DOI: 10.6092/issn.2531-7342/10832

***Research article***

# Map of suitability for the spontaneous growth of *Tuber magnatum* in Emilia-Romagna (Italy)

Nicola Laruccia[1], Vittorio Marletto[2], Pamela Leonardi[3], Federico Puliga[3], Alessandra Zambonelli[3]

1 Regione Emilia-Romagna, Servizio programmazione e sviluppo locale integrato, viale della Fiera 8, 40127 Bologna, Italy
2 Struttura IdroMeteoClima, Arpae Emilia-Romagna, viale Silvani, 6 - 40122 Bologna, Italy
3 Dipartimento di Scienze e Tecnologie Agro-Alimentari, University of Bologna, via Fanin 44, 40127 Bologna, Italy

Corresponding author e-mail: nicola.laruccia@regione.emilia-romagna.it

**Article info**
Received 17/04/2020; accepted 10/06/2020
DOI: 10.6092/issn.2531-7342/10832

## Abstract
In this work we used an inductive and deductive approach to produce the map of Suitability for the Spontaneous Growth of *Tuber magnatum* (white truffle) (SSGT) in Emilia-Romagna region (northern Italy). This map was produced in order to identify the environments where appropriate actions should be applied to protect this threatened truffle species.
The steps used to define the map were: 1) surveying and mapping the actual productive areas (APAs) in two provinces of Emilia-Romagna (Bologna and Modena); 2) identification of some morphological, climatic, vegetational and pedological properties related to *T. magnatum* development; 3) production of digital maps representing the value that each property assumes in discrete land portions; 4) overlapping the map of APAs with each of the thematic maps and selection of the properties showing the strongest relationships with the presence of *T. magnatum*; 5) creating the map of SSGT, covering the whole hilly area of Emilia Romagna region; 6) assessing the reliability of the SSGT map, by overlapping the APAs map and the areas of the provinces of Parma and Piacenza where the mycelium of *T. magnatum* was found using specific PCR.
The relationships found by means of the inductive approach (comparison between selected properties and observed frequency of truffle) demonstrated its effectiveness in predicting deductively the areas with distinct suitability for truffle.

**Keywords**
white truffle; Italy; productive areas; map; soil mycelium detection; land suitability

## Introduction

Truffles are the hypogeous fungi belonging to the genus *Tuber* (Ascomycetes, Pezizales) (Zambonelli and Bonito, 2012). They live in mycorrhizal association with trees and shrubs in a wide range of habitats, including subtropical rain forests, temperate forests, boreal forests, floodplains, tree nurseries, restoration sites and Mediterranean woodlands (Bonito et al., 2010). Their ascomata have the spores sequestered in a more or less spherical mass and at maturity they have a strong aroma, which is used to attract animals for their spore dispersion (Trappe et al., 2009). The pleasant and unique aroma of some *Tuber* species makes them the most appreciated mushroom (Mello et al., 2006). *Tuber magnatum* Picco is the truffle that commands the highest prices, which ranges from 1,000 to 5,000 €/kg or more, depending on the ascoma size and the season (Zambonelli et al., 2020). It is principally found in Italy, for this reason it is also known as "the Italian white truffle" (Hall et al., 2007); recently it was also found in several countries of the Balkan region and in the countries nearby (Bratek et al.,

ISSN 2531-7342 Department of Agricultural and Food Science in partnership with the Italian Mycological Society (UMI).

DOI: 10.6092/issn.2531-7342/10832

2007; Riccioni et al., 2016) and, although more sporadically, in the south-east of France (Hall et al., 1998; http://www.ayme-truffe.com/en/truffes/tuber_magnatum.php) and Switzerland (Büntgen et al., 2019).

Currently, little is known about the complex interactions of *T. magnatum* with the host plant and its micro-ecological requirements. Consequently, its cultivation is not successful yet, unlike for the other precious European *Tuber* species (*Tuber melanosporum* Vittad., *Tuber aestivum* Vittad. and *Tuber borchii* Vittad.) (Hall et al., 2007). In the absence of reliable strategies for *T. magnatum* cultivation, it is important to safeguard the natural productive areas. An Italian ministerial commission of MIPAAFT (Ministry of Agricultural, Food and Forestry Policies and Tourism) has established a truffle-sector plan, which includes the inventory of *T. magnatum* natural productive areas and several measures for protecting them (https://www.politicheagricole.it/flex/cm/pages/ServeBLOB.php/L/IT/IDPagina/11100).

However, mapping all *T. magnatum* truffières is a difficult task because the truffle hunters keep the places where *T. magnatum* is found as jealously guarded secrets passed down from father to son, generation after generation (Hall et al., 2007). Although several attempts were made to map them (Tibiletti and Zambonelli, 2000; Zambonelli and Iotti, 2005), these inventories are not complete and include only the most well-known productive areas or the areas whose productivity has declined in the last years. Despite molecular methods to extract the DNA from soil and to assess the presence of DNA of *T. magnatum* in the soil, or even to quantify it, have been recently perfected (Zampieri et al., 2010; Iotti et al., 2012), these techniques are too expensive and time consuming to map the presence of this truffle at regional level. Their use is generally limited to verifying the presence of truffle in a determined area or to assess the effects of cultivation practices on its development in the soil (Salerni et al., 2014; Iotti et al., 2014, 2018).

Emilia-Romagna region [Nomenclature of territorial units for statistics, level 2 (NUTS 2)] is particularly rich with truffles. All the most precious truffle species that, according to the National Law N. 753/1985, can be harvested and traded in Italy, are present in Emilia-Romagna (Zambonelli and Iotti, 2005). Although *T. magnatum* grows almost everywhere across the region, it is more widespread in broad-leaved forests of hills and low mountains. In the plain areas it is quite rare, because ectomycorrhizal plants are present almost only in urban habitats (e.g. parks, road trees) or in private gardens (Zambonelli and Morara, 1984).

Emilia-Romagna region is located in the northern part of Italy; it consists of nine provinces (NUTS 3: Bologna, Ferrara, Forlì-Cesena, Modena, Parma, Piacenza, Ravenna, Reggio Emilia and Rimini) and covers an area of 22,446 km$^2$. It can be divided into three large environments: the Apennine range, the Po valley and the Adriatic coast: to the east it is bordered by the Adriatic Sea for about 130 km. Nearly half of the region (48%) consists of plains (minimum elevation -3 m) while 27% is hilly and 25% mountainous (maximum elevation 2,165 m). Above about 1,000 m altitude, sandstones and flysches prevail, from which non calcareous soils [Inceptisols, Entisols and Alfisols (Soil Survey Staff, 2014)] have developped. In the hilly and low mountain areas, more or less stratified rocks, often deformed by tectonics and generally calcareous (flysches, clays, marls and sandstones), prevail. In this environment, erosive processes (sheet erosion and landslides) are widespread and rejuvenate soils [Entisols and Inceptisols (Soil Survey Staff, 2014), generally calcareous]. The alluvial plain was recently formed and still the floods continue to rejuvenate the soils [Inceptisols, Entisols and Vertisols (Soil Survey Staff, 2014), generally calcareous (Regione Emilia-Romagna, 1994, 2018)].

Average annual temperatures of Emilia-Romagna range from about 8 °C on the highest mountains to about 14-15 °C in the plain. In winter intense frosts are common, even in the plains, especially in the areas away from the Adriatic Sea, where moreover the highest summer temperatures occur. Annual total precipitation varies from more than 2,000 mm in the highest mountains to less

DOI: 10.6092/issn.2531-7342/10832

than 600 mm in the inland eastern areas of the Romagna plain. The distribution of rainfall shows a main peak in autumn and a secondary peak in spring. The summer water deficit reaches its highest values in the plains and gradually decreases with increasing altitude (Antolini et al., 2017).

Considering the difficulties and limits of mapping the Actual Productive Areas (APAs), the map of Land Suitability (FAO, 1976) for the Spontaneous Growth of *Tuber magnatum* (SSGT) may be more inclusive in order to identify the areas where the ecological conditions are more suitable for *T. magnatum* growth and hence to extend the protective measures to the areas where a high probability of truffle presence is expected. In this work a hybrid inductive and deductive approach was applied to create the SSGT map of Emilia-Romagna region.

## Material and methods

### *Georeferenced information and related databases*

The maps of *T. magnatum* APAs for the provinces of Bologna and Modena used in this study were produced in 2000 and 2003, respectively (Zambonelli unpublished data, Zambonelli and Iotti, 2005). The APAs were indicated by truffle hunters and surveillance agents during various field inspections and delineated on a high-scale topographic map (CTR 1:10,000). The APAs were successively digitalized in ESRI shapefile format.

The geographic and alphanumeric information relating to the main environmental factors, used in this study as basic knowledge, was: i) the regional soil map at 1:50,000 scale (Regione Emilia-Romagna, 2018), which covers 71% of the regional territory, and the soil map at 1:250,000 scale (Regione Emilia-Romagna, 1994), covering the whole regional territory; for both maps the information on soil properties and their distribution in each map unit is stored in a MS Access database, consisting of several related tables; ii) a climatic data set of Emilia-Romagna, which consists of daily records of the main meteorological data, starting from 2001 until 2015, referred to 1,024 regular cells with side of 5 km. This dataset was chosen because it derives from interpolation of recent data from local weather station and accounts for topography as well as for the urban share (ERG5 grid, Antolini et al., 2016); iii) the Digital Elevation Model (DEM) of the regional territory (ESRI Grid format, 10×10 m resolution, available at http://geoportale.regione.emilia-romagna.it/it/download/prodottiraster); furthermore, many ancillary rasters had previously been derived from DEM, namely Slope Factor, Aspect, Topographic Position Index, (https://www.arcgis.com/home/item.html?id=b13b3b40fa3c43d4a23a1a09c5fe96b9), Curvature (http://www.saga-gis.org/saga_tool_doc/2.2.6/ta_morphometry_0.html), Morphological Uneveness Index (Generali M., personal communication), Topographical Wetness Index (http://www.saga-gis.org/saga_tool_doc/3.0.0/ta_hydrology_20.html), Flow Accumulation (http://resources.esri.com/help/9.3/arcgisdesktop/com/gp_toolref/spatial_analyst_tools/flow_accumulation.htm) iv) the forestry map of Emilia-Romagna region (1:10,000 scale) (https://ambiente.regione.emilia-romagna.it/it/parchi-natura2000/foreste/quadro-conoscitivo/sistema-informativo-regionale/cartografia-interattiva-foreste). For each forest type, the two main wooden species (trees or shrubs) are reported, along with some ancillary information.

Except for the DEM and the derived rasters, all the above listed digital maps were available in vector format (ESRI shapefile).

### *Data processing*

All the geographical data, projected and represented in the same Reference System (EPSG:4265), were managed in a GIS environment (ESRI ArcGis 10.3).

The maps of APAs, of soils, of climate and of forestry were converted to raster format with the same cell size (10×10 m) and alignment as the DEM. A filter was applied in order to exclude the

DOI: 10.6092/issn.2531-7342/10832

cells: i) not covered by the forestry map; ii) with elevation ≥ 975 m asl, where *T. magnatum* in Emilia-Romagna is absent (Zambonelli and Morara, 1984; Tibiletti and Zambonelli, 2000); iii) falling within the Po plain, where no topographical analysis could be conducted. The total number of cells analysed amounted to 51,146,887, corresponding to 5,115 km$^2$.

Based on the available literature and on the Authors' personal knowledge on truffle ecology, some morphological (slope, slope curvature, aspect, Topographic Position Index, Topographic Wetness Index, Flow Accumulation Index), climatic (Continentality and Summer Water Stress indexes), vegetational (host plants) and pedological properties (reaction, calcium carbonate content, texture, drainage class), supposed to affect the presence of *T. magnatum*, were selected for further analysis (Zambonelli and Morara, 1984; Tibiletti and Zambonelli, 2000, Zambonelli and Iotti, 2005; Bragato et al., 2006; Hall et al., 2007). When the selected properties were not directly available, they were derived from the primary data.

As far as concerns the climatic indixes, according to data availability, it was only possible to calculate them for the ERG5 cells, which have a very low geographical resolution (5×5 km). In order to allow an analysis of climate as detailed as the other factors considered, we developed an original downscaling procedure to carry the climatic indexes calculated for the ERG5 cells down to the level of every single 10×10 m cell. The downscaling procedure involved the selection of the cells from the ERG5 grid falling within, or immediately surrounding, each province. The selected cells had to honour these requirements: i) average elevation between 30 and 1,400 m; ii) urban share <20%. For each province and for each of the selected climatic indexes, by means of polynomial regression analysis we found the local relationship between elevation and index. Known the difference in elevation between a given 10×10 m cell and the 5x5 km ERG5 cell within which it falls (Δe), the value of a given index for the 5×5 km ERG5 cell was downloaded to the 10×10 m cell, adding or subtracting a corrective value (Δi) to the index. Δi was calculated based on the same relationship previously found, but using, as indipendent variable, Δe instead of the elevation. The sign of Δi depends on the sign of Δe.

Values for the selected properties were assigned to each cell of the whole dateset.

As final outcome of data processing in the GIS environment, a dbf table was realized, which contains, for each cell of the whole dataset, all the proprieties assigned as described above, as well as the Boolean information on presence/absence of *T. magnatum* derived from the APAs map, limited to the subset of 12,752,436 cells falling within the provinces of Bologna and Modena.

This table was imported in a new MS Access database, where data were processed and analized (the database is deposited in an Emilia-Romagna Region's server). The values of continuous properties were subdivided in discrete classes, the number of which depending mainly on the range of values.

In the subset covering the provinces of Bologna and Modena, where the APAs maps were available, alphanumerical elaborations and statistical monovariate analyses were performed in order to find, for each environmental factor, the properties (single or combined in indexes) showing the strongest relationships with the observed presence of *T. magnatum*.

To assess the strength of the relationship for each property, the observed frequency (*OF*) of truffle (see below) was plotted vs the central value of each class or for the value of each index. Indexes could indeed assume only few discrete values, as they were derived from combinations of previously classified individual properties. The coefficient of determination ($R^2$) of the value-*OF* relationship (usually derived from non-linear regression and expressed as a polynomial equation) was adopted as indicator of the strength of the property in describing truffle distribution.

The observed frequency of truffle was calculated as:

$$OFp_i = \frac{tAp_i}{TAp_i} \times 100$$

DOI: 10.6092/issn.2531-7342/10832

where $OFp_i$ is the observed frequency of truffle for value *i* of property *p* (as percentage); $TAp_i$ is the total area for value *i* of property *p*; $tAp_i$ is the area occupied by truffle (according to APAs map) for value *i* of property *p*.

The relationships between environmental properties and *OF*, detected in the provinces of Bologna and Modena (where APAs maps were available), were subsequently used as predictive tools to assess the suitability for truffle growth, expressed in terms of expected frequency (*EF*), in the whole study area, i.e. in each of the 51,146,887 selected cells. The *EF* of a cell corresponds to the expected probability of finding *T. magnatum* in it. The *EF* of a cell was calculated as the average of the expected frequency for each property assigned to the cell:

$$EF = \frac{\Sigma EFp}{Np}$$

where *EF* = overall expected frequency; *EFp* = expected frequency for property *p*; *Np* = number of properties.

If for at least one of the pedological, climatic or morphological properties the *EFp* value resulted equal to 0, the cell was considered unsuitable for truffle, even if all the other properties were favorable for the truffle growth.

### *Soil sampling and DNA extraction*

In the provinces of Parma and Piacenza not covered by APA maps, truffle hunters indicated 35 points (small patches of around 8-10 $m^2$) in various areas where they found ascomata of *T. magnatum* (Fig. 1a). These areas were located in 13 municipalities of Piacenza province (24 areas) and in 7 municipalities of Parma province (11 areas). For each productive point, nine soil cores along two perpendicular lines were taken, using 1.6 cm diameter disposable polyvinyl tubes (Fig. 1b). One extra soil core was also taken from a nearby agricultural field as control. Soil cores were stored at 4 °C and extracted by breaking the tubes within 24 h from sampling. Soil samples from the same productive point were thoroughly mixed. Organic (mainly roots) and coarse fragments were removed. The soil was kept frozen at -80 °C until lyophilization (70 h in a Virtis Benchtop 2-K freeze dryer, SP Industries) and later grounded and stirred in a mortar, sieved (1 mm mesh), and then stored at 4 °C until DNA extraction.

Soil DNA was extracted after Iotti et al. (2012), adapted for 1 g of soil. Crude DNA solutions were then purified using the Nucleospin Plant II kit (Macherey-Nagel, Düren, Germany) following the manufacturer's instructions. Total DNA was quantified by means of a NanoDrop ND-1000 Spectrophotometer (Thermo Scientific) and their quality evaluated by means of an optical density (OD) 260/280 nm and 260/230.

### *Assessment of the presence of* **T. magnatum** *mycelium in the soil*

A nested PCR approach was used to detect *T. magnatum* mycelium in the soil. The soil DNAs were firstly amplified with the fungal ITS universal primers ITS1f/ITS2 (White et al., 1990; Gardes and Bruns, 1993). The mix was composed of 10× buffer (2 ml), 2.5 mM dNTPs (1.5 ml), 10 mM of each primer (0.5 ml), water (11.6 ml) and Red Taq (Sigma) (1.4 ml). DNA (25 ng) was then added and the PCR was run with an initial denaturation at 94 °C for 4 min, followed by 30 cycles of 94 °C for 45 s, 50 °C for 45 s and 72 °C for 90 s, with a final extension at 72 °C for 5 min.

The second PCR round was performed using the specific ITS primer pair for *T. magnatum* TmgI-TmgII (Amicucci et al., 1998). The mix contained 10× buffer (2.5 ml), 2.5 mM dNTPs (2 ml),

DOI: 10.6092/issn.2531-7342/10832


Fig. 1 – Ascomata of *T. magnatum* (a), soil sampling (b).

10 mM of each primer (0.8 ml), water (16.7 ml), Red Taq 1 U (Sigma) (1.2 mL) and DNA template (1 ml of the first PCR product). PCR reactions were performed with the following conditions: 6 min at 94 °C followed by 34 cycles of denaturation at 94 °C for 30 s, annealing at 50 °C for 30 s, extension at 72 °C for 30 s and a final extension at 72 °C for 5 min.

The PCR were carried out using a SimpliAmp thermal cycler (ThermoFisher) and PCR products were run on 2% agarose gel and visualized by staining with ethidium bromide.

## Results and discussion

### *Pedological, morphological, climatic and vegetational properties*

*Soil indexes*

*Tuber magnatum* prefers deep, fresh but well drained, alluvial or colluvial soils, with calcareous and soft topsoil (Bragato et al., 2006). For this reason, the calcium carbonate content/reaction and the texture in the first 30 cm, as well as the drainage class, were chosen as the properties supposed to mostly affect the truffle presence. The soil descriptions linked to the regional soil maps were used as the sources for this information (Regione Emilia-Romagna, 2018). The drainage class showed a poor correlation with the OF, while both the reaction/calcium carbonate content and the texture resulted to be effective in describing the truffle distribution. We found out that the best relationship was achieved combining in a single soil index these two properties: i) the texture class (Soil Survey Staff, 2004) and ii) the combination of empirical classes of calcium carbonate content and of reaction. The empirical classes reflect the actual ranges for soil units described by the regional soil maps.

Each property has been assigned an empirical coefficient, based on the degree of matching with truffle requirements (Table 1).

DOI: 10.6092/issn.2531-7342/10832

**Table 1** - Classes of selected soil proprieties and coefficients, based on the degree of matching with truffle requirements

| **Calcium carbonate content and pH** | **Coefficient** |
|---|---|
| Strongly calcareous (total calcium carbonate >10%) | 1 |
| Calcareous (total calcium carbonate 5-10%) | 0.87 |
| Weakly calcareuos (total calcium carbonate 0-5%) and pH 6.3-7.8. This class was also assigned to highly variable soils | 0.14 |
| Non calcareous and acid (pH 4.5-6) | 0 |
| **Texture classes (Soil Survey Staff, 2004)** | **Coefficient** |
| Sandy Loam | 1 |
| Loam | 0.89 |
| Sandy Clay Loam, Clay Loam, Silty Clay Loam | 0.83 |
| Silty Clay, Sandy Clay, Clay | 0.26 |
| Sand, Loamy Sand | 0 |

The idoneity index of a soil was calculated adding up the values of the coefficients for the two considered properties. If a coefficient had a value of 0, the soil index was set to 0, regardless of the value of the other coefficient. Each soil map unit, due to the low resolution of the available maps, may consist of several soil units, sometimes with very contrasting properties, so we had to choose a method to assign an unique value to the map unit. We found that assigning to each map unit the highest value among the indexes of the individual soil units (after having excluded those occupying less than 10% of the map unit surface) gave the best relationship with the truffle distribution.

In Figure 2a the relationship between the soil index and the observed frequency in the provinces of Bologna and Modena is shown.

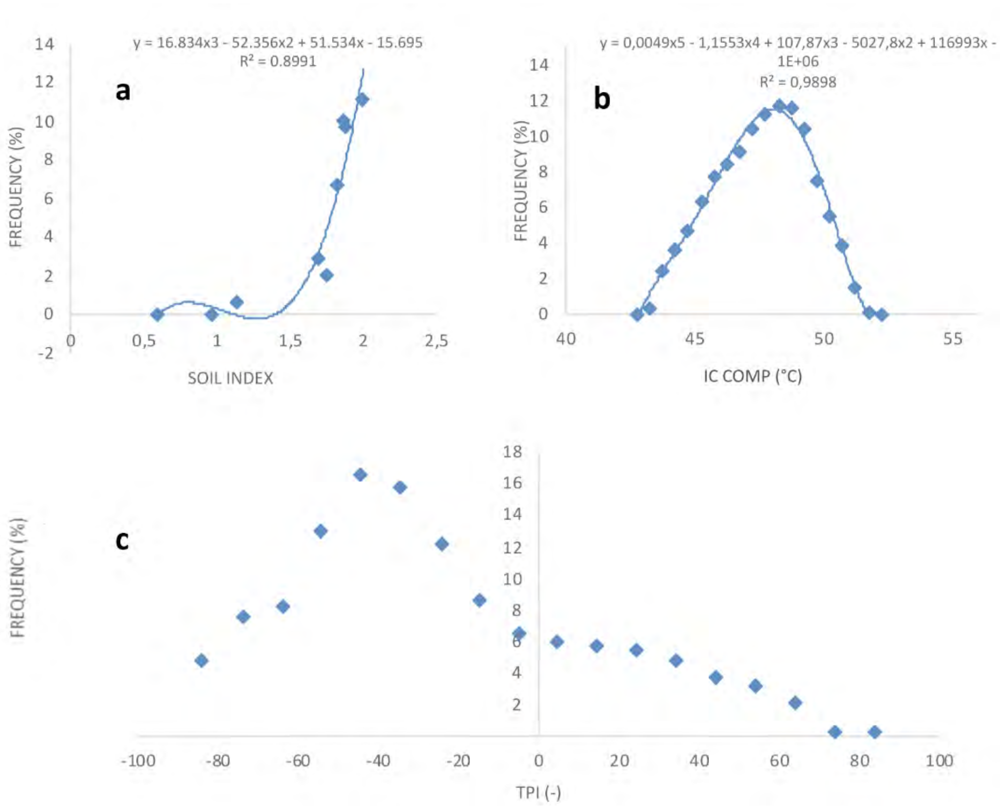


**Fig. 2** - Relationship between pedological, climatic and morphological properties and observed truffle frequency: soil index (a), new compound continentality index (CI_comp) (b), topographic position index (TPI) (c).

DOI: 10.6092/issn.2531-7342/10832

*Morphological indexes*
All the available morphometric properties (namely slope, slope curvature, aspect, Topographic Position Index, Topographic Wetness Index, Fow Accumulation Index) were analyzed. As already stated, *T. magnatum* prefers fresh, calcareous and very porous, highly aerated soils. Where the highly interconnected macroporosity allows this hypogeous fungus to "breathe" and its ascomata to grow in size without physical constraints (Bragato et al., 2004). In the Appennines, these conditions were expected to occur in micro habitats, located on the north-facing slopes and at the feet of slopes, where the accumulation of colluvial deposits, transported by the action of gravity and water, "rejuvenate" the soil, supplying fresh (calcareous) and soft materials.

Despite expectations, the slope aspect did not show any significative correlation with truffle distribution, nor taken by itself neither in combination with other properties, such as elevation or slope factor. The best indication for truffle presence ($R^2$ = 0.9107, with a 5th degree polynomial equation) was given by the Topographic Position Index (TPI).

The Topographic Wetness Index (TWI, adopted by Rellini et al., 2011) was not as strictly correlated to *T. magnatum* as the TPI. Two different algorithms (Tarboton, 1997) were considered in calculating the TWI: a) the D-8 model, considering the direction of flow (and consequently the accumulation of the flow itself) all distributed in the downstream cell towards which there is the greatest slope (TWI_D8); b) the D-Inf model, considering the flow direction (and consequently the accumulation of flow itself) distributed continuously towards the downstream cells as a function of the slope towards each of these (TWI_Dinf). For each cell, the contributing area is calculated adding the contribution of all the 8 surrounding cells.

The shape of the curve of truffle frequency vs TWI_D8 showed some unexplained irregularity, which resulted in a poor reliability of the best regression found (6th degree polynomial equation), despite a high $R^2$ (0.90). The relationship between truffle frequency and TWI_Dinf showed a very clear linear trend, but $R^2$ (0.82) was low if compared to the TPI's one (Fig. 2c).

The TPI measures the relative topographic position of the central cell as the difference between its elevation and the mean elevation within a predetermined neighbourhood (in this study, a radium of 250 m).

Positive TPI values indicate that the cell has an elevation higher than the average of the surrounding cells, while negative values indicate a position lower than the average of the latter.

The highest truffle presence was found in areas with TPI ranging between -50 e -30, which correspond with the areas localized at the foot of the slopes.

Although the relationship between the TPI values and the frequency of the truffle follows a clear and very evident trend, the shape of the regression curve seems to derive from the superposition of two different curves (Fig. 2c). This trend is suggestive of a complex relationship between TPI and APAs, worthy of further investigation.

For this reason we preferred to calculate the expected frequency adopting discrete values for classes of TPI, rather than using a continuous function (Table 2).

*Bioclimatic indexes*
Several bioclimatic indexes were used in previous studies to assess the suitability for *T. magnatum*. For instance, in Abruzzo region (central Italy) monthly cold stress, monthly water stress, summer deficit stress, continentality, thermicity index, yearly ombrothermal index, summer ombrothermal index, potential evapotranspiration were adopted (De Laurentis and Spinelli, 2009).

In this study, we chose to evaluate only the continentality and the summer water stress indexes as, taken together, they can be considered representative of the climatic conditions to which the truffle is particularly sensitive. The continentality is an excellent indicator of the thermal field, as it accounts

DOI: 10.6092/issn.2531-7342/10832

for both the maximum and minimum temperatures during the year: in environments where extreme heat and/or severe frosts occur the growth of the truffle is hindered (Zambonelli et al., 2012). The summer water deficit is well known as a condition that strongly affects the possibility of growth or production of the truffle (Iotti et al., 2018).

**Table 2 -** Observed frequency of truffle in areas with different Topographic Position Index (TPI) in the APAs

| TPI values | Observed frequency (%) |
|---|---|
| TPI < -90 | 0.0 |
| -90 ≥ TPI < -80 | 4.8 |
| -80 ≥ TPI < -70 | 7.5 |
| -70 ≥ TPI < -60 | 8.3 |
| -60 ≥ TPI < -50 | 13.0 |
| -50 ≥ TPI < -40 | 16.5 |
| -40 ≥ TPI < -30 | 15.7 |
| -30 ≥ TPI < -20 | 12.2 |
| -20 ≥ TPI < -10 | 8.7 |
| -10 ≥ TPI < 0 | 6.5 |
| 0 ≥ TPI < 10 | 6.0 |
| 10 ≥ TPI < 20 | 5.8 |
| 20 ≥ TPI < 30 | 5.5 |
| 30 ≥ TPI < 40 | 4.8 |
| 40 ≥ TPI < 50 | 3.7 |
| 50 ≥ TPI < 60 | 3.2 |
| 60 ≥ TPI < 70 | 2.2 |
| 70 ≥ TPI < 80 | 0.3 |
| 80 ≥ TPI < 90 | 0.3 |
| TPI ≥ 90 | 0.0 |

The analysis carried out found that the relationship between the presence of truffles and the summer stress index was poor, while the relationship with the continentality indexes was strong. The continentality indexes seek to express the amplitude of the annual temperature oscillation.

Among the various continentality indexes proposed by Rivas Martinez (2008), the following were tested:

1. **Simple**: averaged difference between the mean temperature of the hottest month and the coldest one;
2. **Amplified**: averaged difference between the mean absolute maximum temperatures of the hottest month and the mean absolute minimum temperatures of the coldest month.

We observed that truffle frequency is well correlated with both indexes, but it is more correlated with the sum of the two. Hence, we assumed this new compound continentality index as the best climatic indicator (CI_comp).

The CI_comp values have been grouped in classes and the relationship with the observed truffle frequency has been formalized by means of a non-linear regression (Fig. 2b).

In the context of Emilia-Romagna region, the continentality indexes indeed resulted very effective in differentiating the climatic conditions, with respect to temperature, between the areas of internal plain (where a really continental climate is recognized, with very high summer temperatures

DOI: 10.6092/issn.2531-7342/10832

and intense winter frosts) from the montaneous areas, where the lower thermic oscillation is due to altitude and winds effectively removing the thermal inversion conditions that are very common in the plains, especially in winter. The optimum values are found in hilly and low-mountainous areas of the Appennine where, in addition, summer water deficit is moderate.

*Vegetation indexes*

*Tuber magnatum* is a hypogean mushroom that lives in symbiosis with the roots of some tree plants. The presence of specimens of these plants is an essential condition for the life of the truffle. The following plants, recorded in the forestry map of Emilia-Romagna region, were considered as the symbiotic plants of *T. magnatum* (Hall et al., 2007): *Carpinus betulus* L., *Corylus avellana* L., *Ostrya carpinifolia* Scop., *Populus alba* L. , *Populus nigra* L., *Populus tremula* L., *Quercus cerris* L., *Quercus frainetto* Ten., *Quercus ilex* L., *Quercus petreae* (Matt.) Liebl., *Quercus pubescens* Willd., *Quercus robur* L., *Salix alba* L., *Salix caprea* L., *Tilia cordata* Mill., *Tilia platyphyllos* Scop. and *Tilia x vulgaris* Hayne.

As expected, forest types in which, according to the forestry map, at least one of the 2 main species is symbiont show on the average a higher frequency of the truffle. However, the frame is not at all homogeneous: as can be seen in Table 3, in the woods with downy oak (*Q. pubescens*) or black hornbeam (*O. carpinifolia*) the greatest occurrence of truffle is observed. Conversely, in the woods with poplars (*P. nigra, P. alba, P. tremula*), white willow (*S. alba*), Turkey oak (*Q. cerris*) and other species with less diffusion, the frequency is comparable or even lower than the average frequency of truffle (Table 3).

**Table 3** - Total area occupied by host plants; absolute and relative area occupied by *T. magnatum*

| **Species** | **Total surface occupied (ha)** | **Sup. with *T. magnatum* (ha)** | **Sup %** |
|---|---|---|---|
| *Q. pubescens* | 60,765 | 5,725 | 9.4 |
| *Q. cerris* | 36,349 | 2,507 | 6.9 |
| *O. carpinifolia* | 35,148 | 3,700 | 10.5 |
| *P. nigra* | 7,413 | 303 | 4.1 |
| *S. alba* | 5,379 | 141 | 2.6 |
| *Quercus* spp. (oaks lacking further specifications) | 1,039 | 199 | 19.1 |
| *P.tremula* | 940 | 31 | 3.3 |
| *P. alba* | 744 | 49 | 6.6 |
| *C.avellana* | 504 | 11 | 2.1 |
| *Q.robur* | 473 | 3 | 0.6 |
| *C.betulus* | 246 | 0.4 | 0.16 |
| *S. caprea* | 229 | 16.5 | 7.2 |
| *Q. petreae* | 61 | 0.17 | 0.3 |
| *Q. ilex* | 58 | 1.1 | 1.9 |
| *Tilia x vulgaris* | 25 | 0 | 0 |
| *Q. frainetto* | 23 | 2 | 8.4 |
| *T. platyphyllos* | 14 | 0 | 0 |
| *T. cordata* | 3 | 0.02 | 0.6 |

DOI: 10.6092/issn.2531-7342/10832

Differently from the generalization procedure adopted for the other factors considered, the forest types present in the map have not been assigned an expected frequency that takes into account the observed frequency for the individual species or for combinations of the two main species reported in the map legend. Instead, it was decided to assign the observed frequency (Table 3) only to the types of the map in which a symbiont species is present. The other types were assigned a value of expected frequency equal to 0.

***Realization of the map of SSGT of Emilia-Romagna region***

As already stated, to realize the SSGT map for the whole regional territory, the relationships between observed truffle frequency and environmental properties, found for the provinces of Bologna and Modena (where APAs maps were available), were applied to the 51,146,887 10×10 m selected cells.

The overall expected frequency (*EF*) corresponds to the average of the 4 *EFp* values (one value for each of the selected properties). If one or more of these values (with the exception of the vegetation-dependent one) assumes a value of 0, the corresponding property is considered as a limiting factor to the point of absolutely preventing the growth of the truffle.

The Suitability classes were defined based on the *EF*, as given in Table 4. Unlike the classic FAO's approach (FAO, 1976), according to which a certain potential yield corresponds to each class of suitability, for the peculiarity of the truffle (which is "searched") it is more significant to express the suitability of a place in terms of probability to find it (*EF*) rather than in terms of predicted yield. In Fig. 3a is reported an example of the resulting SSGT maps.

**Table 4 -** Total area of SSGT classes, expected frequency (EF) and % of the whole study area

| Suitability class | EF | Sup. (ha) | Sup. % |
|---|---|---|---|
| S1 –Very highly suitable | >10% | 7,002 | 5.5 |
| S2 – Highly suitable | 7-10% | 60,598 | 47.5 |
| S3 – Moderately suitable | 5-7% | 42,302 | 33.2 |
| S4 – Marginally suitable | 2-5% | 3,498 | 2.7 |
| N – Not suitable | <2% | 14,124 | 11.1 |

***Detection of* T. magnatum *mycelium in the soil and SSGT map validation***

A first attempt to validate the SSGT map was carried out comparing ex-post the SSGT map with the APAs map available for the provinces of Modena and Bologna (Fig. 3b). The comparison was carried out overlapping the two maps and calculating the share of each SSGT class where *T. magnatum* had been found (according to the APAs map). The results, reported in Table 5, show that the SSGT map has an excellent capacity to define areas with different attitudes to truffle production.

Furthermore, the points where the *T. magnatum* mycelium was found in the provinces of Parma and Piacenza were also overlapped to the SSGT map. Only for samples 8, 9 and 10 this was not possible because they were located in flat areas, not covered by the SSGT map (Table 6). These field points, although deriving from a less systematic survey, are more reliable than the APAs of the provinces of Bologna and Modena, since in the former the presence of mycelium was verified by DNA testing.

As reported in the Table 6, *T. magnatum* mycelium was detected in most of the soil samples (27 out of 35) collected in the areas of Piacenza and Parma provinces where production of *T. magnatum* ascomata in the last two years was referred. *T. magnatum* mycelium was never amplified in soil samples collected in the control areas. The field points where ascomata were declared by truffle hunters not to be found in recent years seem to have lost the presence of *T. magnatum* mycelium

DOI: 10.6092/issn.2531-7342/10832

(samples 11, 34 and 35). *T. magnatum* mycelium was not found also in the field points indicated with high uncertainty by truffle hunters (samples 12, 23 and 24) (Table 6).

These results confirm that the detection of the presence of *T. magnatum* DNA in the soil is a reliable technique to verify the actual productivity of an area. *T. magnatum* mycelium forms broad patches in the soil which can extend up to 100 m far from the host plant (Zampieri et al., 2010). In these patches the ascomata are formed in different positions in subsequent years. However, the mycelial biomass tends to concentrate in the soil surrounding the fruiting body and has a decreasing trend going away from this point (Iotti et al., 2014). Moreover, it is subject to seasonal fluctuation depending on the soil temperature and moisture (Iotti et al., 2018). Prolonged drought or soil disturbance can cause the disappearance of *T. magnatum* mycelium, leading to loss of productivity.

All the points where *T. magnatum* mycelium was recently found correspond to areas of the classes S3 (Moderately suitable; 8 areas), S2 (Highly suitable; 15 areas) or S1 (Very highly suitable: 1 area). The low number of samples falling in the S1 class reflects the low percentages of areas of this class in the provinces of Parma and Piacenza (2%). In these provinces the productivity of *T. magnatum* is lower than in the rest of Emilia-Romagna (Zambonelli and Morara, personal communication). The points in the areas indicated approximately by the truffle hunters, where *T. magnatum* mycelium was not found, fall in S4 (Marginally suitable: 2 areas) and N (Not suitable: 1 area) suitability classes. These areas were probably not correctly indicated by the truffle hunters and have never been productive.

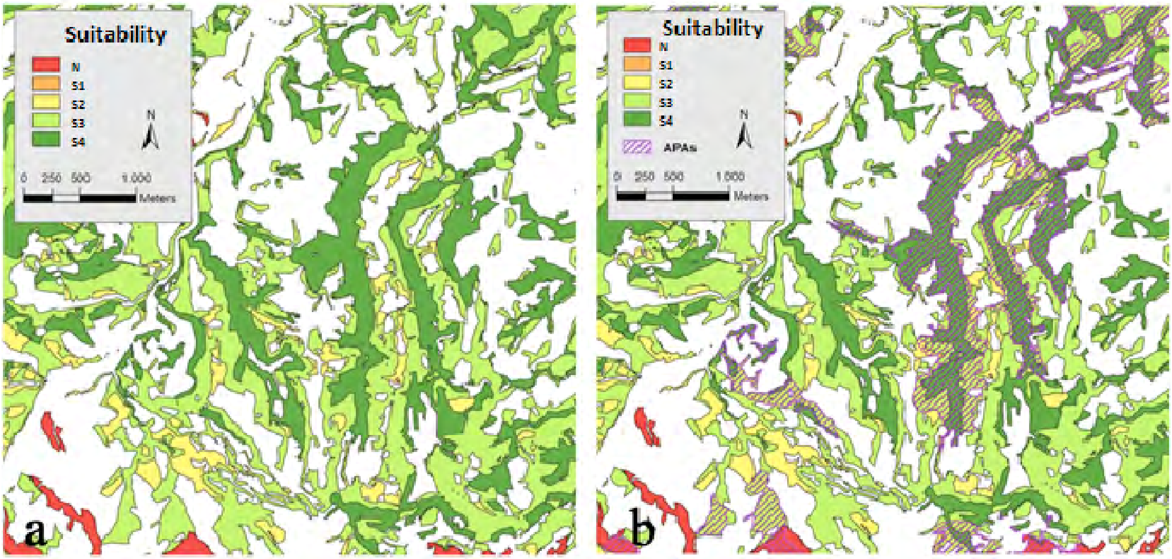


**Fig. 3**- Example of the SSGT map (a); the same area of (a) with the APAs map overlapped on the SSGT map (b): N – Not suitable S1 –Very highly suitable; S2 – Highly suitable; S3 – Moderately suitable; S4 – Marginally suitable.

**Table 5** - Area and area % covered by APAs for each SSGT class

| SSGT class* (EF) | Total area (ha) | APAs' area (ha) | % |
|---|---|---|---|
| S1 ( >10%) | 6,924 | 1,457 | 21.0 |
| S2 (7-10%) | 60,371 | 5,803 | 9.6 |
| S3 (5-7%) | 31,023 | 1,646 | 5.3 |
| S4 (2-5%) | 14,972 | 322 | 2.2 |
| N ( <2%) | 14,141 | 126 | 0.9 |

*S1 –Very highly suitable; S2 – Highly suitable; S3 – Moderately suitable; S4 – Marginally suitable; N – Not suitable

DOI: 10.6092/issn.2531-7342/10832

**Table 6** - *T. magnatum* DNA presence in the analyzed soil samples and the corresponding expected frequency (EF) and suitability class in the SSGT map

| Soil sample | Presence of *T. magnatum* | EF | Suitability class |
|---|---|---|---|
| 1 | yes | 6.4 | S3 |
| 2 | yes | 8.5 | S2 |
| 3 | yes | 7.7 | S2 |
| 4 | yes | 7.7 | S2 |
| 5 | yes | 8.4 | S2 |
| 6 | no | 8.3 | S2 |
| 7 | yes | 9.2 | S2 |
| 8 | yes | na | na |
| 9 | yes | na | na |
| 10 | yes | na | na |
| 11 | no | 8.7 | S2 |
| 12 | no | 1.9 | N |
| 13 | yes | 9.5 | S2 |
| 14 | yes | 9.5 | S2 |
| 15 | yes | 7.0 | S3 |
| 16 | yes | 6.5 | S3 |
| 17 | yes | 6.6 | S3 |
| 18 | yes | 7.0 | S2 |
| 19 | yes | 8.8 | S2 |
| 20 | yes | 6.5 | S2 |
| 21 | yes | 8.5 | S2 |
| 22 | yes | 7.0 | S2 |
| 23 | no | 4.2 | S4 |
| 24 | no | 4.8 | S4 |
| 25 | yes | 10.5 | S1 |
| 26 | yes | 5.4 | S3 |
| 27 | yes | 6.0 | S3 |
| 28 | yes | 6.0 | S3 |
| 29 | no | 5.2 | S3 |
| 30 | yes | 7.2 | S2 |
| 31 | yes | 7.2 | S2 |
| 32 | yes | 7.2 | S2 |
| 33 | yes | 6.5 | S3 |
| 34 | no | 6.5 | S3 |
| 35 | no | 6.6 | S3 |

## Conclusions

In this work we used an inductive and deductive approach to map SSGT in Emilia-Romagna region. The relationships found by means of the inductive approach (comparison between selected properties and observed frequency of truffle) demonstrated to be very effective in predicting deductively the potentiality outside the surveyed provinces: an excellent correspondence between SSGT map and presence of truffles was found. The SSGT map can be used as a valid predictive tool.

It is worth pointing out that the basic information used had variable cartographic resolutions (e.g. from 10×10 m for the DEM to 5×5 km for the climate grid, which means that the former is 250,000 times more detailed than the latter) and different quality and reliability (due to inherently different

DOI: 10.6092/issn.2531-7342/10832

detection and survey methods). Nevertheless, this work resulted in a very satisfying outcome. The gap in resolution between the more detailed and the less detailed maps (i.e. DEM and climate grid) was bridged by a very accurate downscaling operation.

Further elaborations of the available data should include: a) downscaling of soil maps (which currently have a low to very low resolution compared to DEM), using morphometry-based algorithms; b) a multivariate statistical analysis, aimed at exploring the cross-correlations among different factors and properties. These activities would require a lot of computing power, due to the huge number of cells and properties to be analysed.

The method used to produce the SSGT map may be applied in other geographical environments where the presence of *T. magnatum* is well known or is presumable, based on environmental characteristics. Topographic Position Index and continentality were used for the first time as, respectively, topographic and climatic indicators of the potentiality for *T. magnatum* development. The SSGT map allows to identify the environments suitable for the growth of *T. magnatum,* where appropriate protection actions should be applied. In fact, the protection of *T. magnatum* habitats is indispensable, as this truffle cannot be successfully cultivated yet and since several factors, such as intensive harvesting, forest degradation and climate change, are threatening the survival of this prized truffle species.

## Acknowledgments

This work was supported by Regione Emilia-Romagna, Assessorato Agricoltura Caccia e Pesca. The Authors thank Maria Capecchi, Paola Tarocco, Giulio Ercolessi, Marco Pattuelli, Stefano Bassi, Mauro Generali of Regione Emilia-Romagna and Gabriele Antolini of Arpae for the valuable contributions provided in the map construction; Nanì Adelina and Enrico Merli of Regione Emilia-Romagna for the support in the field surveys.

The Authors also thank the truffle hunter associations who indicated the areas of *T. magnatum* production in the provinces of Piacenza and Parma: Associazione Parmense Raccoglitori Tartufo, Associazione Tartufai Parmensi, A.P.T.P. Associazione Provinciale Tartufai Piacentini, Associazione Tartufo Nero di Piacenza, Associazione Nazionale Tartufai Italiani Regione Emilia-Romagna.

## References

Amicucci A, Zambonelli A, Giomaro G, Potenza L, Stocchi V (1998) Identification of ectomycorrhizal fungi of the genus *Tuber* by species-specific ITS primers. Moecular Ecology 7(3):273–277. https://doi.org/10.1046/j.1365-294X.1998.00357.x

Antolini G, Auteri L, Pavan V, Tomei F, Tomozeiu R, Marletto V (2016) A daily high-resolution gridded climatic data set for Emilia-Romagna, Italy, during 1961–2010. International Journal of Climatology 36(4):1970-1986. https://doi.org/10.1002/joc.4473

Antolini G, Pavan V, Tomozeiu R, Marletto V (2017) Atlante climatico dell'Emilia-Romagna 1961-2015. Edizione 2017, Arpae Emilia-Romagna, Bologna. www.arpae.it/clima.

Bonito G, Gryganskyi A, Vilgalys R, Trappe JM (2010) A global meta-analysis of *Tuber* ITS rDNA sequences: Species diversity, host specificity, and long-distance dispersal. Molecular Ecology 19(22):4994–5008. https://doi.org/10.1111/j.1365-294X.2010.04855.x

Bragato G, Gardin L, Lulli L, Raglione M (2006) Tartufi eduli. In: Metodi di valutazione dei suoli e delle terre (Costantini EAC, ed) Cantagalli, Siena. http://lnx.tartufaifvg.it/

Bragato G, Sladonja B, Peršurić D (2004) The soil environment for *Tuber magnatum* growth in Motovun forest, Istria. Natura Croatica 13(2):171–185. https://hrcak.srce.hr/10662

Bratek Z, Gógán A, Halász K, Bagi I, Erdei V, Bujáki G (2007) The northernmost habitats of *Tuber*

DOI: 10.6092/issn.2531-7342/10832

*magnatum* known from Hungary. In: First Hypogean Mushroom Conference (Rabat, Morocco), 6-8 April 2004, Rabat, Morocco, pp 28-30.
Büntgen U, Lendorf H, Lendorf A, Leuchtmann A, Peter M, Bagi I, Egli S (2019) Truffles on the move. Frontiers in Ecology and the Environment 17(4):200-202. https://doi.org/10.1002/fee.2033
De Laurentis G, Spinelli S (2009) Carta della vocazionalità tartuficola della regione Abruzzo risultati dell'indagine conclusiva. ARSSA. Lanciano.
FAO (1976) A framework for land evaluation Soils bulletin No.32. FAO, Rome.
Gardes M, Bruns TD (1993) ITS primers with enhanced specificity for basidiomycetes-application to the identification of mycorrhizae and rusts. Molecular ecology 2(2):113-118. https://onlinelibrary.wiley.com/doi/abs/10.1111/j.1365-294X.1993.tb00005.x
Hall IR, Brown GT, Zambonelli A (2007) Taming the truffle: the history, lore, and science of the ultimate mushroom. Timber Press, Portland.
Hall IR, Zambonelli A, Primavera F (1998) Ectomycorrhizal fungi with edible fruiting bodies. *Tuber magnatum*, Tuberaceae. Economic Botany 52(2):192–200. https://doi.org/10.1007/BF02861209
Iotti M, Leonardi M, Lancellotti E, Salerni E, Oddis M, Leonardi P, Perini C, Pacioni G, Zambonelli A (2014) Spatio-temporal dynamic of *Tuber magnatum* mycelium in natural truffle grounds. PLoS One 9:e115921. https://doi.org/10.1371/journal.pone.0115921
Iotti M, Leonardi M, Oddis M, Salerni E, Baraldi E, Zambonelli A (2012) Development and validation of a real time PCR assay for detection and quantification of *Tuber magnatum* in soil. BMC Microbiology 12(1):93. https://doi.org/10.1186/1471-2180-12-93
Iotti M, Leonardi P, Vitali G, Zambonelli A (2018) Effect of summer soil moisture and temperature on the vertical distribution of *Tuber magnatum* mycelium in soil. Biology and Fertility of Soils 54(6):707–716. https://doi.org/10.1007/s00374-018-1296-3
Mello A, Murat C, Bonfante P (2006) Truffles: much more than a prized and local fungal delicacy. FEMS Microbiology Letters 260(1):1–8. https://doi.org/10.1111/j.1574-6968.2006.00252.x
Regione Emilia-Romagna (1994) I suoli dell'Emilia-Romagna servizio cartografico. Ufficio Pedologico. https://geo.regione.emilia-romagna.it/cartpedo/
Regione Emilia-Romagna (2018) Carta dei suoli della pianura, del basso e del Medio Appennino emiliano-romagnolo in scala 1:50.000. Regione Emilia Romagna, Bologna. http://geo.regione.emilia-romagna.it/gstatico/documenti/dati_pedol/carta_suoli_50k.pdf
Rellini I, Pavarino M, Scopesi C, Zotti M (2011) Physical land suitability map for *Tuber magnatum* Pico in Piana Crixia municipality territory (Liguria-Italy). Journal of Maps 7(1):353-362. https://doi.org/10.4113/jom.2011.1180
Riccioni C, Rubini A, Belfiori B, Gregori G, Paolocci F (2016) *Tuber magnatum*: The Special One. What Makes It so Different from the Other *Tuber* spp.? In: True Truffle (*Tuber* spp.) in the World (Zambonelli A, Iotti M, Murat C, eds), Soil Biology series, vol 47. Springer International, Cham. https://doi.org/10.1007/978-3-319-31436-5_6
Rivas-Martínez S (2008) Global bioclimatics (Clasificación Bioclimática de la Tierra). Phytosociological Research Center. http://www.globalbioclimatics.org/book/bioc/global_bioclimatics-2008_00.htm
Salerni E, Iotti M, Leonardi P, Gardin L, D'Aguanno M., Perini C, Pacioni G, Zambonelli A (2014) Effects of soil tillage on *Tuber magnatum* development in natural truffières. Mycorrhiza 24(1):79–87. https://doi.org/10.1007/s00572-013-0543-6
Soil Survey Staff (2004) Soil survey laboratory methods manual. Version 4.0. USDA–NRCS. U.S. Gov. Print. Office, Washington, DC.
Soil Survey Staff (2014) Keys to Soil Taxonomy, 12th edition, USDA - NRCS, Washington DC.
Tarboton DG (1997) A new method for the determination of flow directions and upslope areas

DOI: 10.6092/issn.2531-7342/10832

in grid digital elevation models. Water Resources Research 33(2):309-319. https://doi.org/10.1029/96WR03137

Tibiletti E, Zambonelli A (2000) I tartufi della provincia di Forlì-Cesena. Patron, Bologna.

Trappe JM, Molina R, Luoma DL, Cázares E, Pilz D, Smith JE, Castellano MA, Miller SL, Trappe M (2009) Diversity, Ecology, and Conservation of Truffle Fungi in Forests of the Pacific Northwest. Gen. Tech. Rep. PNW-GTR-772. US Department of Agriculture, Forest Service, Pacific Northwest Research Station, Portland.

White TJ, Bruns T, Lee S, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: PCR protocols a guide to methods and applications (Innis A, Gelfand DH, Sninsky JJ, White TJ, eds). Academic Press, San Diego, pp 315-322.

Zambonelli A, Bonito G (2012) Edible ectomycorrhizal mushrooms, current knowledge and future prospects. Soil Biology series, vol 34. Springer-Verlag, Berlin Heidelberg.

Zambonelli A, Iotti M (2005) Appennino Modenese terre da tartufo. Giorgio Mondadori, Milano.

Zambonelli A, Iotti M, Puliga F, Hall IR (2020) New biotechnologies for enhancing the cultivation of white truffles. In: Advances in Plant Breeding Strategies (Al-Khayri JM et al., eds), vol 3. Springer, (in press).

Zambonelli A, Morara M (1984) Le specie di Tuberales dell'Emilia Romagna, ecologia e distribuzione. Natura e Montagna, 4: 9-32.

Zampieri E, Murat C, Cagnasso M, Bonfante P, Mello A (2010) Soil analysis reveals the presence of an extended mycelial network in a *Tuber magnatum* truffle ground. FEMS Microbiology Ecology 71(1):43-49. https://doi.org/10.1111/j.1574-6941.2009.00783.x

Zambonelli A, Perini C, Pacioni G (2012) Progetto MAGNATUM. Monitoraggio delle Attività di Gestione delle tartufaie NAturali di TUber Magnatum. Alimat, Cesena. https://agricoltura.regione.emilia-romagna.it/caccia/temi/tartufi/pubblicazioni/libro-magnatum.pdf