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Virtual Truffle Hunting—A New Method of Burgundy Truffle (*Tuber aestivum* Vittad.) Site Typing

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Abstract: The aim of this study was to enable searches for truffles (*Tuber* spp.), particularly the Burgundy truffle (*T. aestivum* Vittad.), to be carried out in forests based on a method that has been constantly developed since 2007 by the Forest Research Institute. The method is termed "Virtual Truffle Hunting" and it takes 12 parameters into account: bedrock, soil pH, Ca+ and $CaCO_3$ content in soil, C/N ratio, soil structure, altitude of terrain, type of forest site, forest structure, the Burgundy truffle host trees, and the presence of particular species including orchids and insects. A simple "Virtual Truffle Hunting" software has also been developed, which makes the use of the method easy, fast, and effective. This method is to ascertain the truffle potential for all areas in which digital maps are not available. In 2015, the method was tested in 20 sites, representing forests in 5 Polish macroregions. Hunting for hypogeous fungi was conducted from June to October with the help of trained dogs. Thanks to this method, 14 new truffle sites were found. The knowledge of environmental conditions conducive to the Burgundy truffle growth enabled us to form an effective tool in order to identify new sites of truffle presence.

Keywords: bioindicators; hypogeous fungi; soil parameters; software; *Tuber aestivum*; valorization

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1. Introduction

Truffles are one of the most economically valued non-wood forest products owing to their taste and aroma. These subterranean mushrooms are especially appreciated in countries that have been associated with their cultivation for many decades, including France, Italy, and Spain. Prices of the highly esteemed truffle species, for example, white truffle (*Tuber magnatum* Picco) and black truffle (*Tuber melanosporum* Vittad.), can even reach 2000–3000 Euro per kilogram [1,2].

In Poland, the most common species is the Burgundy truffle (*Tuber aestivum* Vittad.) [3–5]. In 2012, the location of a population of the smooth black truffle (*Tuber macrosporum* Vittad.) was found [6], and the presence of winter (*Tuber brumale* Vittad.) and whitish (*Tuber borchii* Vittad.) truffles has also been confirmed [7–10]. Several other species of truffles that have no commercial value, including *T. maculatum* Vittad., *T. excavatum* Vittad., and *T. rufum* Picco, have been noted in various regions of the country [3,11]. In Poland, the distribution of sites suitable for individual species is poorly known. Despite the relatively rich tradition of using truffles in Polish cuisine, the fungi have disappeared from the table for many years, but today, they are slowly coming back into favor [4,5].

Truffles are the subterranean fruiting bodies of *Tuber* (Ascomycotina, Pezizales), a mycorrhizal genus of fungi that needs host plants and appropriate environmental conditions for development [12–15]. The process of fruiting body formation is affected by many biotic and abiotic factors, and research on this aspect has been conducted in many countries [16–19]. In Europe, *Tuber* spp. form a mycorrhizal symbiosis with many species of deciduous and coniferous trees and shrubs [20]. Truffles can also form mycorrhizas with some bushes, including representatives of the Mediterranean shrubs belonging to the family Cistaceae known as "rock roses". Members of this family associated with several truffle species include *Cistus* L., commonly known as purge, as well as *Helianthemum* Mill. and *Fumana procumbens* (Dunal) Gren. et Godr. [21,22].

Previous studies have shown that orchids belonging to the genera *Epipactis* Zinn, *Cephalanthera* Rich, and *Cypripedium* L. also coexist with the Burgundy truffle [14,23]. Orchids have been reported to form mycorrhizal symbiosis with many species of truffles [24]. For example, mycelia of *T. maculatum* were isolated from the roots of *Epipactis helleborine* (L.) Crantz and *Cephalanthera damasonium* (Mill.) Druce, and mycelial *T. excavatum* was isolated from *Epipactis microphylla* (Ehrh.) Sw. root tissues [25]. Moreover, the orchid species and truffles mentioned here occupy a similar ecological niche. The species have similar soil preferences, and the key factors to their development are high calcium content in soil and high soil pH. For example, *Cypripedium calceolus* L. grows in soils with a pH range of 6.6–7.5 [26]. Thus, although the presence of orchid mycorrhiza does not guarantee fructification of truffles, several orchid species can be very helpful in indicating truffle sites.

Truffle species show different preferences for habitat, particularly for two environmental factors: soil pH and annual mean temperature [27]. Some species of truffles also have a high degree of specialization and selectivity towards plant partners [28]. On the other hand, the largest ecological generalists within the genus *Tuber* are considered whitish and Burgundy truffles, which form mycorrhizas with many species of angiosperms and gymnosperms, including plant species that are aliens in Europe, e.g., pecan nut (*Carya illinoinensis* (Wangenh.) K. Koch) [29]. Biotic and abiotic factors connected with presence of *T. aestivum* in Europe have been broadly investigated [14,15,17–19,30–34].

For many animals, including mammals (rodents, deer, wild boars), birds, and snails, truffles are a nutritious food source [35,36], but for some arthropods, most notably those from the orders of Coleoptera (beetles) and Diptera (flies), these fungi are the basis of their diet [16,37]. The fauna of mycophagic beetles associated with truffles are represented by *Leiodes cinnamomea* Panzer and *L. oblonga* Erichson (Coleoptera: superfamily Staphylinoidea) [38–40]. Adult females of round fungus beetles (*Leiodes* Latreille) are attracted by flavors emitted by the fruiting bodies of truffles at an early stage of their development, as even immature fungi can attract the beetles [41]. For the *Leiodes* beetles, some truffle species (*T. melanosporum*, *T. aestivum*, and *T. excavatum*) form a favorable environment for growth [38,42–45]. Flies belonging to the genus *Suillia* Robineau-Desvoidy [40] and the dor beetles (family Bolboceratidae Mulsant) (I.A. *Bolbelasmus unicornis* Schrank and *Odonteus armiger* Scopoli) can also feed and develop on truffles [46].

Factors affecting the life cycle of truffles can be divided into several groups, but it should be emphasized that they all interact with each other. The development of the fruiting bodies is shaped by the internal characteristics of the population (e.g., physiological factors) and environmental elements (biotic and abiotic) [47]. Soil and weather conditions as well as interactions with the plants are of the greatest importance among this group of factors. Büntgen et al. [47] also emphasize the role of other elements and processes, including terrain features (e.g., elevation, exposition), the way matter circulates in the ecosystem (particularly carbon, water, and nutrients), the presence of mycophagous fauna, and biotic disorders (e.g., defoliation of trees). Moreover, numerous biotic and abiotic factors mentioned here that drive truffle fructification are interrelated: soil chemistry depends on bedrock as well as land-use, while terrain elevation influences plant species composition, as well as weather conditions, and so on [47].

Truffles usually prefer well aerated, calcareous soils with an alkaline pH and an appropriate amount of organic matter (e.g., about 7.5 $\pm$ 3% for *T. aestivum*) [14,48]; however, moisture and temperature preferences vary depending on the truffle species. For example, *T. melanosporum* is a thermophilic species and prefers highly-drained soils, while *T. macrosporum* can grow in colder climates and in periodically flooded soils [49]. Truffle species also differ as a result of preferences in soil pH. The Burgundy truffle can occur in neutral soils, and the whitish truffle tolerates a pH of 5.0 [50].

Soil properties are shaped by many factors, including geological structure and altitude, climate, vegetation, soil organisms, and soil tillage [17]. The basic chemical and physical features of the soil, on the basis of which the soil is classified, stem from the geological history and the type of bedrock on which they were formed. They affect soil pH, content of macroelements such as exchangeable calcium and magnesium cations, soil structure, and other parameters important for the development of truffle fruiting bodies [14,15,32]. In the analysis of soils from natural sites and from truffle orchards, their granulometric composition is taken into account [33,34,51–53], because the share of individual fractions determines aeration and soil permeability.

Most truffle species occur in temperate regions and prefer a mild climate, free from extreme heat in summer on the one hand, and severe frosts on the other [54]. Büntgen et al. [47] list the five most important climate features shaping the life cycle of truffles: precipitation, temperature, snow cover length, number of sunny days per year, and distribution of negative temperatures throughout the year. The Burgundy truffle is an ecologically plastic species with high ecological amplitude, higher in terms of resistance to climatic anomalies, hydrological changes, and other abiotic stress than *T. melanosporum* [17,43,47,51,55].

The aim of this work was to create a comprehensive method of typing truffle sites based on knowledge obtained during ten years of research conducted by the authors and to check the effectiveness of the method in forest stands. An additional goal was to search for new truffle sites in Poland. This method could be successfully adopted in various areas of Europe to ascertain the truffle potential for the areas where there is a lack of digital maps based on the computer multilayer analysis [30,56,57]. Based on the results of the study conducted since 2007 and literature data, a comprehensive method of truffle site typing has been proposed. This method is named "Virtual Truffle Hunting" and is presented in the form of a practical table (Table 1) that can be easily applied with suitable free software.

Table 1. "Virtual Truffle Hunting"—a method for typing truffle sites.

	Possibility of Truffle Fruiting Body Occurrence On Site (Preference Level)			
Characteristics of Forest Site	**High (Optimal Conditions) 3 pts**	**Medium (Conditions within Tolerance Limit) 2 pts**	**Low (Conditions at the Tolerance Limit) 1 pts**	**None (Conditions Outside the Tolerance Limit) 0 pts**
Bedrock	Chalk Corallian limestones Gypsum Limestone Lithothamnium Limestones Marly limestone Oolite Pelagic limestones Shelly limestones	Encrinite Marlstone Nummulite limestone Seekreide Terra rossa Travertine	Calcareous gaizes Dolomite Marly schist Other rocks containing calcium	Rocks not containing any form of calcium
Soil pH in H_2O	>7.2	6.8–7.1	6.7–5.7	<5.7
Ca [cmol(+)/kg]	30–60	15–30 or >60	<15	Not present
$CaCO_3$ [%]	>5	1–5	<1	Not present
C/N	>15	10–15	5–10	<5
Soil structure	Clay loam Loam Sandy clay loam Silty clay Silty clay loam	Clay	Sandy loam Sandy clay Silt loam	Sand Loamy sand Silt

Table 1. *Cont.*

	Possibility of Truffle Fruiting Body Occurrence On Site (Preference Level)			
Characteristics of Forest Site	**High (Optimal Conditions) 3 pts**	**Medium (Conditions within Tolerance Limit) 2 pts**	**Low (Conditions at the Tolerance Limit) 1 pts**	**None (Conditions Outside the Tolerance Limit) 0 pts**
Altitude (m.a.s.l.)	<600	600–1000	1000–1600	>1600
Forest habitat type (Fr.—fresh, Mo.—moist, U—upland, BF—broadleaved forest, M—mixed)	Fr. BF; Fr. UBF	Fr. MBF; Fr. UMBF	Mo. BF; Mo. UBF; Mo. MBF; Mo. UMBF	Other forest habitat types
Forest structure	Open canopy closure, presence of gaps, paths or tracks	Moderate canopy closure	Full canopy closure, some shrubs are present	Full canopy closure, dense shrub layer
Tree and shrub mycorrhizal partners of *Tuber aestivum*	More than 2 species in tree layer, presence of *Corylus avellana*	At least 1 species in tree layer and at least one tree or shrub species in lower forest layer	At least 1 species of tree or shrub in tree or shrub layer.	Lack of tree or shrub mycorrhizal partners of *Tuber aestivum*
Orchids presence (*Cephalanthera*, *Cypripedium* or *Epipactis* genera)	More than one species (3 pts)	One species (2 pts)	No species of *Cephalanthera*, *Cypripedium* or *Epipactis* genera (0 pts)	
Insects associated with truffle	Three points (3 pts) should be added to the final equation, if at least one of the insect species occurs in the subarea (not dependent on number of species or their abundance): - “truffle beetles” from Leiodidae family; - dor beetles: *Bolbelasmus unicornis* or *Odonteus armiger*; - “truffle flies” from genus *Suillia*.			

2. Materials and Methods

The method of “Virtual Truffle Hunting” is a scale for showing the possibility of the occurrence of the Burgundy truffle fruiting bodies based on chosen environmental factors and indicators. The concept of the method is similar to the assumptions of other commonly used natural scales, e.g., the Extended Biotic Index for water quality assessment (IBE) developed by Ghetti & Chierici [58].

The method included 12 biotic and abiotic parameters helpful in determining the Burgundy truffle sites (Table 1). Six parameters relate to soil conditions: bedrock, soil pH in H_2O, calcium exchangeable cation content and calcium carbonate content in soil, soil carbon to nitrogen ratio, and soil granulometric structure, according to the soil texture triangle [34]. The information concerning bedrock (necessary for the method validation) was taken from the Central Geological Database [59] or the Forest Data Bank [60]. In order to determine other soil parameters, it was necessary to take soil samples from the sites and perform laboratory analyses in accordance with the methodology of Hilszczańska et al. [14]. Each parameter was assessed from 0 to 3 points (Table 1). Other important factors were altitude (m.a.s.l), forest habitat type and structure, and the presence and number of *T. aestivum* mycorrhizal partners (Table 2). Each of these traits is awarded a maximum of 3 points. The valorization was also based on the known bioindicators of truffles: orchids and mycophagous insects. Points are awarded (max. 3) for the presence of orchids at the site as well as for truffle-associated insect occurrence (3 points). To sum up, for each of 10 parameters, a four-step characteristic was proposed, according to which from 0 to 3 points are awarded. In the case of bioindicators (orchids and insects), the scale was modified and the criterion for awarding points is given in Table 1. The maximum number of points for one forest division is 36. The following classification thresholds were proposed: 31–36 pts.—high possibility of fruiting body occurrence, 25–30 pts.—medium possibility, 19–24 pts.—low possibility, and below 19 pts.—zero possibility. The calculation is facilitated by the software "Virtual Truffle Hunting" [61], which was created to speed up the calculation.

Table 2. The most common trees and shrubs species forming mycorrhiza with the Burgundy truffle (*Tuber aestivum* Vittad.) in Poland [14,17,19,21,23].

Trees and Shrubs Taxa	
Angiospermae	**Gymnospermae**
Betula pendula *Carpinus betulus* *Corylus avellana* *Fagus sylvatica* *Quercus* spp. *Populus* spp. *Tilia* spp. *Ulmus* spp.	*Abies alba* *Picea abies* *Pinus nigra* *Pinus sylvestris*

In 2015, a comprehensive method for finding truffle sites using "Virtual Truffle Hunting" was tested in 20 selected forest divisions in five macro-regions of Poland (Figure 1). The choice of area was based on the Forest Data Bank database [60]. The basic characteristics of 20 selected test sites are given in Table 3. At the selected sites, truffle fruiting bodies and accompanying hypogeous fungi were searched for from June to the end of October 2015 with the help of a trained dog. Additionally, the following parameters were recorded at each site:

- bedrock;
- chemical and granulometric composition analysis of soil samples (in the laboratory) [14];
- altitude;
- forest habitat type;
- the structure of the site and the undergrowth layer;
- description of trees and shrubs species that were associated with truffle mycorrhizae;
- information on the presence of orchids and indicator insects included in the method "Virtual Truffle Hunting".

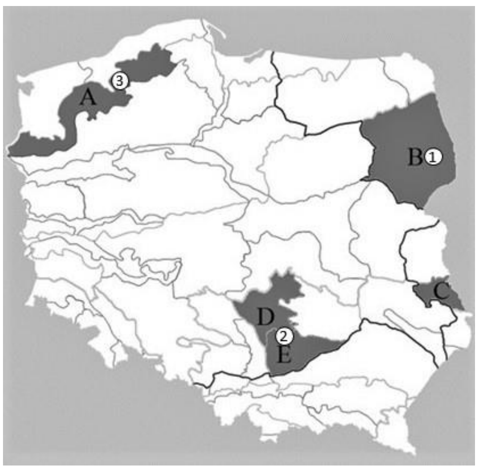

Figure 1. Location of five Polish macroregions where the "Virtual Truffle Hunting" method was tested (A—West Pomeranian Lake District, B—North Podlasie Lowland, C—Volhynia Polesie, D—Przedbórz Upland, and E—Nida Basin) and three meteorological stations (1—Białystok, 2—Jędrzejów, and 3—Szczecinek).

Table 3. Characteristics of 20 investigated forest sites (Fr. BF—fresh broadleaved forest, Fr. UBF—fresh upland broadleaved forest, Fr. MBF—fresh mixed broadleaved forest, Fr. UMBF—fresh upland mixed broadleaved forest).

Plot No.	Macroregion	Bedrock (Age)	Altitude (m.a.s.l.)	Tree Species Dominant (Age) and Accompanying Tree Species	Forest Habitat Type
1.	West Pomeranian Lake District	Glacial till (Weichselian glaciation)	20–75	*Fagus sylvatica* (155 yr.), *Quercus petraea*, *Acer pseudoplatanus*, *Pinus sylvestris*	Fr. BF
2.	West Pomeranian Lake District	Terminal moraine till (Weichselian glaciation)	135–158	*Fagus sylvatica* (148 yr.), *Carpinus betulus*, *Pinus sylvestris*, *Picea abies*	Fr. MBF
3.	West Pomeranian Lake District	Travertine (Late Carboniferous)	96–118	*Fagus sylvatica* (98 yr.), *Quercus robur*	Fr. BF
4.	North Podlasie Lowland	Sandur sand and gravel (Riss glaciation)	174–175	*Tilia cordata* (150 yr.), *Picea abies*	Fr. MBF
5.	North Podlasie Lowland	Glacial till (Riss glaciation)	179–180	*Picea abies* (175 yr.), *Quercus robur*, *Pinus sylvestris*	Fr. MBF
6.	Volhynia Polesie	Chalk (Late Cretaceous)	241–243	*Quercus robur* (98 yr.), *Carpinus betulus*, *Pinus sylvestris*	Fr. BF
7.	Volhynia Polesie	Chalk (Late Cretaceous)	219–230	*Quercus robur* (73 yr.), *Carpinus betulus*	Fr. BF
8.	Przedbórz Upland	Limestone (Late Jurassic)	295–320	*Quercus robur* (23 yr.), *Fagus sylvatica*, *Pinus sylvestris*, *Betula pendula*	Fr. UMBF
9.	Przedbórz Upland	Glacial till (Riss glaciation)	210–215	*Pinus sylvestris* (100 yr.), *Quercus robur*	Fr. BF
10.	Nida Basin	Gypsum (Miocene)	244–246	*Quercus petraea* (126 yr.), *Carpinus betulus*, *Tilia cordata*	Fr. BF
11.	Nida Basin	Gypsum (Miocene)	219–228	*Fagus sylvatica* (48 yr.), *Quercus petraea*, *Carpinus betulus*	Fr. BF
12.	Nida Basin	Marly limestone (Late Cretaceous)	301–303	*Quercus robur* (92 yr.), *Carpinus betulus*	Fr. UBF
13.	Nida Basin	Marly limestone (Late Cretaceous)	300–305	*Quercus robur* (84 yr.), *Pinus sylvestris*, *Carpinus betulus*	Fr. UBF
14.	Nida Basin	Marly limestone (Late Cretaceous)	297–301	*Acer pseudoplatanus* (32 yr.), *Tilia cordata*, *Carpinus betulus*	Fr. UBF
15.	Nida Basin	Lithothamnium limestones (Miocene)	291–295	*Quercus robur* (65 yr.), *Carpinus betulus*	Fr. MBF
16.	Nida Basin	Lithothamnium limestones (Miocene)	300–301	*Quercus robur* (70 yr.), *Carpinus betulus*	Fr. MBF
17.	Nida Basin	Lithothamnium limestones (Miocene)	294–295	*Carpinus betulus* (65 yr.), *Quercus robur*	Fr. MBF

Table 3. *Cont.*

Plot No.	Macroregion	Bedrock (Age)	Altitude (m.a.s.l.)	Tree Species Dominant (Age) and Accompanying Tree Species	Forest Habitat Type
18.	Nida Basin	Lithothamnium limestones (Miocene)	295–296	*Quercus robur* (77 yr.)	Fr. UBF
19.	Nida Basin	Marly limestone (Late Cretaceous)	251–252	*Quercus robur* (147 yr.)	Fr. UBF
20.	Nida Basin	Marly limestone (Late Cretaceous)	250–251	*Quercus robur* (147 yr.), *Tilia cordata*, *Carpinus betulus*, *Acer pseudoplatanus*	Fr. UBF

On the basis of information obtained in forest sites and the results of soil analyses, each of the 20 test areas was estimated for potential truffle presence in accordance with the proposed method for truffle typing site using "Virtual Truffle Hunting".

3. Results

The results of sites' valorization, as well as the scores for each parameter and the final assessment and the list of fruiting bodies of hypogeous fungi at each site, are presented in Tables 4–7. Based on the method used, two sites were classified as unfavorable for the growth of truffles (zero probability of occurrence) and there was no fruiting of truffles or other species of hypogeous fungi. Five sites were classified as sites with a low probability of truffle occurrence. However, fruiting bodies were not confirmed on only one of the sites. At the other four sites, fruiting bodies of the genera *Elaphomyces*, *Melanogaster* and *Genea* and two species of truffles (*Tuber ferrugineum* and *T. excavatum*) were observed. Most of the tested forest districts (8) obtained a score indicating an average probability of truffle occurrence. In each of these forest districts, fruiting bodies of *Tuber aestivum*, *T. excavatum*, and *T. rufum* were found. According to the method used, five areas were characterized by a high probability of truffle fruiting bodies occurrence. On each of them, fruiting bodies of Burgundy truffle were found (from 3 to 38 fruiting bodies).

Table 4. Characteristics of 20 investigated forest sites (Fr. BF—fresh broadleaved forest, Fr. UBF—fresh upland broadleaved forest, Fr. MBF—fresh mixed broadleaved forest, Fr. UMBF—fresh upland mixed broadleaved forest) (for sites 1–5).

Characteristics of Forest Sites	Site Number				
	1	2	3	4	5
Bedrock	Glacial till (0 pts)	Terminal moraine till (0 pts)	Travertine (2 pts)	Sandur sand and gravel (0 pts)	Glacial till (0 pts)
Soil pH in H_2O	7.8 (3 pts)	7.7 (3 pts)	7.7 (3 pts)	7.5 (3 pts)	5.3 (0 pts)
Ca [cmol(+)/kg]	18.87 (2 pts)	32.25 (3 pts)	45.59 (3 pts)	9.24 (1 pts)	1.63 (1 pts)
$CaCO_3$ [%]	7.23 (2 pts)	42.7 (3 pts)	60.24 (2 pts)	3.78 (2 pts)	0 (0 pts)
C/N	16.2 (2 pts)	22.1 (1 pts)	25.2 (1 pts)	13.7 (3 pts)	13.9 (3 pts)
Soil structure	Sandy loam (1 pts)	Sand (0 pts)	Loamy sand (0 pts)	Loamy sand (0 pts)	Loamy sand (0 pts)
Altitude (m.a.s.l.)	20–75 (3 pts)	135–158 (3 pts)	96–118 (3 pts)	174–175(3 pts)	179–180 (3 pts)
Forest habitat type	Fr. BF (3 pts)	Fr. MBF (2 pts)	Fr. BF (3 pts)	Fr. MBF(2 pts)	Fr. MBF (2 pts)
Forest structure	Open canopy closure, presence of gaps, paths or tracks (3 pts)	Moderate canopy closure (2 pts)	Full canopy closure, some shrubs are present (1 pts)	Open canopy closure, presence of gaps, paths or tracks (3 pts)	Full canopy closure, dense shrub layer (0 pts)

Table 4. *Cont.*

Characteristics of Forest Sites	Site Number				
	1	**2**	**3**	**4**	**5**
Tree and shrubs mycorrhizal partners of *Tuber aestivum*	More than 2 species in tree layer, presence of *Corylus avellana* (3 pts)	More than 2 species in tree layer, presence of *Corylus avellana* (3 pts)	At least 1species in tree layer and at least one tree or shrub species in lower forest layer (2 pts)	At least 1 species of tree or shrub in tree or shrub layer (1 pts)	More than 2 species in tree layer, presence of *Corylus avellana* (3 pts)
Orchids presence (*Cephalanthera*, *Epipactis* or *Cypripedium* genera)	1 species (*Cephalanthera damasonium*) (2 pts)	Not present (0 pts)	Not present (0 pts)	Not present (0 pts)	Not present (0 pts)
Insects associated with truffle	Not reported (0 pts)	Not reported (0 pts)	Not reported (0 pts)	*Odonteus armiger* (3 pts)	*Odonteus armiger* (3 pts)
Final note (possibility of occurrence)	26/36 (medium)	20/36 (low)	20/36 (low)	21/36 (low)	15/36 (none)
Presence of hypogeous fungi	*Rhizopogon roseolus* (1)	*Tuber ferrugineum* (1)	*Elaphomyces muricatus* (2)	*Melanogaster broomeianus* (19)	Not found

Table 5. Characteristics of 20 investigated forest sites (Fr. BF – fresh broadleaved forest, Fr. UBF—fresh upland broadleaved forest, Fr. MBF—fresh mixed broadleaved forest, Fr. UMBF—fresh upland mixed broadleaved forest), (for sites 6–10).

Characteristics of Forest Sites	Site Number				
	6	**7**	**8**	**9**	**10**
Bedrock	Chalk (3 pts)	Chalk (3 pts)	Limestone (3 pts)	Glacial till (0 pts)	Gypsum (3 pts)
Soil pH in H_2O	7.1 (2 pts)	7.2 (3 pts)	7.9 (3 pts)	4.3 (0 pts)	7.6 (3 pts)
Ca [cmol(+)/kg]	7.85 (1 pts)	12.72 (1 pts)	2.36 (1 pts)	0.83 (1 pts)	38.88 (3 pts)
$CaCO_3$ [%]	0.25 (1 pts)	0.17 (1 pts)	1.93 (2 pts)	0 (0 pts)	0.88 (1 pts)
C/N	13.3 (3 pts)	13.6 (3 pts)	11.6 (3 pts)	16 (2 pts)	10.9 (3 pts)
Soil structure	Sandy loam (1 pts)	Loamy sand (0 pts)	Loamy sand (0 pts)	Loamy sand (0 pts)	Loam (3 pts)
Altitude (m.a.s.l.)	241–243 (3 pts)	219–230 (3 pts)	295–320 (3 pts)	210–215 (3 pts)	244–246 (3 pts)
Forest habitat type	Fr. BF (3 pts)	Fr. BF (3 pts)	Fr. BF (3 pts)	Fr. BF (3 pts)	Fr. BF (3 pts)
Forest structure	Full canopy closure, some shrubs are present (1 pts)	Full canopy closure, some shrubs are present (1 pts)	Full canopy closure, dense shrub layer (0 pts)	Full canopy closure, dense shrub layer (0 pts)	Open canopy closure, presence of gaps, paths or tracks (3 pts)
Tree and shrubs mycorrhizal partners of *Tuber aestivum*	More than 2 species in tree layer, presence of *Corylus avellana* (3 pts)	More than 2 species in tree layer, presence of *Corylus avellana* (3 pts)	More than 2 species in tree layer, presence of *Corylus avellana* (3 pts)	More than 2 species in tree layer, presence of *Corylus avellana* (3 pts)	More than 2 species in tree layer, presence of *Corylus avellana* (3 pts)

Table 5. *Cont.*

Characteristics of Forest Sites	Site Number				
	6	7	8	9	10
Orchids presence (*Cephalanthera*, *Epipactis* or *Cypripedium* genera)	More than 1 species (*Cypripedium calceolus*, *Cephalanthera damasonium*) (3 pts)	1 species (*Cephalanthera damasonium*) (2 pts)	Not present (0 pts)	Not present (0 pts)	More than 1 species (*Cypripedium calceolus*, *Cephalanthera damasonium*) (3 pts)
Insects associated with truffle	Not reported (0 pts)	*Leiodes oblonga* (3 pts)	Not reported (0 pts)	Not reported (0 pts)	*Leiodes oblonga; L. cinnamomea* (3 pts)
Final note (possibility of occurrence)	24/36 (medium)	26/36 (medium)	21/36 (low)	12/36 (none)	34/36 (high)
Presence of hypogeous fungi	*Tuber excavatum* (17), *Genea verrucosa* (1), *Elaphomyces* sp. (1)	*Tuber rufum* (5), *Genea* spp. (4), *Hymenogaster* sp. (1)	Not found	*Elaphomyces* sp. (2)	*Tuber aestivum* (6)

Table 6. Characteristics of 20 investigated forest sites (Fr. BF—fresh broadleaved forest, Fr. UBF – fresh upland broadleaved forest, Fr. MBF—fresh mixed broadleaved forest, Fr. UMBF—fresh upland mixed broadleaved forest). (for sites 11–15).

Characteristics of Forest Sites	Site Number				
	11	12	13	14	15
Bedrock	Gypsum (3 pts)	Marly limestone (3 pts)	Marly limestone (3 pts)	Marly limestone (3 pts)	Lithothamnium limestones (3 pts)
Soil pH in H_2O	7.5 (3 pts)	5.6 (0 pts)	7.5 (3 pts)	7.5 (3 pts)	7.3 (3 pts)
Ca [cmol(+)/kg]	40.6 (3 pts)	20.78 (2 pts)	33.56 (3 pts)	30.35 (3 pts)	30.63 (3 pts)
$CaCO_3$ [%]	20.24 (3 pts)	0.33 (1 pts)	16.77 (3 pts)	38.04 (3 pts)	6.77 (3 pts)
C/N	16.3 (2 pts)	15.3 (2 pts)	17.4 (2 pts)	23.7 (1 pts)	12.7 (3 pts)
Soil structure	Sandy loam (1 pts)	Silt loam (1 pts)	Loam (3 pts)	Silt loam (1 pts)	Sandy loam (1 pts)
Altitude (m.a.s.l.)	219–228 (3 pts)	301–303 (3 pts)	300–305 (3 pts)	297–301 (3 pts)	291–295 (3 pts)
Forest habitat type	Fr. BF (3 pts)	Fr. UBF (3 pts)	Fr. UBF (3 pts)	Fr. UBF (3 pts)	Fr. MBF (2 pts)
Forest structure	Open canopy closure, presence of gaps, paths or tracks (3 pts)	Open canopy closure, presence of gaps, paths or tracks (3 pts)	Open canopy closure, presence of gaps, paths or tracks (3 pts)	Full canopy closure, some shrubs are present (1 pts)	Open canopy closure, presence of gaps, paths or tracks (3 pts)
Tree and shrubs mycorrhizal partners of *Tuber aestivum*	More than 2 species in tree layer, presence of *Corylus avellana* (3 pts)	More than 2 species in tree layer, presence of *Corylus avellana* (3 pts)	More than 2 species in tree layer, presence of *Corylus avellana* (3 pts)	More than 2 species in tree layer, presence of *Corylus avellana* (3 pts)	More than 2 species in tree layer, presence of *Corylus avellana* (3 pts)
Orchids presence (*Cephalanthera*, *Epipactis* or *Cypripedium* genera)	Not present (0 pts)	Not present (0 pts)	1 species (*Cypripedium calceolus*) (2 pts)	More than 1 species (*Cypripedium calceolus*, *Epipactis* sp., *Cephalanthera damasonium*) (3 pts)	More than 1 species (*Cypripedium calceolus*, *Epipactis* sp., *Cephalanthera damasonium*) (3 pts)

Table 6. *Cont.*

Characteristics of Forest Sites	Site Number				
	11	12	13	14	15
Insects associated with truffle	*Leiodes oblonga*, *L. cinnamomea* (3 pts)	*Leiodes oblonga* (3 pts)	*Leiodes oblonga* (3 pts)	*Leiodes oblonga* (3 pts)	Not reported (0 pts)
Final note (possibility of occurrence)	30/36 (medium)	24/36 (low)	34/36 (high)	30/36 (medium)	30/36 (medium)
Presence of hypogeous fungi	*Tuber aestivum* (38)	*Tuber aestivum* (7)	*Tuber aestivum* (29)	*Tuber aestivum* (3), *T. excavatum* (24)	*Tuber aestivum* (20)

Table 7. Characteristics of 20 investigated forest sites (Fr. BF—fresh broadleaved forest, Fr. UBF—fresh upland broadleaved forest, Fr. MBF—fresh mixed broadleaved forest, Fr. UMBF—fresh upland mixed broadleaved forest). (for sites 16–20).

Characteristics of Forest Sites	Site Number				
	16	17	18	19	20
Bedrock	Lithothamnium limestones (3 pts)	Lithothamnium limestones (3 pts)	Lithothamnium limestones (3 pts)	Marly limestone (3 pts)	Marly limestone (3 pts)
Soil pH in H_2O	7.3 (3 pts)	7.5 (3 pts)	7.5 (3 pts)	5.9 (1 pts)	7.4 (3 pts)
Ca [cmol(+)/kg]	45.3 (3 pts)	30.63 (3 pts)	28.02 (2 pts)	31.07 (3 pts)	46.37 (3 pts)
$CaCO_3$ [%]	17.76 (3 pts)	6.77 (3 pts)	3.14 (2 pts)	1.82 (2 pts)	14.62 (3 pts)
C/N	15.3 (2 pts)	13.0 (3 pts)	13.2 (3 pts)	12.1 (3 pts)	14.5 (3 pts)
Soil structure	Sandy loam (1 pts)	Loamy sand (0 pts)	Sand (0 pts)	Sandy loam (1 pts)	Loam (3 pts)
Altitude (m.a.s.l.)	300–301 (3 pts)	294–295 (3 pts)	295–296 (3 pts)	251–252 (3 pts)	250–251 (3 pts)
Forest habitat type	Fr. MBF (2 pts)	Fr. MBF (2 pts)	Fr. UBF (3 pts)	Fr. UBF (3 pts)	Fr. UBF (3 pts)
Forest structure	Open canopy closure, presence of gaps, paths or tracks (3 pts)	Open canopy closure, presence of gaps, paths or tracks (3 pts)	Open canopy closure, presence of gaps, paths or tracks (3 pts)	Open canopy closure, presence of gaps, paths or tracks (3 pts)	Moderate canopy closure (2 pts)
Tree and shrubs mycorrhizal partners of *Tuber aestivum*	More than 2 species in tree layer, presence of *Corylus avellana* (3 pts)	More than 2 species in tree layer, presence of *Corylus avellana* (3 pts)	At least 1species in tree layer and at least one tree or shrub species in lower forest layer (2 pts)	At least 1species in tree layer and at least one tree or shrub species in lower forest layer (2 pts)	More than 2 species in tree layer, presence of *Corylus avellana* (3 pts)
Orchids presence (*Cephalanthera*, *Epipactis* or *Cypripedium* genera)	More than 1 species (*Cypripedium calceolus*, *Epipactis* sp., *Cephalanthera* sp.) (3 pts)	1 species (*Cephalanthera damasonium*) (2 pts)	1 species (*Cephalanthera damasonium*) (2 pts)	1 species (*Cephalanthera damasonium*) (2 pts)	1 species (*Cephalanthera damasonium*) (2 pts)
Insects associated with truffle	Not reported (0 pts)	Not reported (0 pts)	Not reported (0 pts)	*Leiodes oblonga* (3 pts)	*Leiodes oblonga* (3 pts)
Final note (possibility of occurrence)	29 /36 (medium)	28/36 (medium)	26/36 (medium)	29/36 (medium)	34/36 (high)
Presence of hypogeous fungi	*Tuber excavatum* (14), *T. aestivum* (13)	*Tuber excavatum* (6), *T. aestivum* (1), *Genea* sp. (2)	*Tuber aestivum* (6)	*Tuber aestivum* (3)	*Tuber aestivum* (24)

4. Discussion

The selection of truffle sites based on soil and vegetation analysis works especially well with Burgundy truffle (*T. aestivum*) [17–19,32,33]. Based on data in the literature and our own research [3–6,14,40], it was possible to create a new, methodical tool for typing sites conducive to locating truffles in Poland.

It should be noted that the proposed method is particularly adapted at a national level as it takes into account geological formations typical of Poland, as well as native species of flora and entomofauna. The comprehensive method for selecting truffle occurrences using "Virtual Truffle Hunting" is a pioneering tool and has no counterpart in other countries. Compared with the GIS (geographic information system)-based methods developed for the identification of potential naturally-occurring truffle areas [62,63], the advantage of this methodology is that it is much easier to apply. It does not require a high level of computer skills and the availability of thematic maps in ruster or vector format, and can thus also be applied in areas that have not been well studied in terms of georeferencing and by people who are not experts in numerical cartography.

The effectiveness and efficiency of "Virtual Truffle Hunting" are determined by both the number and the variety of biotic and abiotic parameters selected to create the method. The multiplicity of environmental factors affecting the development of truffle fruiting bodies means that the developed method focuses on key factors. For example, as previously mentioned, truffles can fruit in soils with a granulometric composition significantly different from the preferred soils, as long as the soil has an optimal calcium content. The "Virtual Truffle Hunting" method allows balancing the estimation of parameters while taking into account their variability.

It should be emphasized that the selection of precipitations conducive to the development of truffle fruiting bodies can take place regardless of the meteorological conditions prevailing in a given season; however, the search for fruiting bodies on selected sites should be carried out only in years favorable for truffle development. Optimal conditions for verification of field typing should be considered in those years in which the growing season is preceded by mild winters. Heavy rainfall in summer (July–August) seems to be conducive to fructification of truffles, hence the best search date is the period from mid-July to the first frost [64].

The results obtained in our work, as well as the development of new research methods, allow us to point out favorable conditions for the development of truffles in forest stands. Thanks to new information on the environmental preferences of truffles and by combining the various methods of searching, it is possible to accurately indicate those regions in which environmental conditions favor the occurrence of these fungi. Identification of their sites can be multifaceted—based on geological data, soil, and meteorological analyses, as well as vegetation features.

Given the precipitations (Figure 2) and temperatures (Figure 3) typical of European countries, the differences are not as high as could be expected. During the period June through September, precipitations in Poland are at the similar level as precipitations in France (Burgundy region) [33]. When we compare the period January through May in Huesca [65], precipitations are much higher than in Poland. Hence, taking into account weather and plasticity of *T. aestivum*, we are of the opinion that this method with some implementations could be used outside of Poland.

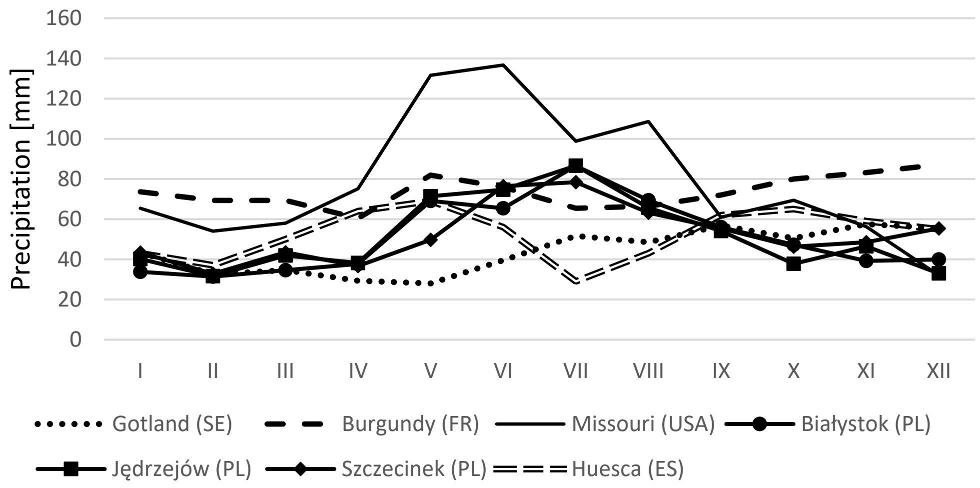

Figure 2. Monthly mean precipitation (long—term average) for the chosen localization of five countries [33,34,65,66].

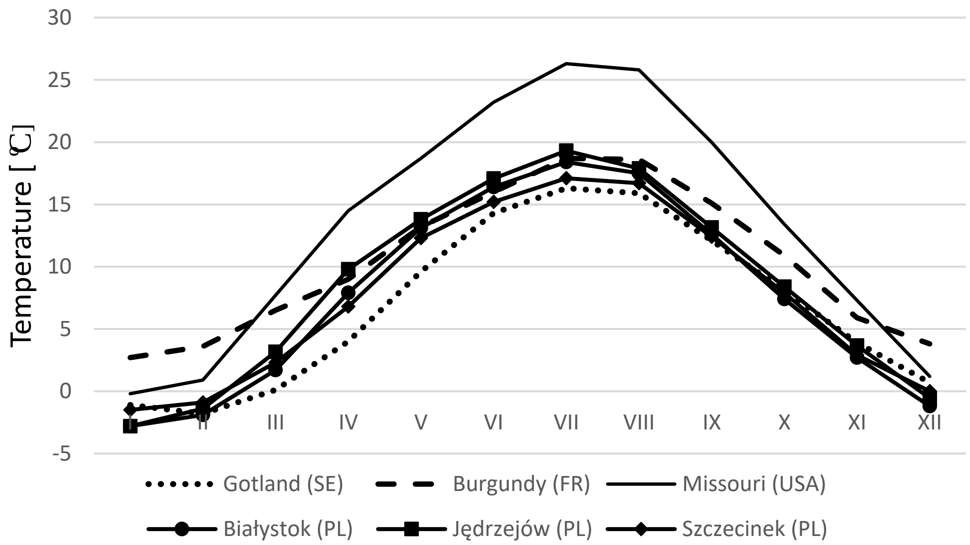

Figure 3. Monthly mean temperature (long—term average) for the chosen localization of countries [33,34,66].

Soil conditions conducive to the development of truffles in Poland can be found in stands located mainly in the Uplands of Southern and South-Eastern Poland: Silesia-Cracow, Lesser Poland, and Lublin-Lviv. The macroregion of the Nida Basin is considered to be particularly conducive to truffles owing to soil and climate conditions, similar to those prevailing in some regions of France and Italy [15]. However, potentially favorable conditions for truffle development also exist in some locations in the north of Poland. The comprehensive "Virtual Truffle Hunting" truffle typing method can be particularly useful in determining potential truffle sites in these regions of the country. For example, in the Szczecin Landscape Park "Beech Woods", although brown soils predominate, there are places, including sites in the vicinity of the Emerald Lake, with a soil rich in calcium carbonate, formed from chalk limestone. The geographical location and movement of atmospheric air masses from the Atlantic Ocean make this region, like the Nida Basin, one of the warmest in the country. There are some stands with photophilous xerothermic oak forest features and calciphilus plants [67], which are helpful in selecting truffle stands. It can thus be assumed that, despite many unique features, stands potentially conducive to the development of fruiting bodies of truffles are more numerous in Poland than was previously thought, and these valuable sites require discovery and examination.

5. Conclusions

Knowledge of the environmental requirements (especially soil) of truffles (*Tuber* spp.) has enabled the development of an effective tool for identifying new locations of these fungi in Poland. A comprehensive method of typing the truffle sites using "Virtual Truffle Hunting" has allowed us to indicate as many as 14 new forest divisions as the sites of four valuable truffle species (*Tuber aestivum*, *T. excavatum*, *T. rufum*, and *T. ferrugineum*). The "Virtual Truffle Hunting" method requires further estimation throughout Poland, which will probably allow us to widen the list of favorable sites in various parts of our country.

It seems that, owing to the similarities of weather conditions (rainfall and temperature) in France, Sweden, and Poland (Figures 2 and 3), this method may be used in some applications in Central and Northern Europe. We are of the opinion that this method should be tested especially in neighboring countries (Germany and Czech Republic), and we hope that, with this article, we encourage studies conducting discussions and providing suggestions for the method's development.

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