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Effects of forest management and climatic variables on the mycelium dynamics and sporocarp production of the ectomycorrhizal fungus *Boletus edulis*

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ABSTRACT

The effect of forest management practices (cutting for timber production and intensive seasonal sporocarp collection) on extraradical soil mycelium and sporocarp production of the king bolete (*Boletus edulis* Bull.) has been evaluated in a *Pinus sylvestris* L. forest in Soria (central Spain) from 2011 to 2015. Extraradical soil mycelium of *B. edulis* was quantified by real-time PCR from soil samples taken in plots submitted to clearcutting and partial cutting in 2012, and in undisturbed areas. In a parallel experiment, the *B. edulis* mycelium biomass was also measured in fenced plots where all the fruiting bodies of this species were removed weekly along the fruiting season of five years (2011–2015), and compared to plots in which no collection of *B. edulis* was done.

Clearcutting and partial cutting of trees resulted in a sharp decrease of *B. edulis* mycelium biomass in all the sampled plots. The differences between control and cut plots were significant from the 7th month after cutting until the last measurement in September 2015. The cut plots showed a reduced amount of *B. edulis* mycelium biomass as compared to undisturbed plots and no recovery was observed three years after cutting. No significant differences in fungal biomass between clearcutting and partial cutting plots were observed.

Intensive collection of *B. edulis* sporocarps along four productive seasons (September to November) did not significantly affect the soil mycelium biomass as compared to non-collected plots. The mean mycelium biomass varied significantly among the different sampling times but no significant interaction between the sporocarp collection and sampling time was detected.

The obtained dataset allowed to establish correlations between soil mycelium biomass, sporocarp production, and climatic parameters (monthly mean precipitation and temperature). Monthly mean precipitation measured two, three, four and five months before sampling was negatively correlated with *B. edulis* mycelium biomass. No correlations were detected between monthly mean temperature and mycelium biomass. Mean precipitation in autumn (September to November) was significant and positively correlated with sporocarp productivity. No significant effects of mean precipitation in previous summer, spring and winter on sporocarp production were detected. No significant effect of temperature in the fruiting of *B. edulis* was observed.

We concluded that mycelium surveys are useful indicators of the fungal response to management practices and may provide complementary information to sporocarps surveys towards an integrated forest management considering sustainability and multiproductive criteria.

1. Introduction

The social and economic value of wild edible fungi (WEF) has been recognized in many regions of the world (Boa, 2004). Most of the marketed WEF cannot be cultivated and have to be collected in natural forests. Harvests of several edible mushrooms have declined over the past century, partly because of changes in their natural environment caused by various natural and social factors (Wang and Hall, 2004). Mushrooms are an important non-timber forest product which may exceed the value of timber in Mediterranean areas (De Román and Boa, 2006; Palahí et al.,

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2009) providing local employment in rural forest areas through picking regulations, gastronomy and tourism (Martínez-Peña et al., 2011).

Boletus edulis is a valued ectomycorrhizal fungus consumed worldwide with a strong, distinctive flavour, occurring in temperate forests throughout the Northern Hemisphere (Boa, 2004). This species is symbiotically associated with a large number of trees and scrubs and can be commonly found in *Pinus sylvestris* forests of the Iberian Peninsula (Águeda et al., 2008) where it can produce more than 40 kg of sporocarps/ha/yr in average (Martínez-Peña, 2009). Sporocarp production has been linked to climate variability and phenology (Gange et al., 2007; Kauserud et al., 2008, 2010; Büntgen et al., 2012b), but also to stand structure and local site characteristics (Martínez-Peña et al., 2012a; Mengiste Taye et al., 2016). Stand basal area (Martínez-Peña et al., 2012a), age class (Martínez-Peña et al., 2012b) and temperature (Hernández-Rodríguez et al., 2015) have been described as significant drivers for sporocarp production of *B. edulis* in northern Spain.

Many of the studies on mushroom productivity have been based on sporocarp surveys. However, most of the life cycle of ectomycorrhizal fungi occurs belowground in association with the host plants forming mycorrhizas and extended mycelia to increase water and nutrient absorption (Smith and Read, 1997; Ekblad et al., 2013). The detection and quantification of extraradical mycelium of the target fungal species in the soil may be a valuable information to complete the data obtained from the irregular sporocarp production and ectomycorrhiza sampling for tracking the fungal presence along the whole biological cycle. Because of the methodological difficulties for mycelium detection in soil, this is the most poorly understood phase of the symbiosis in spite of being the most metabolically active (Read, 1992; Horton and Bruns, 2001; Leake et al., 2004).

Promoting multifunctional forest management to maximize mushroom yield may result in a higher economic profit in Mediterranean forest ecosystems (Palahí et al., 2009). Clearcutting or heavy thinning can reduce drastically the fruiting of ectomycorrhizal fungi (Luoma et al., 2004; Norvell and Exeter, 2004; Durall et al., 2006), but it has been long recognized that sporocarp surveys do not always reflect the presence of the fungus underground (Gardes and Bruns, 1996). Sporocarp production by many species can be irregular and infrequent, often fruiting every several years giving a false negative conclusion for the surveys (Molina, 2008; Van Norman and Huff, 2012). Gordon and Van Norman (2014) studied the persistence of the mycorrhizal genus *Phaeocollybia* in a clearcut area. Unexpectedly, they detected the presence of the fungus in the soil 12 years after clearcutting without evidence of sporocarps. They concluded that some fungal species may have a robust survival capacity once established in the environment, and that prolonged periods without sporocarp production may be a normal part of the life trajectory of some fungi as they respond to local environmental changes. Thus, pre and post disturbance surveys of fungal presence in the soil may give us the clues for an effective management of key fungal species for conservation and sustainable production.

The increase of harvesting pressure on WEF in many parts of the world (Boa, 2004), as well as the decrease of some ectomycorrhizal species diversity (Arnolds, 1991; Wang and Hall, 2004) have motivated a concern on the effects of fungal picking on the production of key edible fungi. The highly used and revered 'matsutake' (*Tricholoma matsutake*) in Japan suffered a severe decline considering historical productions (Hosford et al., 1997). A combination of climatic, socioeconomic, and biological factors in Japan were considered to explain the production losses. A change in the way communities use local forests also had adversely affected the health of pine forests and, indirectly, matsutake productivity. This situation has led to the implementation of harvesting regulations in the production areas (Amend et al., 2010). Similarly, the natural production of the Perigord black truffle (*Tuber melanosporum* Vitt.), one of the most appreciated edible fungus, has steadily decreased in France since the beginning of the twentieth century (Büntgen et al., 2012a,b) and this decline has been attributed to several causes: rural desertification, improper truffle orchards management and climate changes. Le Tacon et al. (2014) found that the decrease of black truffle production in France from 1965 to 1987 could be attributed to the decrease of land surface devoted to truffles and abandoned management rather than to climate changes. None of these examples of mushrooms/truffles decline with long recordings seem to be related to intensive harvesting. A long-term study carried out in Switzerland (Egli et al., 2006) showed that intensive sporocarp collection reduced neither the future yields of fruit bodies nor the species richness. However, forest floor trampling reduced the total number of sporocarps as compared to non-trampled plots but soil mycelium was not apparently damaged.

The purpose of this study is to test the hypotheses that tree cutting and intensive sporocarp collection significantly affects the persistence and dynamics of *B. edulis* mycelium. We have used molecular techniques based on the real-time PCR, to establish the relationships between sporocarp productivity, climatic variables and mycelium biomass. The experiment has been set up in a *P. sylvestris* forest area in northern Spain with historical data on sporocarp production available since 1995. Timber, mushroom picking and mycological tourism are one of the main local economic resources. The results are discussed in relation to the management strategies to maximize the ecosystem services provided in the study area.

2. Materials and methods

2.1. Study area

The study site known as 'Pinar Grande' is located in a homogeneous *Pinus sylvestris* L. stand with an extension area of 12,533 ha in the Sistema Ibérico mountains, located in the northeast zone of the Iberian Peninsula. Accompanying vegetation includes *Erica vagans*, *E. tetralix*, *Agrostis* sp., *Nardus stricta*, *Cynosurus crystatus*, *Lotus* sp. and *Brachypodium* sp. Altitude varies between 1097 and 1543 m a.s.l. with dominating west and east orientations. Soils are acidic brown earths or alluvial, with acid pH (4–5), sandy loam to sandy texture, low holding capacity, organic matter between 4 and 15% and low fertility. Annual rainfall is 865 mm/year, 69 mm/year falling in July and August and 132 mm/year in September and October. Medium annual temperature is 8.8 °C and the warmest month is July with an average temperature of 17.4 °C. Frosts are frequent in late spring and early autumn. Forest management consists of periodic clearcutting in mosaics with a rotation of 100 years.

Eighteen fenced permanent plots of 175 m^2 were established in the area for the periodic study of the fruiting mushrooms, with a sampling area of 150 m^2 divided in six adjacent subplots of 5 × 5 m. Total sporocarp production (epigeous macromycetes with a diameter larger than 1 cm) has been inventoried in all the plots from the 35 to the 50 week of the year from 1995 to date. Ectomycorrhizal biomass in the study area represents a 93.4% of the total sporocarps collected, being *B. edulis* the species which contributes to the highest biomass (26.6%) (Martínez-Peña, 2009).

Climatic variables (mean monthly temperature and precipitation) throughout the study period were obtained from the automatic weather station named 'La Cuerda del Pozo', code 2-011, located in the Soria province, next to the experimental site (02°-42′-W, 41°-53′-N, altitude: 1150 m). Additional information on