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Insights into the dynamics of *Boletus edulis* mycelium and fruiting after fire prevention management

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ABSTRACT

Boletus edulis Bull. is among the most valuable mushroom species worldwide. *Cistus ladanifer* L. scrublands host an extraordinarily high production of this profitable fungus. Its fructification varies greatly among years depending on factors such as stand age and density along with climatic variables. Important information is missing, however, on the dynamics of this species, particularly at the below-ground level and its correlation with sporocarp production. We sought to improve understanding of the ecology of this species that could allow prediction of *B. edulis* production using faster and less expensive procedures under forest management scenarios.

Under the hypothesis that fire prevention management influences the presence of *Boletus edulis* mycelium and thus, the production of sporocarps, different management treatments were performed. These consisted of different levels of fuel reduction: (controlled burning, total clearing, 50% clearing, and uncleared), established in scrublands of different ages/origins (young-fire, young-cleared and senescent). To analyse *B. edulis* mycelium in the soil, soil samples were taken at three different times during the year, quantifying *B. edulis* DNA by real-time PCR using specific primers and Taq-man probes. To analyse *B. edulis* production, all sporocarps were collected and weighed on a weekly basis during the autumn season.

Our results confirmed that management significantly influence quantities of *B. edulis* mycelium in soil and sporocarp yields that were highest in the uncleared plots and lowest in total clearing plots. Fifty percent clearing plots showed a significant recovery for both mycelium quantity and *B. edulis* yields after three years.

Concerning the origin of the stand, young-fire plots had the highest amounts of *B. edulis* mycelium in the soil. A positive correlation was detected between *B. edulis* fresh weight production and the amount of mycelium in the soil suggesting the ability to predict *B. edulis* yields using faster and less costly methods than weekly inventories carried out for several autumn seasons.

1. Introduction

Boletus edulis Bull. is among the most valuable forest mushrooms worldwide (Boa, 2004), and its trade has become an important complementary economic activity in many regions (Cai et al., 2011; Martínez-de-Aragón et al., 2011), especially in geographic areas where other benefits from forest products are difficult to obtain (de la Varga et al., 2012). *B. edulis*, is one of the highest priced species, reaching up to 40–50 €/kg (Hernández-Rodríguez et al., 2015b). However, the availability of this highly sought mushroom, as with the majority of ectomycorrhizal forest fungi, depends on natural fructification, since no controlled production has been achieved to date (Mediavilla et al., 2016). Thus, harvesting this species in natural forests is the only way to obtain this resource (de la Varga et al., 2013) which can only be collected when habitat and climatic conditions are adequate for its fructification (Liu et al., 2016).

A large number of variables are related to mushroom productivity that require systematic quantitative analyses to test the influence of each one (Martínez-Peña et al., 2012b). Studies have been conducted along these lines, focused on the influence of aspects such as: i) climatic factors: the mean temperature of the autumn is crucial for *B. edulis* fructification (Hernández-Rodríguez et al., 2015a); ii) stand structure:

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best yields of *B. edulis* are reached when the stand basal area is 40–45 m^2 ha^{-1} (Martínez-Peña et al., 2012b); iii) local site characteristics: they have an important impact on mushroom yield (Egli, 2011); iv) stand age: most *Boletus* fruit in association with mature forests, with highest production at around 51–70 years old (Martínez-Peña et al., 2012a).

Surprisingly, *B. edulis* has been also reported associated with extraordinarily young forest systems dominated by *Cistus ladanifer* shrubs as young as 5-years-old (Martín-Pinto et al., 2006). Therefore, management of these scrublands focused on increasing *B. edulis* yields is of special interest. *Cistus* scrublands dominate large ecosystems distributed over the Mediterranean basin in Europe and the West coast in U.S.A., with *Cistus ladanifer* L. (Guzmán and Vargas, 2005) as one of the most abundant species. *Cistus* species occur in poorly developed, nutrient deficient, acidic soils (Rossini-Oliva et al., 2016) and grow under diverse and extreme climates, withstanding cold stress, drought, and high temperatures (Devesa, 2008).

These pyrophytic ecosystems are frequently affected by fire, causing significant economic loss. Stand management is required to reduce the negative impact of fire on fungal yields. In this context, a previous study tested different fuel reduction treatments for effects on the production and diversity of fungal communities (Hernández-Rodríguez et al., 2015b), and concluded that 50% clearing was the most appropriate practice for production of mycorrhizal species.

Knowledge of correlations between *B. edulis* mycelium in the soil and sporocarp production under different management options is crucial to understanding the biology of this economically valuable fungal species and may enable prediction of *B. edulis* yields using faster and less expensive methods than the weekly inventories throughout autumn seasons.

The aims of this study were i) to test different silvicultural management treatments for *C. ladanifer* shrublands on the presence of *B. edulis* mycelium in the soil, and ii) to identify correlations between the dynamics of soil mycelium quantities and sporocarp yields.

2. Materials and methods

2.1. Study site

The study area is located in Zamora province (North-western Spain) and exclusively dominated by *C. ladanifer* shrubs. This region is characterized by a sub-Mediterranean climate with a dry season during the summer months and cold winters. The mean annual rainfall is 450–700 mm, and mean temperatures range from 14.5 to 15.8 °C. These climatic data were provided by the closest meteorological station (Alcañices, 0724617 Longitude-UTM, 4618218 Latitude-UTM, 29T Grid and 806 m above sea level, Spanish Meteorological Agency). The soils in this zone are characterized by stoniness, acidity (pH 5.0–5.5), and lack of calcium and phosphorous. Nitrogen and potassium availability are variable, with good levels of humification. Further information can be accessed in Hernández-Rodríguez et al. (2015b).

2.2. Fuel reduction treatments

The study site is comprised for three areas with different ages and origins: a) an eight-year-old stand whose origin was a wildfire, from now on “young-fire”; b) an eight-year-old stand whose origin was a clearing of the previous stand, from now on “young-clear”; c) a 20-year-old stand whose origin was a wildfire, from now on “senescent”. These three areas belong to the same stand, and share similar environmental conditions. Specifically, young-fire and young clear areas are contiguous, while the senescent area is a few meters apart.

Silvicultural treatments were applied depending on their feasibility in accordance with the age of the stands and vegetation characteristics. In the 8-year-old areas (a and b), the fuel reduction treatments were: 1) uncleared i.e. control; 2) 50% clearing; and 3) total clearing. In the 20-year-old stand (c), the treatments were: 1) uncleared i.e. control; 2) total clearing; and 3) controlled burning (Fig. 1).

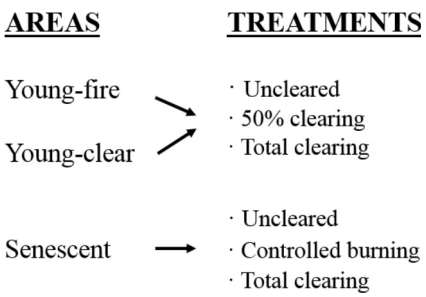

Fig. 1. Diagram of the fuel reduction treatments applied in each area.

Total clearing was performed using a tractor with a brush thrasher mower, while 50% clearing was carried out manually removing half of the plants with a brush cutter. Both clearing treatments were carried out in spring 2010. Controlled burning was performed with the help of Zamora EPRIF (Integral Fire Prevention Team) (Ministry of Agriculture, Food and Environment) in October 2010 under favourable weather conditions that allowed ignition without the risk of uncontrolled fire.

In summary, we studied three different areas with three treatments per area, and three plots per treatment, resulting in a total of twenty-seven plots sampled.

2.3. Sampling

Sampling plots consisted of transects of 2 m × 50 m, established in accordance with previous studies (Luoma et al., 1991; Smith et al., 2002). To analyse extra-radical mycelium, soil samples were taken at three different times: December 2013, April 2014 and July 2014. Using a 250 cm^3 soil extractor (3.5 cm diameter and 26 cm deep), five soil samples were taken in each plot at 5 m intervals along the longitudinal axis of the plot (Taylor, 2002). To analyse *B. edulis* production, all *B. edulis* sporocarps were collected and weighed on a weekly basis during the autumn mushroom season (from late October to late December) from 2010 to 2014. Sampling began the first autumn production season after the treatments had been implemented (Hernández-Rodríguez et al., 2015b).

2.4. Soil DNA extraction

Soil was prepared prior to DNA extraction. It was dried at room temperature and then sifted with a 1 mm sieve in order to discard stones and coarse elements. The five soil samples of each plot were pooled resulting in a composite soil sample for each plot.

Soil DNA extractions were carried out with the PowerSoil® DNA Isolation Kit (MoBio Laboratories Inc., Carlsbad, CA, USA) from 0.25 g of soil per sample according to manufacturer’s instructions. A total of 81 samples were processed. The extracted DNA was stored at −20 °C until use.

2.5. Quantification of extra-radical soil mycelium by real-time PCR

B. edulis DNA was amplified by real-time PCR (qPCR) in an Applied Biosystems® 7500 Real-Time PCR System (Applied Biosystems, Mannheim, Germany), using the kit qPCR *Boletus edulis*-VK provided by Vacunek S.L. (Bilbao, Spain), and according to the manufacturer’s instructions for a final reaction volume of 25 µl. The kit provides the necessary reagents and enzymes, mixed in a single master mix. The kit uses a Taqman probe marked with FAM-BHQ1® and also a positive internal control with primers and a Taqman probe marked with JOE-BHQ1®, which allows detection of false negatives caused by inhibition.

One microliter of soil DNA was added as a template mixed with four microliters of Tris-HCl pH8 (10 mM) in order to avoid inhibitions. An