important for the stimulation of truffle hyphal growth than the complete soil has been made [9].

Here we emphasize soil properties of the surface horizons in four stands with fruiting bodies of *T. aestivum*, to find out which soil factors shape the fructification of truffles the most. To our best knowledge it is the first work done on the subject in Poland. The results are expected to be very useful for truffle plantation owners and managers, whose number is increasing every year in Central and Eastern European countries.

Material and Methods

Study Area

The study area is located in the Nida Basin in southern Poland. Four stands representing mixed deciduous forest were chosen. The stands are indicated with symbols: PO, WR, GR, and SA, and their altitudinal gradients are 247, 290, 264, and 319 m a.s.l., respectively. At each stand three plots (100 m^2) were established to study the occurrence of truffles, physic-chemical properties of soil, and type of vegetation. All those forests are situated close together in a basin sharing similar topographic and microclimatic conditions; geographic coordinates are: 50°25′-50°28′N and 20°19′-20°48′E. Average annual precipitation is 600 mm, with a low yearly average temperature of 8.0ºC [14]. Its lithology comprises Jurassic and Cretaceous limestone and marlstone, and soils are rendzic leptosols. The forests represent the geo-botanical classification type of Tillio-Carpinetum typicum [15].

Soil Snalysis

In total, five samples of soil were taken at each plot. The analysis was done for one mixed sample for each plot, so twelve samples of soil were analysed. The soil was sampled by removing the litter and vegetation layers and then collecting approximately 0.5 kg of soil down to a depth of 20-30 cm, depending on the rockiness of the soil. The soil analyses were performed in the laboratory of the Polish Centre for Accreditation (No. AB740). The soil pH in water and essential nutrient contents (Table 1) were measured according to ISO 10390 (1997) and PB-14ed.2 of 1 January 2010 (using inductively coupled argon-plasma spectrometry following mine-ralisation in chloric (VII) acid), a percentage of N and total organic carbon (TOC) - ISO13878 (2002) and PN-ISO 10694 (2002), content of carbon calcium (percent) -Scheibler's method (ISO 10693, 1994), and exchangeable cations (Ca, Mg, K, Na) - ISO 11260 (2011), respectively. The soil texture was measured based on three particle-sized fractions: <2 μm (clay), 2-63 μm (silt), and 63-2000 μm (sand) (ISO 11277, 2005).

Truffle Harvest and Taxonomic Studies

The truffle localities at the stands were found using trained truffle dogs in collaboration with researchers from the Agricultural University in Nitra (Slovakia). Inventories were made in 2012-2014, and 532 fruiting bodies were found for all sites. Species of truffles were identified on the basis of microscopic features and compared to the criteria by Granetti et al. [3] and Molinier et al. [16]. Samples of fruiting bodies were also taken for molecular identification. The sequences are deposited in Genbank NCBI (KJ524517–KJ524528).

Statistical Analysis

Data were analysed using Statistica v. 10 (Dell, 2016). Normality was checked using the Shapiro-Wilk test. The homogeneity of variance was also checked by using the Levene test and the Brown-Forsythe test. Principal component analysis (PCA) was done with 11 soil variables of 12 soil samples (Table 1) to show the variation patterns of those elements potentially associated with the presence of *Tuber aestivum* in four natural stands. Data regarding the number of *T. asetivum* fruiting bodies and the value of $CaCO_3$ were log-transformed to meet the requirements of parametric correlation (Pearson test) with the p-value ≤ 0.05.

Results

Soil physicochemical characteristics are a key factor for truffles' fruiting bodies development. Even when a tree or shrubs are well mycorrhizaed and the climate is favourable, fruiting cannot take place if certain soil parameters are inappropriate.

Table 1 shows investigated soil parameters. The soils have silty clay loam, loam, and clay loam texture. Most of the investigated soils have pH above 7.0 and a low

Table 1. Texture and chemical composition of the analysed soils.

No.	Sand	Silt	Clay	pH	$CaCO_3$	TOC	N^+	Ca^{2+}	Mg^{2+}	K^+	Na^+
PO	9.2	40.5	50.3	7.5	31.2	5.41	0.45	44.8	1.51	1.27	0.041
PO	8.4	47.12	44.48	7.4	2.53	4.64	0.39	46.6	1.3	0.68	0.064
PO	6.69	37.91	55.4	6.3	0.26	2.7	0.218	27.5	1.17	0.62	0.064
WR	23.71	52.0	24.29	7.4	8.62	4.97	0.46	46.1	1.22	0.72	0.04
WR	16.88	53.05	30.07	7.4	17.61	4.99	0.47	43.5	1.35	0.64	0.038
WR	25.08	47.7	27.22	7.6	36.56	3.38	0.302	30.0	0.57	0.58	0.03
SA	13.22	41.47	45.31	7.3	1.26	2.68	0.22	31.8	1.08	0.53	0.072
SA	23.6	42.4	34.0	7.4	6.74	3.79	0.325	38.9	1.31	0.34	0.087
SA	23.71	52.0	24.29	6.8	8.62	2.38	0.205	25.5	1.22	0.72	0.04
GR	39.37	30.45	30.18	7.4	0.94	3.64	0.346	40.8	1.97	0.44	0.045
GR	27.23	44.25	28.52	7.2	2.16	4.4	0.428	40.6	2.14	0.56	0.053
GR	46.0	25.22	28.78	6.0	0.03	3.36	0.329	19.6	1.98	0.36	0.044

Sand, silt, and clay are expressed in percentages, active $CaCO_{3,}$ TOC (total organic carbon), and N are in percentages, and exchangeable cations are expressed in cmol x kg^{-1}.

percentage of total calcium carbonate. Organic carbon levels are moderate and the C/N ratio is above 10.

The chemical soil variables analysed with PCA were used to investigate the overall impact of the top soil horizon on the presence of *T. aestivum* fruiting bodies. The first three factors account for 78.02% of the variance contained in the original matrix (Fig. 1). PC1 accounts for 37.99% of the variance and focuses on the differences between soils with a higher amount of Ca, K, N, TOC, $CaCO_3$, pH, and silt (Table 2). The second factor PC2 represents 22.68% of the variance and indicates the differences between soils with a higher percentage of clay and sand as well as the amount of Mg. The third factor, PC3, accounts for 17.35% of the variance and shows differences between soils with accounts of Na (Table 2).

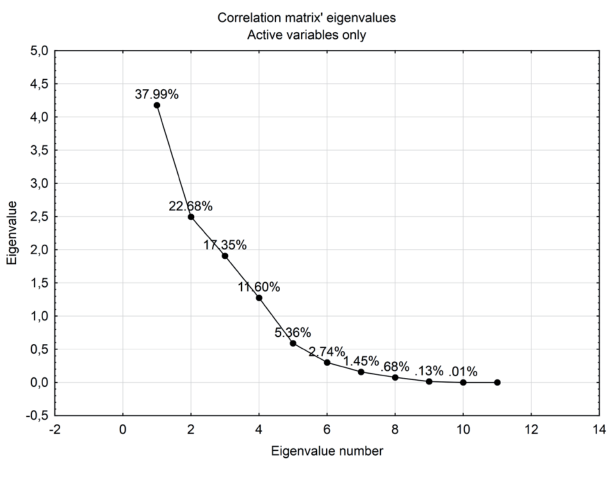

Fig. 1. Scree plot of principal component analysis (PCA).

Fig. 2 shows correlations between variables. Positively correlated are the variables whose vectors lay close to themselves, for example K, silt, $CaCO_3$, and pH. These variables are negatively correlated with Mg and sand. Amounts of Na are negatively correlated with Ca, TOC, and N. Most analysed soils were characterized by rather low concentrations of $CaCO_3$ (Table 1, Fig. 3). The soils with a higher percentage of $CaCO_3$ showed positive correlation with the number of fruiting bodies of *T. aestivum* (Fig. 3).

Table 2. Coefficients of soil variables according to PCA (factors loadings).

Variables	Factor 1 (PC1)	Factor 2 (PC2)	Factor 3 (PC3)
Sand	0.489	-0.798	0.269
Silt	-0.631	0.279	0.355
Clay	-0.046	0.677	-0.588
pH	-0.773	0.012	0.043
$CaCO_3$	-0.635	0.129	0.566
TOC	-0.828	-0.403	-0.306
N	-0.735	-0.588	-0.238
Ca^{2+}	-0.825	-0.177	-0.427
Mg^{2+}	0.233	0.732	-0.511
K^+	-0.698	0.251	0.001
Na^+	0.301	0.408	-0.670

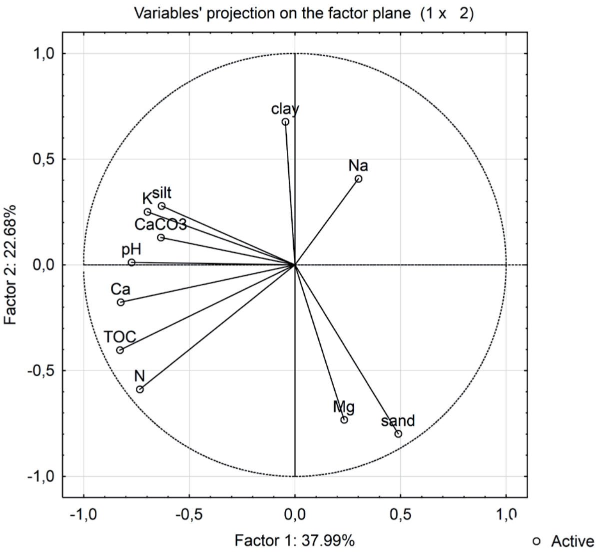

Fig. 2. Relationship between variables of soils typical of *T. aestivum*.

Discussion

Data on *T. aestivum* ecology are still rather sparse due to the fact that the species is less valued (economically and culinary) than, for example, *T. melanosporum*. Although *T. aestivum* is widely distributed throughout Europe [1, 17], researchers have given it less attention. Our data showed that the development of Burgundy truffle fruiting bodies is influenced by content of carbonates. Both forms of the carbonates, namely $CaCO_3$ and exchangeable Ca^{2+}, regulate pH, the latter form being more relevant for truffle development (Fig. 2, Table 2). An increase of $CaCO_3$ content positively affected a number of *T. aestivum* fruiting bodies (Fig. 3). This corresponds to the broad opinion that truffles in general, and Burgundy truffle in particular, require high calcium availability in the soils [18].

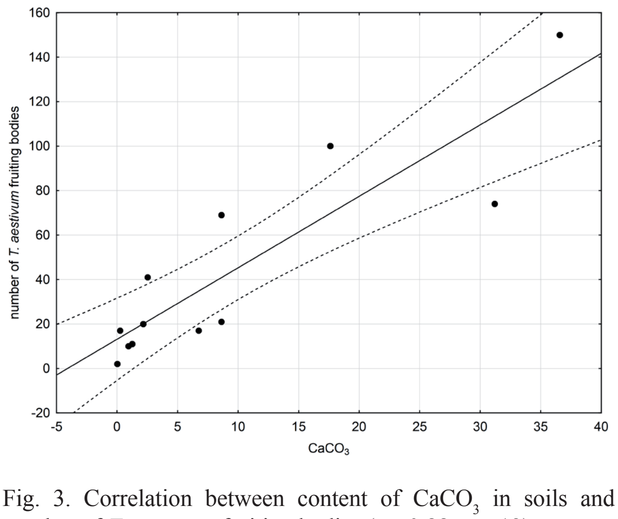

Fig. 3. Correlation between content of $CaCO_3$ in soils and number of *T. aestivum* fruiting bodies (r = 0.88, n = 12).

Moreover, Gryndler et al. [9] suggest that high bioavailability of calcium stimulates the development of truffle mycelium. The importance of carbonates for proper development of three truffle species – *T. melanosporum*, *T. aestivum*, and *T. mesentericum* – has been highlighted by some authors [11, 19, 20]. The role of active carbonate in the formation of truffle fruiting bodies was observed clearly in the case of *T. melanosporum* [11]. In a review by Chevalier and Sourzat [20] dedicated to soil requirements of *T. aestivum*, the authors showed variability of calcium carbonate levels in truffle orchards in France, Italy, Hungary, and Sweden. For example, in France the level of limestone is highly variable and ranges from 0.4% to 52%. In Italy, the level of calcium carbonate in *T. aestivum* soils is just as variable, although generally it is relatively low and ranges between 0.9% and 12%. However, in the region of Parma, it can be as high as 52.9%, with an average of 21.9%. In Hungary most of the soils contain little (5–8%) or only traces of lime (0.1-5%), while in Sweden total limestone can be from just a trace to 10.5%. Our data on this aspect (Table 2) seems to fit perfectly into these findings. The ranges of other nutrients (C, N, Ca, and K) that are very important for *T. aestivum* development were similar to those found at Swedish and French truffle orchards [20]. For our *T. aestivum* the pH is generally weakly alkaline or neutral, or possibly mildly acidic (6.0-7.6), and in this context resembling the soils in Hungary and Sweden [21, 22] more than the soils in France and Italy, where due to the constant presence of limestone from the surface down, the pH is always above 7 and ranges between 7.1 and 8 in the A1 horizon [20]. However, Thomas [23] found that the lowest optimal pH for mycorrhiza of *T. aestivum* development is 7.51.

Analysing the structure of investigated soils, we found that sandy textures are less conducive to truffle development (Fig. 2). The result is in accordance with findings by Chevalier and Sourzat [20]. At *T. aestivum* sites in natural forests in Poland, the soil texture is highly variable, ranging from silty-clay to clayey-silt and more rarely silty, silty-sandy, or clayey. The analysed soils varied from “heavy” (up to 55.4% clay) to “light” (up to 46% sand). The great diversity in soil texture at *T. aestivum* sites is well documented in the literature [11, 19, 20, 24, 25]. The “light” soils are generally thought to be less favourable for truffle cultivation. However, even excessively sandy soils can support truffle development if they are sufficiently rich in calcium [20]. Thus, *T. aestivum* appears to have a wide tolerance for soil texture as it develops in sedimentary terrain of various geological ages, from Palaeozoic to Quaternary and recent alluvia. Moreover, *T. aestivum* fruit bodies are able to grow on alkaline volcanic substrates [26]. This diversity of substrates (and the fact that *T. aestivum* is less thermophilic than *T. melanosporum*) explains the broad geographic distribution of this species – from Morocco to Sweden and from Ireland to Azerbaijan [2]. In Poland, as well as in France and Italy [20], the soils where *T. aestivum* occurs are typically rendzinas on terrain derived from sedimentary rocks (marly limestone) from the Mesozoic (Cretaceous).

The ecological and pedo-climatic requirements of the Burgundy truffle allows the fungus to adaptat to different environments typical of the Mediterranean region and in northern and eastern Europe as well. The main challenge of research about *T. aestivum* soils is to estimate very precisely the optimal conditions favourable for fruiting bodies' growth. It is also important to characterize conditions that facilitate the colonization and persistence of *T. aestivum* mycorrhizas on root systems. This knowledge is a key factor for establishing best management practices in truffle orchards, such as irrigation, soil tillage, supplies of organic matter, and nutrients. Our results are a new input of knowledge in this aspect and are important for truffle growers, especially in new areas outside the Mediterranean region.

Conclusions

The data show that content of calcium is crucial for fructification of *T. aestivum* fruiting bodies together with the soil's pH level and the share of the silt fraction.

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Conflict of Interest

The authors declare no conflict of interest.

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