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Ecosystems supporting *Tuber magnatum* Pico production in Serbia experience specific soil environment seasonality that may facilitate truffle lifecycle completion

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ABSTRACT

The production of *Tuber magnatum* Pico, the most prized and ecologically the most complex, constantly declines in natural habitats, while the success in plantation lacks. Contrary to the data from its habitats in Mediterranean sites, in Serbia this truffle colonizes typical developed alluvial forests, implying that dynamics of soil parameters and nutrient availability, rather than presence of specific ectomycorrhizal (EMC) plant host species or climate, might be crucial for supporting truffle life cycle. In order to reveal specific relationship generating soil microenvironments in productive and unproductive sites, soil water content (SWC) and temperature (*T*) were measured periodically in two depths, in a typical *T. magnatum* habitat in Western Serbia and compared to the atmospheric parameters (precipitation, air temperature). In three key time-points (spring soil water saturation, summer drought and autumn ascocarp production) soil was sampled through the profile and analyzed for nutrients that might be important for plant/fungal growth and ectomycorrhiza establishment. Results revealed that specific dynamics of soil water, aeration, available P and N, and possibly vegetation phenology, might be determining productivity of the microsites within the productive area. The hypothesis on soil water and nutrient availability seasonal dynamics, which might be controlling truffle establishment and life cycle completion in investigated area, was proposed.

1. Introduction

Tuber magnatum Pico is the most prized among truffles, but also ecologically the most puzzling one among the economically important species. The decades long trials on cultivation gave no significant results so far (Bencivenga et al., 2009), and the results of the concurrently conducted research could not explain the environmental requirements for life cycle completion of this precious fungal species. The white truffle life cycle starts with spore germination, continues to haploid mycelia proliferation, ECM establishment and is completed by ascocarp formation and spore maturation (Paolloci et al., 2006). It is assumed that the mycelia start to develop in spring (Iotti et al., 2014), while ascocarps are usually collected in mid September–end December period (Hall et al., 2007). Long lasting problems of detecting white truffle mycelia or ECM in the soil were solved very recently with development of efficient protocols based on molecular methods (Murat et al., 2005; Zampieri et al., 2010; Iotti et al., 2012), but with puzzling results. Often, mycelia or ECM could not be detected even in highly productive sites, while they were simultaneously detected in unproductive areas (Murat et al., 2005; Zampieri et al., 2010; Iotti et al., 2012; Leonardi et al., 2013). Apparently, mycelia or ECM presence is no clear sign of site productivity and ascocarp appearance remains the most trustful and undeniable evidence of the truffle life cycle completion *in situ*. The exact biological processes that characterize changes from haploid mycelia growth, to symbiotic state or transformation toward ascocarp formation of *T. magnatum in situ* is hardly possible to follow. However, the symbiotic state of entire process of truffle ascocarp formation and maturation was proved by Zeller et al. (2008) and Le Tacon et al. (2013), implying that ascocarp presence must be accompanied by the presence of mycelia and functional ECM. The newest results obtained by Iotti et al. (2014) in different habitats in Italy revealed that in early spring, the mean quantity of mycelium tends to increase and redistributes within

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the fruiting patches, while in autumn it concentrates only in the close vicinity of the ascocarps. *T. magnatum* inhabits areas with more or less expressed climatic seasonality, which may imply that the seasonal changes in soil environment in productive sites could determine changes in mycelia transformation toward different life cycle stages, which would be in line with the results of Iotti et al. (2014).

From the research done in Italian habitats, it was known that, for ascocarp production, *T. magnatum* requires older ECM plant host (most often different oaks, poplars, lindens, hornbeams and others), very aerated, light, weakly alkaline (pH 7.5–8.5), young soils with active $CaCO_3$, abundant annual precipitation with very short dry periods and often sub-Mediterranean hilly habitats (Bencivenga and Urbani, 1992; Lulli et al., 1993). Recently, the first attempts to describe *T. magnatum* habitats in Serbia revealed some discrepancies from the Italian data. *T. magnatum* areas of mid-Balkan Peninsula are very often located in the wide alluvial plains of the south Pannonia ridge. Their soils are much heavier (even 50% clay), well developed, often with no traces of $CaCO_3$ and a lower pH (6.8–7.5). (Marjanović et al., 2010, 2011). In this area, soil hydrology is strongly influenced by snow melt in Alps and Dinaric Massif, dictating the formation of a shallow water table, and consequently high soil moisture content of upper soil layers. However, very continental climate with dry, hot and long summers often causes water deficit in the soil surface. Therefore, natural forest vegetation in such habitats is dominated by flood tolerant, but also tap root forming, *Quercus robur* L. and *Populus sp*. L. or, if the water table is very near to the surface, *Fraxinus angustifolia* Vahl. This end-succession stage vegetation type is common and widespread in the most fertile sites in alluvial zones of European rivers (Schnitzler, 1997), but no white truffles have ever been reported from there. However, in Serbia, which is positioned in similar latitude as Italy, but with the climate clearly different (much lower precipitation rates, low winter temperatures with snow and often long, dry summers), *T. magnatum* appears exactly in such riparian forests (Marjanović et al., 2010).

Soils in riparian ecosystems, experiencing cycles of saturation and aeration, are especially characterized by the strong cyclic variation in P and N availability and speciation (Hefting et al., 2004). While P is regarded as a relatively stationary nutrient in forest soils, N is the first oxido-reduction susceptible soil compound to react to changes in soil oxidation level. Enhanced aeration triggers chemical changes in N-species and increases the number of nitrifying bacteria (Marschner, 1995). On the other hand, soil aeration has often been underlined as a basic requirement for ascocarp production of *T. magnatum* (Bragato et al., 2004, 2010; Lulli and Primavera, 2001). Here we aimed to determine if the seasonal variations in soil moisture and aeration in the typical riparian site hosting *T. magnatum* in Serbia might be important for its productivity. To emphasize the possible specificities of riparian ecosystems in Serbia, we also evaluate how the atmospheric factors influence habitat pedo-climate by calculating correlations of the measured soil variables, soil temperature and water content, with actual air temperatures and precipitation of the area. As P and N limitation are thought to favor the establishment of ectomycorrhizal symbiosis (Buscot et al., 2000), we hypothesised that, within the productive area, soil moisture and aeration and direct influence of their seasonal changes on the availability of P and N, might be the key factors responsible for temporal changes of truffle mycelia through developmental phases: vegetative proliferation, ECM establishment and ascocarp production (Paolocci et al., 2006). Few seasons before the experiments were performed, the production of *T. magnatum* was followed in investigated area to detect distinctive production sites, while the measurements of soil parameters in investigated season were accompanied by harvesting ascocarps in chosen investigated plots.

2. Material and methods

2.1. Investigated area

The studied forest is situated in the alluvial plain between rivers Kolubara and Tamnava (North-West Serbia) in municipality of the town Ub (the forest owner did not agree with publication of exact coordinates). The forest covers up app. 5 ha of a flat terrain (app. 100 m a.s.l.), with shallow ditches and elevations, not more than 1 m deep/high (respectively). According to the owner, until the seventies of the last century when the downstream riverbeds were regulated, this site was on the edge of a natural floodplain and depressions inside the forest were occupied by permanent ponds. Since then, the site has been gradually drying, but the water table remained high, as reflected by formation of shallow ponds in the time of snow melt, which usually disappear in July. The forest is characterized as a typical *Querco-Ulmetum*, with also typical absence of *Ulmus sp*., and remnants of *Populus* sp. (Sznitzler, 1997). In general, it is dominated by *Q. robur, Populus alba* L. and *F. angustifolia* and mostly arbuscular mycorrhizal (AM) shrub layer (*Cornus sp., Acer sp., Crategus sp*., *Pirus sp*...) forming a closed canopy. Apart from the ditches and *F. angustifolia* dominated parts, *T. magnatum* ascocarps have been collected by experienced truffle hunters in the entire forest, years before and after the present investigation, but with an uneven distribution pattern (A. Glišić, personal data). In closed canopy forest domain (90–100% canopy cover), formed by a mixed stand of *Q. robur*, *P. alba*, already mentioned shrubs and a less abundant, but persistent herbaceous layer that is drying out in summer only partially, *T. magnatum* ascocarps have been regularly collected every year (productive sites). Partially open patches (app. 30–50% canopy cover) that were formed by wood exploitation are dominated by rare *Q. robur* and covered with a very bulky and diverse herbaceous layer that dies out in summer. In such patches, *T. magnatum* ascocarps appear in the seasons characterized by high precipitation and very scarcely in dry seasons (conditionally productive sites). *F. angustifolia* dominated patches, with some *P. alba* present, formed around the ditches with periodically standing water produce very poor herbaceous layer later in summer and no truffle ascocarps (unproductive parts). Accordingly, all soil measurements were performed in 5 × 5 m plots, one per site, in three sites, chosen as representative: (i) the driest, conditionally productive site 1; (ii) highly productive site 2; (iii) the wettest, unproductive site 3. In sites 1 and 2, truffles were usually found at a depth of 20–30 cm, or even more, in the period of mid September to late December. All ascocarps collected during investigated season in investigated plots were measured, for getting evidence on the difference in site productivity.

The climate of the area was characterized as continental with Mediterranean influences. The data collected from the nearest meteorological station (Valjevo, app. 30 km away) showed an average yearly precipitation of 787.4 mm and average yearly temperature of 11.4 °C. The warmest/coolest months are July and January, with an average temperature of 21.9 °C and 0.6 °C, respectively (http://www.hidmet.gov.rs). Precipitation is distributed throughout the year, mostly in the warmer period, with the driest months being February and October. The Kolubara basin has a continental pluviometric regime that is characterised by a maximum rainfall at the beginning of summer and a minimum in winter (Dragićević et al., 2012).

2.2. Soil physical properties

For the soil morphological features five sampling locations were randomly selected within an area of about 0.5 ha, within each of the three investigated sites (plots) to characterize soil morphology features. The 15 locations were augered to a depth of 100 cm.