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

Olaya Mediavilla[a,b,*], María Hernández-Rodríguez[a], Jaime Olaizola[b], Luis Santos-del-Blanco[a,b], Juan Andrés Oria-de-Rueda[a], Pablo Martín-Pinto[a]

[a] *Sustainable Forest Management Research Institute, Fire and Applied Mycology Laboratory, Departments of Agroforestry Sciences and Vegetal Production and Natural Resources, University of Valladolid (Palencia), Avda. Madrid 44, 34071 Palencia, Spain*
[b] *IDForest – Biotecnología Forestal Aplicada, Calle Curtidores, 17, 34004 Palencia, Spain*

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:

best yields of *B. edulis* are reached when the stand basal area is 40–45 m² 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-
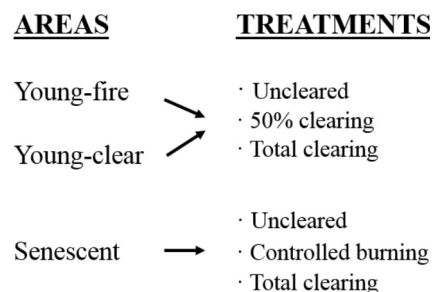

AREAS   **TREATMENTS**

Young-fire → · Uncleared
Young-clear → · 50% clearing
· Total clearing

Senescent → · Uncleared
· Controlled burning
· Total clearing

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

year-old stand (c), the treatments were: 1) uncleared i.e. control; 2) total clearing; and 3) controlled burning (Fig. 1).

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 cm³ 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

aliquot of 100 µl of *B. edulis* mycelial suspension mixed with 0.25 g of soil of similar characteristics to that of our study site served as positive control. The soil used for this positive control was previously checked by conventional PCR for the absence of *B. edulis*.

The analyses were performed in 96-well plates (MicroAmp® Fast Optical 96-Well Reaction Plate). Real-time PCR cycling conditions were 10 min at 95 °C, 45 cycles at 95 °C for 15 s and 60° for 60 s. Three replicates of each sample were included in the analysis, as well as a negative control (using deionized water instead of DNA template). A standard curve with 5 points and three replicates per point using known amounts of mycelium in the soil was generated. This curve was used to translate the outputs from the qPCR System into amount of mycelium in soil. Ten-fold serial dilutions were performed from 11,400,000 to 1140 ng of mycelium/g of soil.

2.6. Data analysis

To analyse the amount of *B. edulis* mycelium in the soil, a linear mixed model was fitted, considering two between-subjects factors (stand origin with three levels, treatment with four levels), and one within-subject factor (time, with three levels). The mathematical formulation of the model was:

$$Y_{ijk;t} = \mu + \alpha_i + \beta_{j(i)} + \gamma_t + \alpha\gamma_{it} + \beta\gamma_{jt(i)} + \varepsilon_{ijk;t}$$

With $i = 1, 2, 3$ for stand type (8 year-old clear, 8 year-old burnt, senescent plots, respectively); $j = 1, 2, 3$ for treatments within each level of stand type (uncleared, 50% clearing and total clearing) for the young stands; and uncleared, total clearing and controlled burning for the senescent stands), $k = 1, 2, 3$ for the replicates of plots within treatment, $t = 1, 2, 3$ for time (December, April and July), where:

Y_{ijkt} = observed value of the dependent variable (logarithm of *B. edulis* mycelium concentration) for plot k with treatment j nested within stand type i at time t.

μ = general main effect.

α_i = main effect of stand type i.

$\beta_{j(i)}$ = main effect of treatment j nested within stand type i.

γ_t = main effect of time t

$\alpha\gamma_{it}$ = interaction effect between origin i and time t.

$\beta\gamma_{jt(i)}$ = interaction effect between treatment j, nested within origin i, and time t.

ε_{ijkt} = random error of the dependent variable for plot k with treatment j nested within stand type i in time t.

Model parameters were estimated using the restricted maximum likelihood method (REML). In addition, *t*-tests and individual contrasts were used for the comparisons between least square means.

To analyse the *B. edulis* fresh weight production, a linear mixed model of analysis of variance with repeated measurements was used, including two between-subjects factors (origin with three levels and treatment with four levels) and one within-subjects factor (year, with five levels). The mathematical formulation of the model was given by:

$$Y_{ijk;t} = \mu + \alpha_i + \beta_{j(i)} + \gamma_t + \alpha\gamma_{it} + \beta\gamma_{jt(i)} + \varepsilon_{ijk;t}$$

With $i = 1, 2, 3$ for stand type (8 year-old clear, 8 year-old burnt, senescent plots, respectively); $j = 1, 2, 3$ for treatments within each level of stand type (uncleared, 50% clearing and total clearing) for the young stands; and uncleared, total clearing and controlled burning for the senescent stands), $k = 1, 2, 3$ for the replicates of plots within the treatment, $t = 1, 2, 3, 4, 5$ for the years (2010, 2011, 2012, 2013 and 2014, respectively), and being:

Y_{ijkt} = observed value of the dependent variable (logarithm of *B. edulis* fresh weight production) for k plot with treatment j nested within stand type i at year t.

μ = general main effect.

α_i = main effect of stand type i.

$\beta_{j(i)}$ = main effect of treatment j nested within stand type i.

γ_t = main effect of time t

$\alpha\gamma_{it}$ = interaction effect between origin i and time t.

$\beta\gamma_{jt(i)}$ = interaction effect between treatment j, nested within origin i, and time t.

ε_{ijkt} = random error in the dependent variable for plot k with treatment j nested within stand type i in time t.

Model parameters were estimated using minimum variance quadratic unbiased estimation (MIVQUE). In addition, *t*-tests and individual contrasts were used for the comparisons between least square means.

The procedure MIXED in SAS software was used for the statistical analysis of the model. When necessary, data were transformed to $\log_{10} + 1$ to meet the normality of residuals assumption. The relationships between soil mycelial concentration, and plot productivity were determined by Pearson correlation analysis using R version 3.3.2 (R Core R Core Team, 2016).

3. Results

3.1. Extra-radical B. edulis mycelium

The standard curve for quantification of extra-radical *B. edulis* mycelium in the soil fulfilled the requirements for real-time PCR in terms of efficiency ($R^2 = 0.99$) (Fig. 2). Negative controls, taken from a nearby area with no production of *B. edulis*, and with similar soil characteristics, did not show amplification.

Amplification of *B. edulis* DNA was found in all the treatments and was confirmed in 78 out of the 81 composite soil samples collected. The three negative samples belonged to plots where total clearing was performed. In these plots, *B. edulis* sporocarps were not found during the previous collection season (Autumn 2013).

The lowest amount of *B. edulis* detected was $4.48 \cdot 10^{-6}$ mg of mycelium per gram of soil, found in the total clearing plots, and the maximum limit of detection was 0.084 mg of mycelium per gram of soil, recorded in the 50% clearing plots.

Differences were found based on the origin of the stand (P = 0.019; Table 1). Young-fire plots had significantly more *B. edulis* mycelium in soil relative to both, young-clear (P = 0.018) and senescent plots (P = 0.011; Fig. 3).

The treatments nested within stand origin affected *B. edulis* mycelial concentration (P = 0.014; Table 1). Every treatment within each origin followed the same trend regarding the amount of mycelium in the soil. Controls showed the highest amounts of *B. edulis* mycelium and total clearing had a significant negative effect (Fig. 4). Despite following the same trend, not all the treatments evolved at the same rate. For the
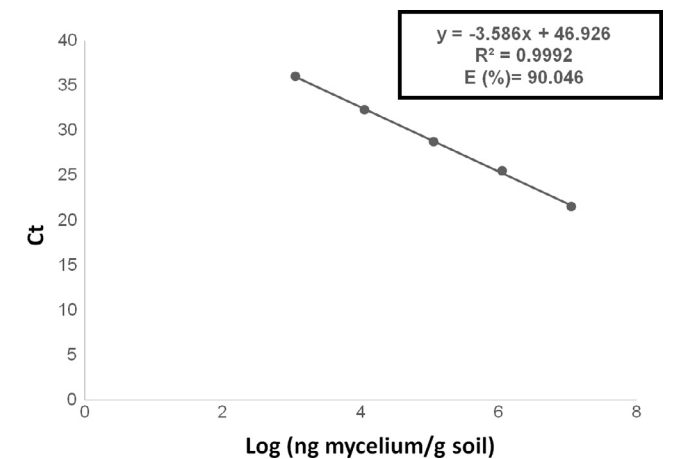

Fig. 2. Standard curve obtained for mycelium quantification of *B. edulis* by real-time PCR. Curve was generated by plotting the Ct values against the logarithm of known amounts of mycelium. The efficiency of the real-time PCR (E) is calculated as $E = (10^{-1/slope} - 1) \times 100$.

Table 1
Analysis of the variance of the amount of *B. edulis* mycelium in the soil (log transformed).

Effect	Num DF	Den DF	F ratio	Prob > F
Origin	2	18	4.96	0.0192
Origin (treatment)	6	18	3.71	0.0141
Time	2	34	1.98	0.1538
Origin * time	4	34	1.31	0.2855

plots whose origin was a clearing of the previous stand, 50% clearing plots showed significant differences when compared to control plots. On the other hand, for young-fire plots and senescent plots, both originating after a wildfire, there were no significant differences between 50% clearing plots and control plots in the case of the young-fire area, nor between controlled burning plots and control plots in the case of the senescent area (Fig. 4).

Regarding the seasonal dynamics of mycelium in the soil, there was suggestive evidence that the estimated amount of mycelium in the soil during the fructification season (December) was higher than in April (P = 0.06) but did not differ from July (P = 0.32; Fig. 5).

3.2. Fresh weight B. edulis production

The autumnal average production from 2010 to 2014 for all the plots was 24.71 kg/ha, whilst the average production for non-managed plots (controls) was 50.10 kg/ha.

Regarding the origin of the stand, significant differences were observed (P < 0.001; Table 2). Both, young-fire and young-clear, were significantly more productive than senescent stands (P = 0.007 and P < 0.001, respectively) (Fig. 3). Consequently, age had a negative effect on *B. edulis* sporocarp production. This trend was also observed when comparing the young vs. senescent controls disregarding the origin (P < 0.001).

Also, treatments nested within stand origin significantly affected *B. edulis* sporocarp yields (P < 0.001; Table 2). Within every origin, treatment followed the same pattern. Lowest production occurred in the treatments where vegetation was completely removed (i.e. total clearing) and controlled burning plots, compared with the control plots (Fig. 6).

Fresh *B. edulis* sporocarp production followed a similar trend as the amount of *B. edulis* extra-radical mycelium for each of the treatments (Fig. 4, Fig. 6). As a result of this similar behaviour between *B. edulis* fresh weight production and amount of mycelium in the soil, a statistically significant positive correlation was obtained (r = 0.40, P = 0.037).

4. Discussion

Widespread presence of *B. edulis* mycelium was detected in soil,

showing amplification by qPCR in all the treatments. In the treatments where the host was initially eliminated (controlled burning and total clearing plots), concentration of *B. edulis* mycelium was the lowest. This finding was expected since *B. edulis* is a mycorrhizal species, whose growth and survival depends on a host plant (Dahlberg, 2002). The occurrence of *B. edulis* mycelium in these plots three years after performing the treatments, could be explained by two factors. First, there are resistant fungal propagules in the soil after fire that play a key role in fungal recovery (Buscardo et al., 2010). Second, *C. ladanifer* rapidly colonize degraded areas, especially following fire because high soil temperatures stimulate seed germination (Bastida and Talavera, 2002), followed by the forming of extensive and dense root systems (Rivest et al., 2011). This rapid vegetation recovery allows fungi to recolonize the new root systems favouring their survival.

Three years after carrying out the treatments, mycelium recovery differed between total clearing and controlled burning plots, where all the vegetation was removed. On the one hand, total clearing plots did not show a recovery of the *B. edulis* mycelium in the soil. This finding is likely due to the fact that elimination of the host produces a dramatic decrease in the extra-radical mycelium in the soil (Durall et al., 2006). Parladé et al. (2017) also reported a sharp decrease of *B. edulis* mycelium biomass in the soil after cutting a *P. sylvestris* stand. In their case, not only did mycelium not recover three years after practices but neither did they find *B. edulis* sporocarps (Parladé et al., 2017). Interestingly, some of the total clearing plots produced *B. edulis* sporocarps three years after the clearing practises in our study, and almost all of them showed sporocarp fructification four years after carrying out the treatments. On the other hand, in the case of controlled burning plots, the amount of mycelium recovered three years after fire.

Half-clearing plots resulted in similar amounts of *B. edulis* mycelium and fresh weight production as control plots. Previous studies have shown the benefits of partial clearings. Bonet et al. (2012) observed an increased *Lactarius deliciosus* sporocarp production one year after the stands were thinned. Egli and Ayer (1997) also reported that a partial clearing in a mixed forest in Switzerland increased edible mushroom production up to sixfold. Performing clearing treatments may enable a better accessibility to mushroom harvesting, decreasing at the same time wildfire intensity and facilitating its extinction (Hernández-Rodríguez et al., 2015b).

Regarding the origin of stands, young-fire plots had the highest amounts of mycelium compared to young-clear and senescent plots. This could be due to two facts: the pyrophytic character of *C. ladanifer* (Comandini et al., 2006), and the higher photosynthetic activity of young stands. In this sense, when we compared plots of the same age but of different origin (young-fire and young-clear plots), highest amounts of mycelium were recorded in plots coming from a wildfire. On the other hand, when we compared plots with the same origin but different age (young-fire and senescent plots), the greater amount of mycelium in young-fire plots could be related to the higher
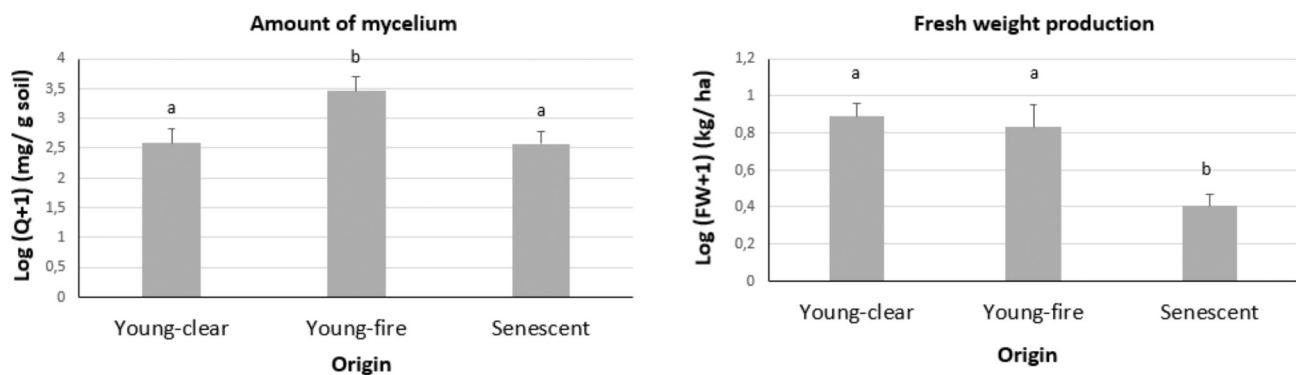

Fig. 3. Amount of *B. edulis* extra-radical mycelium in the soil and *B. edulis* fresh weight production according to different origins. Lower case letters indicate differences between different origins (p-value < 0.05). *t*-test was used for the comparisons of least square means within the same stand type. Error bars denote ± 1 standard error.
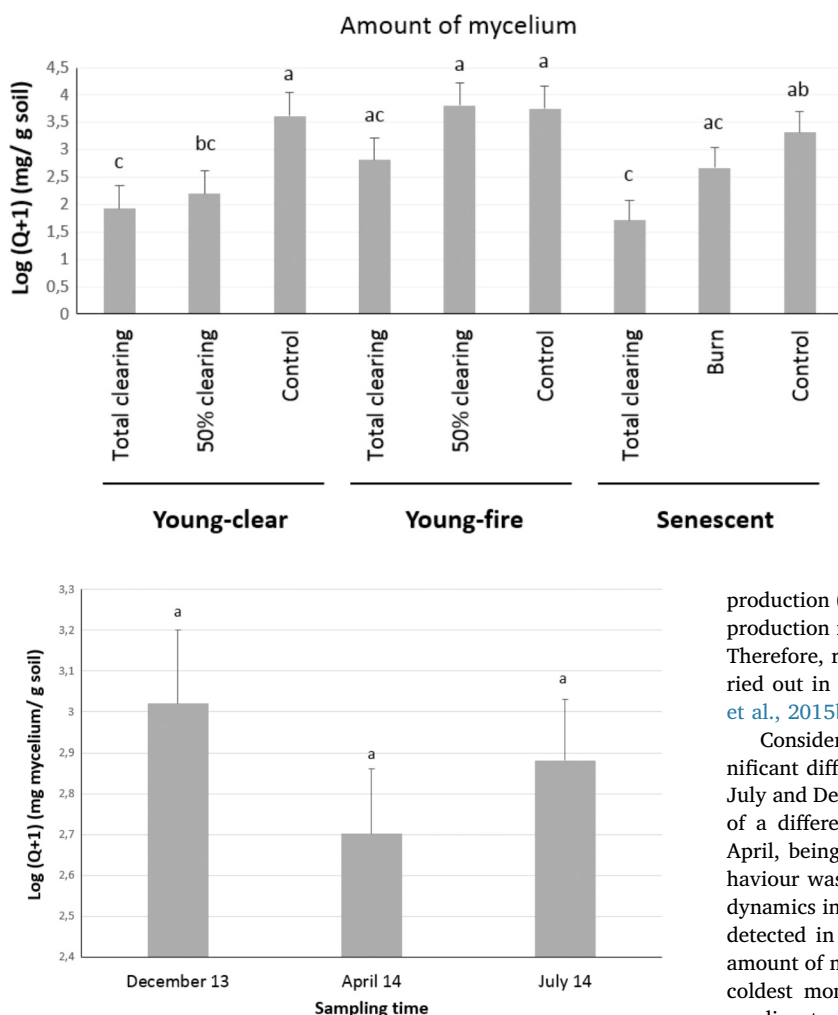
Amount of mycelium

Fig. 4. Amount of *B. edulis* extra-radical mycelium in the soil in the different silvicultural treatments. The data are plot means ± 1 standard error. Data was log-transformed (Log$_{10}$ [Quantity + 1]) for statistical analysis. Lower case letters indicate differences between silvicultural treatments (p-value < 0.05). *t*-test was used for the comparisons of least square means within the same stand type.

Fig. 5. Amount of *B. edulis* extra-radical mycelium at three different sampling times in the year (December 2013, April 2014, July 2014). The data are time means ± standard error. Data was log-transformed (Log$_{10}$ [Quantity + 1]) for statistical analysis. Lower case letters indicate differences between sampling times (p-value < 0.05).

Table 2
Analysis of the variance of the *B. edulis* fresh weight production (log transformed).

Effect	Num DF	Den DF	F ratio	Prob > F
Origin	2	18	13.57	0.0003
Origin (treatment)	6	18	11.21	< 0.0001
Year	4	72	23.58	< 0.0001
Origin * year	8	72	0.56	0.8105

photosynthetic activity of young plots. The development of ectomycorrhizal fungi depends on photosynthetically fixed carbon by their host species (Egli, 2011), and senescent plots display lower photosynthetic activity than younger ones (Hernández-Rodríguez et al., 2013).

Also, a significant decrease in *B. edulis* production was observed in senescent plots compared with young plots. *Cistus* shrubs are short-lived, and they reach their last stage of succession, when 18–20 years old (Oria-de-Rueda et al., 2008). *B. edulis* despite being traditionally considered as a late-stage species, behaves as a pioneer fungus when associated to *C. ladanifer* (Hernández-Rodríguez et al., 2013). In this sense, at 3 years of age, *Cistus* shrubs produce their first *B. edulis* yields, and at 20 years of age, production of *B. edulis* diminishes (Oria-de-Rueda et al., 2008). In addition, previous studies have reported that *Cistus* shrub age has a significant influence on *B. edulis* sporocarp

production (Hernández-Rodríguez et al., 2015b), generally with higher production in younger stands than in mature ones (Bonet et al., 2004). Therefore, rejuvenation of C. *ladanifer* senescent stands should be carried out in order to foster *B. edulis* production (Hernández-Rodríguez et al., 2015b).

Considering the seasonal dynamics of *B. edulis* mycelium, no significant differences in amounts were observed when comparing April, July and December samplings. However, there was suggestive evidence of a difference in the amount of mycelium between December and April, being nearly significantly abundant in December. A similar behaviour was observed by Parladé et al. (2017) for *B. edulis* mycelium dynamics in a *Pinus sylvestris* stand, with highest amounts of mycelium detected in winter. De la Varga et al. (2013) also reported a higher amount of mycelium in the soil for *B. edulis* and *L. deliciosus* during the coldest months. This was hypothesized to be due to the mycelium needing to increase its absorption surface to compensate for the low activity of the host during the cold months (de la Varga et al., 2013). Since the study was carried out in an area with Sub-Mediterranean type climate, it could also be due to an increment of fungus biomass when the soil is wet in winter because of a higher nutrient mineralization than during the hot dry summers.

In addition, the pattern drawn for our amounts of mycelium followed the same trend as the dynamics of mycelium drawn by Parladé et al. (2017). The amount of mycelium was higher in December, decreased in April and increased again in July. In our case, no differences were observed between December and July samplings. This could be due to the sharp increase of mycelium in July towards autumn 2014 when an extremely high mushroom production was observed (Alday et al., 2017).

5. Conclusions and implications

The results obtained confirmed an abundance of *B. edulis* mycelium in the study plots and a positive correlation with fresh weight production. The highest abundance of *B. edulis* was found in control plots and 50% clearing plots from young stands. Our findings support active forest management of these areas, traditionally considered unproductive, focused on conserving and optimizing *B. edulis* yields. This can provide complementary incomes for rural populations in these depressed areas. The proposed management plan could consist of creating a mosaic landscape including partially cleared young stands in order to optimize production and to facilitate access, reducing at the same time, the potential risk and negative effects of forest fires. Finally, the positive correlation found between soil mycelium and sporocarp production suggest that soil mycelium sampling may be a faster and less
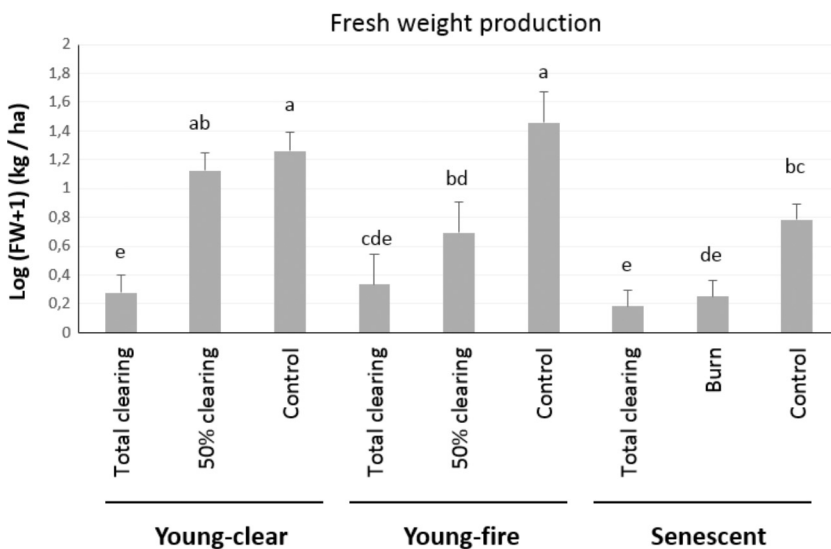
Fig. 6. *B. edulis* fresh weight production in the different silvicultural treatments. The data are plot means ± 1 standard error. Data was log-transformed (Log$_{10}$ [Fresh Weight + 1]) for statistical analysis. Lower case letters indicate differences between silvicultural treatments (p-value < 0.05). *t*-test was used for the comparisons of least square means within the same stand type.

expensive method to predict *B. edulis* yields than the costly weekly inventories carried out for multiple autumn seasons. However, further research will be needed to produce a reliable prediction tool.

References

Alday, J.G., Bonet, J.A., Oria-de-Rueda, J.A., Martínez-de-Aragón, J., Aldea, J., Martín-Pinto, P., De-Miguel, S., Hernández-Rodríguez, M., Martínez-Peña, F., 2017. Record breaking mushroom yields in Spain. Fungal Ecol. 1–3. http://dx.doi.org/10.1016/j.funeco.2017.01.004.

Bastida, F., Talavera, S., 2002. Temporal and spatial patterns of seed dispersal in two *Cistus* species (Cistaceae). Ann. Bot. 89, 427–434. http://dx.doi.org/10.1093/aob/mcf065.

Boa, E., 2004. Wild edible fungi: a global overview of their use and importance to people. In: Non-Wood Forest Products No17. FAO, Rome, pp. 1–147.

Bonet de-Miguel, J.A., Martínez de Aragón, S., Pukkala, J., Palahí, M., 2012. Immediate effect of thinning on the yield of *Lactarius* group *deliciosus* in *Pinus pinaster* forests in Northeastern Spain. For. Ecol. Manage. 265, 211–217. http://dx.doi.org/10.1016/j.foreco.2011.10.039.

Bonet, J.A., Fischer, C.R., Colinas, C., 2004. The relationship between forest age and aspect on the production of sporocarps of ectomycorrhizal fungi in *Pinus sylvestris* forests of the central Pyrenees. For. Ecol. Manage. 203, 157–175. http://dx.doi.org/10.1016/j.foreco.2004.07.063.

Buscardo, E., Rodríguez-Echeverría, S., Martín, M.P., De Angelis, P., Pereira, J.S., Freitas, H., 2010. Impact of wildfire return interval on the ectomycorrhizal resistant propagules communities of a Mediterranean open forest. Fungal Biol. 114, 628–636. http://dx.doi.org/10.1016/j.funbio.2010.05.004.

Cai, M., Pettenella, D., Vidale, E., 2011. Income generation from wild mushrooms in marginal rural areas. For. Policy Econ. 13, 221–226. http://dx.doi.org/10.1016/j.forpol.2010.10.001.

Comandini, O., Contu, M., Rinaldi, A.C., 2006. An overview of *Cistus* ectomycorrhizal fungi. Mycorrhiza 16, 381–395. http://dx.doi.org/10.1007/s00572-006-0047-8.

Dahlberg, A., 2002. Effects of fire on ectomycorrhizal fungi in Fennoscandian boreal forests. Silva Fenn. 36, 69–80.

de la Varga, H., Águeda, B., Ágreda, T., Martínez-Peña, F., Parladé, J., Pera, J., 2013. Seasonal dynamics of *Boletus edulis* and *Lactarius deliciosus* extra-radical mycelium in pine forests of central Spain. Mycorrhiza 23, 391–402. http://dx.doi.org/10.1007/s00572-013-0481-3.

de la Varga, H., Águeda, B., Martínez-Peña, F., Parladé, J., Pera, J., 2012. Quantification of extra-radical soil mycelium and ectomycorrhizas of *Boletus edulis* in a Scots pine forest with variable sporocarp productivity. Mycorrhiza 22, 59–68. http://dx.doi.org/10.1007/s00572-011-0382-2.

Devesa, J.A., 2008. Vegetación y flora de Extremadura. Universitas Editorial, Badajoz, España.

Durall, D.M., Gamiet, S., Simard, S.W., Kudrna, L., Sakakibara, S.M., 2006. Effects of clearcut logging and tree species composition on the diversity and community composition of epigeous fruit bodies formed by ectomycorrhizal fungi. Can. J. Bot. 84, 966–980. http://dx.doi.org/10.1139/b06-045.

Egli, S., 2011. Mycorrhizal mushroom diversity and productivity - an indicator of forest health? Ann. For. Sci. 68, 81–88. http://dx.doi.org/10.1007/s13595-010-0009-3.

Egli, S., Ayer, F., 1997. Est-il possible d'améliorer la production de champignons comestibles en forêt ? L'exemple de la réserve mycologique de la Chanéaz en Suisse. Rev. For. Française 49, 223–243.

Guzmán, B., Vargas, P., 2005. Systematics, character evolution, and biogeography of *Cistus* L. (Cistaceae) based on ITS, trnL-trnF, and matK sequences. Mol. Phylogenet. Evol. 37, 644–660. http://dx.doi.org/10.1016/j.ympev.2005.04.026.

Hernández-Rodríguez, M., de Miguel, S., Pukkala, T., Oria-de-Rueda, J.A., Martín-Pinto, P., 2015a. Climate-sensitive models for mushroom yields and diversity in *Cistus ladanifer* scrublands. Agric. For. Meteorol. 213, 173–182. http://dx.doi.org/10.1016/j.agrformet.2015.07.001.

Hernández-Rodríguez, M., Oria-de-Rueda, J.A., Martín-Pinto, P., 2013. Post-fire fungal succession in a Mediterranean ecosystem dominated by *Cistus ladanifer* L. For. Ecol. Manage. 289, 48–57. http://dx.doi.org/10.1016/j.foreco.2012.10.009.

Hernández-Rodríguez, M., Oria-de-Rueda, J.A., Pando, V., Martín-Pinto, P., 2015b. Impact of fuel reduction treatments on fungal sporocarp production and diversity associated with *Cistus ladanifer* L. ecosystems. For. Ecol. Manage. 353, 10–20. http://dx.doi.org/10.1016/j.foreco.2015.05.007.

Liu, B., Bonet, J.A., Fischer, C.R., Martínez-de-Aragón, J., Bassie, L., Colinas, C., 2016. *Lactarius deliciosus* Fr. soil extra-radical mycelium correlates with stand fruitbody productivity and is increased by forest thinning. For. Ecol. Manage. 380, 196–201. http://dx.doi.org/10.1016/j.foreco.2016.08.053.

Luoma, D.L., Frenkel, R.E., Trappe, J.M., 1991. Fruiting of hypogeous fungi in Oregon Douglas-Fir forests: seasonal and habitat variation. Mycologia 83, 335–353.

Martín-Pinto, P., Vaquerizo, H., Peñalver, F., Olaizola, J., Oria-de-Rueda, J.A., 2006. Early effects of a wildfire on the diversity and production of fungal communities in Mediterranean vegetation types dominated by *Cistus ladanifer* and *Pinus pinaster* in Spain. For. Ecol. Manage. 225, 296–305. http://dx.doi.org/10.1016/j.foreco.2006.01.006.

Martínez-de-Aragón, J., Riera, P., Giergiczny, M., Colinas, C., 2011. Value of wild mushroom picking as an environmental service. For. Policy Econ. 13, 419–424. http://dx.doi.org/10.1016/j.forpol.2011.05.003.

Martínez-Peña, F., Ágreda, T., Águeda, B., Ortega-Martínez, P., Fernández-Toirán, L.M., 2012a. Edible sporocarp production by age class in a Scots pine stand in Northern Spain. Mycorrhiza 22, 167–174. http://dx.doi.org/10.1007/s00572-011-0389-8.

Martínez-Peña, F., de-Miguel, Pukkala, S., Bonet, T., Ortega-Martínez, J.A., Aldea, P., Martínez de Aragón, J., 2012b. Yield models for ectomycorrhizal mushrooms in *Pinus sylvestris* forests with special focus on *Boletus edulis* and *Lactarius* group *deliciosus*. For. Ecol. Manage. 282, 63–69. http://dx.doi.org/10.1016/j.foreco.2012.06.034.

Mediavilla, O., Olaizola, J., Santos-Del-Blanco, L., Oria-De-Rueda, J.A., Martín-Pinto, P., 2016. Mycorrhization between *Cistus ladanifer* L. and *Boletus edulis* Bull is enhanced by the mycorrhiza helper bacteria *Pseudomonas fluorescens* Migula. Mycorrhiza 26, 161–168. http://dx.doi.org/10.1007/s00572-015-0657-0.

Oria-de-Rueda, J.A., Martín-Pinto, P., Olaizola, J., 2008. Bolete productivity of Cistaceous scrublands in Northwestern Spain. Econ. Bot. 62, 323–330.

Parladé, J., Martínez-Peña, F., Pera, J., 2017. Effects of forest management and climatic variables on the mycelium dynamics and sporocarp production of the ectomycorrhizal fungus *Boletus edulis*. For. Ecol. Manage. 390, 73–79. http://dx.doi.org/10.1016/j.foreco.2017.01.025.

Rivest, D., Rolo, V., López-Díaz, L., Moreno, G., 2011. Shrub encroachment in Mediterranean silvopastoral systems: *Retama sphaerocarpa* and *Cistus ladanifer* induce

contrasting effects on pasture and *Quercus ilex* production. Agric. Ecosyst. Environ. 141, 447–454. http://dx.doi.org/10.1016/j.agee.2011.04.018.

Rossini-Oliva, S., Mingorance, M.D., Monaci, F., Valdés, B., 2016. Ecophysiological indicators of native *Cistus ladanifer* L. at Riotinto mine tailings (SW Spain) for assessing its potential use for rehabilitation. Ecol. Eng. 91, 93–100. http://dx.doi.org/10.1016/j.ecoleng.2016.01.078.

Smith, J.E., Molina, R., Huso, M.M.P., Luoma, D.L., Mckay, D., Castellano, M.A., Lebel, T., Valachovic, Y., 2002. Species richness, abundance, and composition of hypogeous and epigeous ectomycorrhizal fungal sporocarps in young, rotationage, and old-growth stands of Douglas-fir (*Pseudotsuga menziesii*) in the Cascade Range of Oregon, USA. Can. J. Bot. 80, 186–204.

Taylor, A.F.S., 2002. Fungal diversity in ectomycorrhizal communities: sampling effort and species detection. Plant Soil 244, 19–28.

Team, R.C., 2016. R: A Language and Environment for Statistical Computing.