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DOCTORAL DISSERTATION/ TESIS DOCTORAL

Management of Mediterranean pyrophytic ecosystems dominated by *Cistus ladanifer* in order to increase mushroom production and prevent forest fires

Manejo de ecosistemas pirófitos mediterráneos dominados por *Cistus ladanifer* para incrementar la producción micológica y prevenir incendios forestales

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Contents

Abstract

Cistus species are pyrophytic shrubs widely distributed around the Mediterranean basin. *Cistus ladanifer* L. is within this genus, the most abundant species. It is associated with numerous fungal species, some of which reach a high economic value, such as *Boletus* group. Fungal diversity associated with these ecosystems and the production of these high-value species are threatened by the frequent occurrence of wildfires that put an important source of income for the rural population at risk. A proper management of these areas may contribute to decreasing fire risk and increasing economic benefit derived from mushroom production. Thus, the main objective of this thesis is to find the best management practices in order to increase mushroom production and prevent forest fires in Mediterranean pyrophytic ecosystems dominated by *C. ladanifer*.

In order to achieve this objective, we firstly studied the natural succession of fungal communities after fire. We pretended to understand the natural behavior of these ecosystems in the absence of management, creating a necessary knowledge for the following studies of the thesis. The second study assesses the effect of different fuel reduction treatments on sporocarp production and diversity. Due to the great variability of fungal fruiting, we sampled mushroom production for four years in order to have reliable results of the short-term effect of the treatments. From data about fungal yields and diversity collected in this study, we could develop empirical models that allowed us to predict the evolution of production and diversity of fungi over time, taking into account climatic variables and vegetation characteristics. In addition to the total production and diversity models, a specific model for the production of *Boletus edulis* was also developed, being the most economically important fungal species of this ecosystem. With the models developed in the previous chapter, we could carry out an economic assessment where we valued the profitability of different treatments, as well as their optimal rotation. In this study we took into account *B. edulis* production and the biomass obtained by cutting the bush. All the studies mentioned above are focused on the production of fruiting bodies. However, the fungal species fruiting aboveground are only a partial fraction of the total number of fungal species present in soil. Thus, in order to provide a full understanding of these ecosystems, we have

conducted a final study of soil fungal diversity with New Generation Sequencing techniques.

The results of these studies demonstrate that there is a high fungal diversity associated with these ecosystems, supporting the interest of conservation of these habitats, especially in extremely stony and poor soils. Mosaic management of these areas including patches of different ages and different fuel reduction treatments, may help to keep a high fungal richness associated with *C. ladanifer* succession at the same time that forest fires are prevented resulting from the interruption of fuel continuity. Although these treatments create new conditions that alter fungal fruiting, we found that they do not produce a permanent negative impact on fungal communities since the mycelium of the different species is able to remain in the soil after the treatments. From an economic point of view, the management practice associated with higher yields, and thus, the one that provides the greatest economic benefit is performing one total clearing in the final stage of *C. ladanifer* life cycle. On the other hand, if the management aim is more ecologically oriented (maintaining or increasing fungal diversity), controlled burning may be a better option. Adequate management of these areas would derive important economic benefits from mushroom harvesting in ecosystems that are traditionally considered unproductive, generally located in poor and economically disadvantaged rural areas.

Resumen

Las especies del género *Cistus* son matorrales pirófitos ampliamente distribuidos alrededor de la cuenca del Mediterráneo. Dentro de este género, *Cistus ladanifer* es la especie más abundante. Está asociada a numerosas especies de hongos, alguno de los cuales alcanzan un elevado valor económico como las pertenecientes al grupo *Boletus*. La diversidad fúngica asociada a estos ecosistemas y la producción de estas especies de elevado valor se ven amenazadas por la frecuente presencia de incendios que ponen en riesgo una importante fuente de ingresos para la población rural. Un adecuado manejo de estas zonas contribuiría a disminuir el riesgo de incendio y aumentar el beneficio económico derivado del aprovechamiento de hongos. Por lo tanto, el principal objetivo de esta tesis es encontrar las mejores prácticas de manejo de ecosistemas mediterráneos dominado por *C. ladanifer* para incrementar la producción micológica y prevenir los incendios forestales.

Para llevar a cabo este objetivo, estudiamos, en primer lugar, la sucesión natural de las comunidades fúngicas tras el fuego. Con este trabajo se pretende comprender el comportamiento natural de estos ecosistemas en ausencia de manejo, creando un conocimiento base necesario para los siguientes estudios de la tesis. El segundo capítulo evalúa el efecto de diferentes tratamientos de reducción de combustible en la producción y diversidad de carpóforos. Debido a la gran variabilidad en la fructificación de los hongos, se realizaron muestreos durante cuatro años, para conseguir unos resultados fiables del efecto a corto plazo de los tratamientos. A partir de los datos de producciones y diversidad fúngica recogidos en este estudio, se desarrollaron modelos empíricos que permitieron predecir la evolución de la producción y la diversidad a lo largo del tiempo, teniendo en cuenta variables climáticas y características de la vegetación. Se modelizaron tanto las producciones y diversidades totales para cada grupo trófico como la producción específica de *Boletus edulis*, al ser la especie con mayor importancia económica del ecosistema. A partir de estos modelos, se pudo llevar a cabo una evaluación económica donde evaluamos la rentabilidad de los diferentes tratamientos, así como su rotación óptima. En este estudio se tuvieron en cuenta tanto la producción de *B. edulis* como la biomasa obtenida al cortar el matorral. Todos los estudios anteriores están centrados en la

producción de carpóforos. Sin embargo, las especies de hongos que fructifican produciendo carpóforos epigeos son sólo una parte del número total de especies fúngicas presentes en el suelo. Debido a esto, se analizaron las comunidades de hongos del suelo mediante técnicas de secuenciación masiva, que complementa la información recogida en los estudios anteriores proporcionando un conocimiento completo sobre estos ecosistemas.

Los resultados de estos estudios demuestran la elevada diversidad fúngica asociada a estos ecosistemas, respaldando el interés de conservación de estos hábitats que generalmente aparecen en suelos muy pedregosos y pobres. Un manejo en mosaico de estas áreas, incluyendo parches de diferentes edades y distintos tratamientos de combustible, ayudaría a mantener la elevada riqueza fúngica asociada a las distintas etapas sucesionales de *C. ladanifer*. Al mismo tiempo se previenen los incendios forestales como consecuencia de la interrupción de la continuidad de combustible. Aunque estos tratamientos crean nuevas condiciones ambientales que alteran la fructificación de los hongos, en el estudio de las comunidades presentes en el suelo se constató que, al realizar los tratamientos, no se produce un impacto negativo permanente en las comunidades fúngicas, ya que el micelio de los hongos es capaz de permanecer en el suelo. Desde un punto de vista económico, la práctica de manejo asociada a mayores producciones y por tanto, la que produce mayores beneficios económicos es realizar un desbroce total cercano al final del ciclo de vida de *C. ladanifer*. Por otro lado, si el objetivo de manejo tiene una orientación más ecológica (mantener o aumentar la diversidad fúngica), la quema controlada puede ser la mejor opción. El manejo adecuado de estas áreas produciría importantes beneficios económicos derivados del aprovechamiento micológico en estos ecosistemas que tradicionalmente han sido considerados improductivos y generalmente se encuentran en zonas rurales económicamente desfavorecidas.

List of original articles

This thesis is based on five original works, which are referred to in the text with Roman numerals (**I - V**), published or under revision in different international journals. Authors, coauthors, and the stage of the publication are presented below:

I. 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. Forest Ecology and Management 289 (2013) 48–57.

II. Hernández-Rodríguez, M.; Oria-de-Rueda, J.A.; Pando, V.; Martín-Pinto, P. (2015). Impact of fuel reduction treatments on fungal sporocarp production and diversity associated with *Cistus ladanifer* L. ecosystems. Forest Ecology and Management 353 (2015) 10–20.

III. Hernández-Rodríguez, M.; de-Miguel, S.; Pukkala, T.; Oria-de-Rueda, J.A.; Martín-Pinto, P. (2015). Climate-sensitive models for mushroom yields and diversity in *Cistus ladanifer* scrublands. Agricultural and Forest Meteorology 213 (2015) 173–182.

IV. Hernández-Rodríguez, M.; Martín-Pinto, P.; Oria-de-Rueda, J.A.; Diaz-Balteiro, L. (2016). Optimal management of *Cistus ladanifer* shrublands for biomass and *Boletus edulis* mushroom production. Agroforestry Systems (accepted with minor revision).

V. Hernández-Rodríguez, M.; Geml, J.; Eberhart, J.; Olaizola, J., Oria-de-Rueda, J.A.; Martín-Pinto, P. (2016). Soil fungal community composition in a Mediterranean shrubland is primarily shaped by history of major disturbance, less so by current fire fuel reduction treatments. Manuscript in preparation.

Outline of the thesis

This thesis is divided into five studies focused on finding the best way of handling Mediterranean shrublands of *C. ladanifer* so that the benefit obtained by the mycological production increases while the occurrence of forest fires is reduced or prevented.

The first article of the compendium **(I)** is a study of the natural succession of fungal communities after fire. The objective of is study is to determine the natural behavior of these ecosystems in the absence of management, creating a necessary knowledge for the following studies of the compendium.

After knowing the natural behavior of the fungal community following fire, the second article **(II)** assesses the effect of different fuel reduction treatments on fungal production and diversity. Due to the great variability of fungal fruiting, we sampled mushroom production for 4 years in order to have reliable results of the short-term effect of the treatments.

From production and diversity data of several years, empirical models can be developed to predict the evolution of production and diversity of fungi over time, taking into account climatic variables and vegetation characteristics. This is the objective of the third article of the compendium **(III)**. In addition to the total production and diversity models, a specific model for the production of *Boletus edulis* was also developed, being the most economically important fungal species of this ecosystem.

With the models developed in the previous chapter, we could carry out the economic assessment presented in the fourth chapter of the compendium **(IV)**. In this study we valued the profitability of different treatments, as well as their optimal rotation. In this study we took into account *B. edulis* production and the biomass obtained by cutting the bush.

All the articles mentioned above are focused on the production of fruiting bodies. However, the fungal species fruiting aboveground are only a partial fraction of the total number of fungal species present in soil. Thus, in order to provide a full understanding of these ecosystems, we have conducted a study of soil fungal diversity with New Generation Sequencing techniques, presented in the fifth article of the compendium **(V)**.

A concept map of the thesis is shown in   Fig. 1.
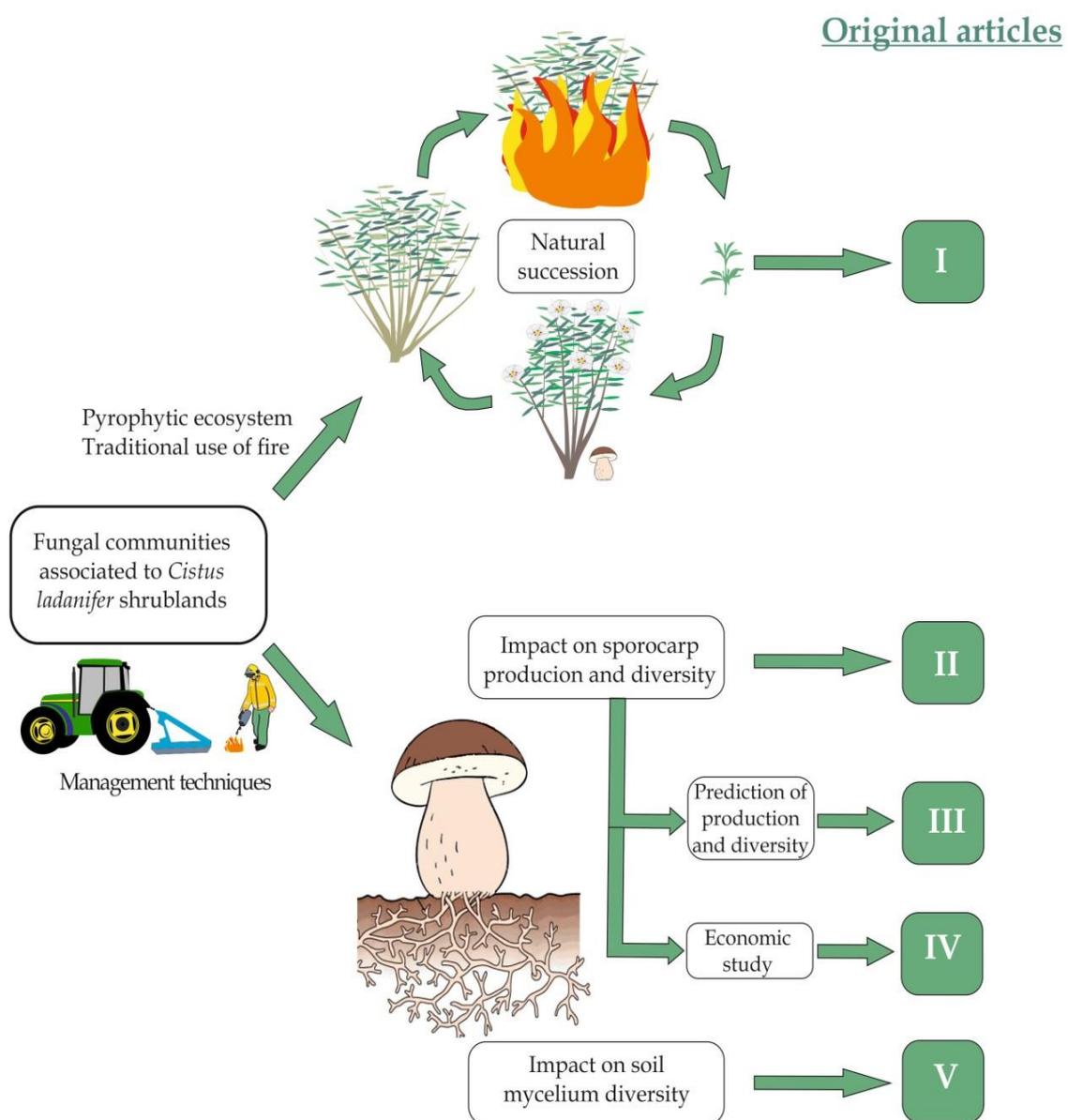
Fig. 1: Concept map of the thesis including all the original articles.

1. Introduction

1.1. *Cistus ladanifer* L. ecosystems

Cistus (Cistaceae) is one of the most characteristic genera of the Mediterranean shrub flora. It is mainly distributed around the Mediterranean basin (Fig. 2a) and it is represented in the Iberian Peninsula by 12 shrub species (Agueda et al., 2008).

Cistus ladanifer L. is within this genus, the most abundant species. It is an obligate-seeder shrub species widely distributed in the Western Mediterranean Region, including the Iberian Peninsula, southern France and North of Morocco and Algeria (Guzmán and Vargas, 2009) (Fig. 2b). In Spain, *C. ladanifer* has a distribution area of about 3 million hectares. It is present in 15% of the forest area of the country (Martín Morgado et al., 2005).
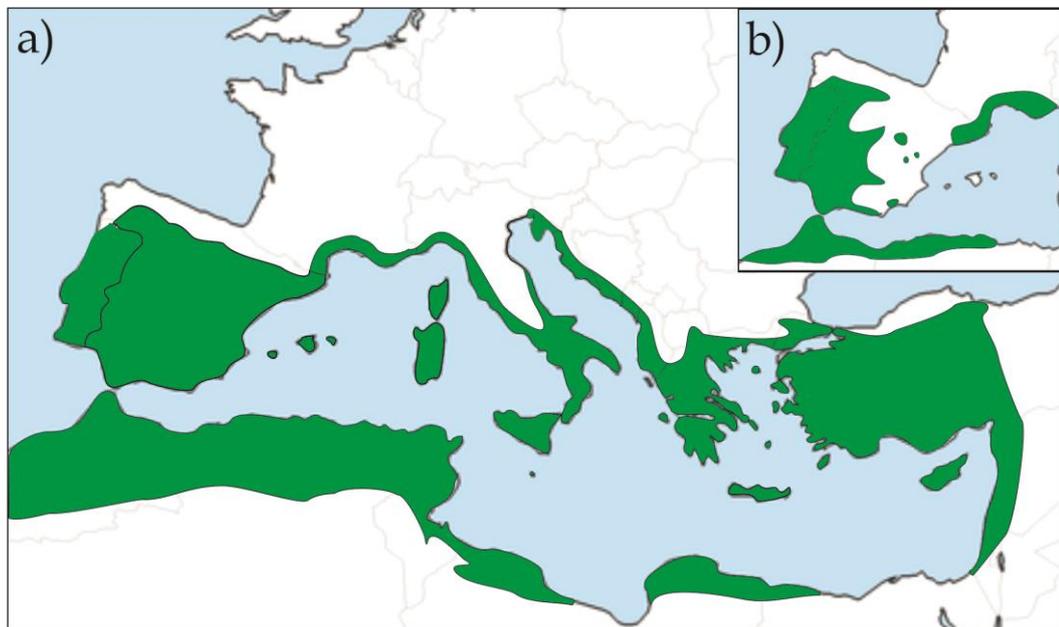
Fig. 2: Distribution of map of *Cistus* species (a) (based on Guzmán and Vargas, 2005) and *C. ladanifer* (b) (based on Guzmán and Vargas, 2009).

It is a pyrophytic species that constitute early successional stages in Mediterranean ecosystems. Senescent stands have a high flammability with high amounts of dead organic matter, stems covered by lichens and very flammable papery bark (Fig. 3). These characteristics make these areas optimal for the ignition and spread of fire and the virulence of the forest fires in these shrublands is very high difficultting their extinction. *C. ladanifer* can colonize highly degraded areas after fire as high temperatures generated by the perturbation in the top layers of the soil trigger seed germination (Bastida and Talavera, 2002). Thus, they often form pure stands in vast areas heavily subjected to fire and/or grazing (Agueda et al., 2008).

Fig. 3: Aspect of a senescent *C. ladanifer* shrubland (left) and detail of the papery bark in mature *C. ladanifer* stem (right).

During the last few decades, this type of shrub communities have been spreading in Spain and other parts of Europe due to the abandonment of both croplands and pasture lands and to the superposition of recurrent fire events (Mendes et al., 2015). Shepherds commonly use periodic burning as a management method to halt the proliferation of woody species and maintain pasturage. This practice continues in some areas at present in spite of the decrease in livestock and the lack of later use (Tárrega et al., 2001).

C. ladanifer shrublands have traditionally produced very scarce economic benefit, derived only from hunting and the use of firewood obtained from fire prevention clearing treatments. Therefore, these areas have not been paid attention in forest

management. However, production of edible wild mushrooms can provide potential economic benefits. *Cistus* species may form both ectomycorrhizae and vesicular arbuscular mycorrhizae and they are associated with more than 200 ectomycorrhizal fungal species of 40 different genera (Comandini et al., 2006). The most valuable fungal species associated with these ecosystems is *Boletus edulis* Bull., which is highly prized culinary mushroom in many countries (Boa, 2004), and perceived as a luxury good in the main Spanish markets (Voces et al., 2012). However, significant fruiting of these fungi in forest occurs only in mature stands (30-40 years old) (Oria-de-Rueda et al., 2008). Since fruiting in *C. ladanifer* ecosystems occurs at a much earlier age (3-4 years old), *C. ladanifer* scrubland conservation could be of interest in areas with poor and degraded soils, where the productivity of forested areas is very low and economic benefit scarce. According to this, Oria-de-Rueda et al. (2008) suggested that promotion and expansion of scrublands for bolete picking can be a valuable stimulant to local rural economies in these low productive areas. In addition, these species are ecologically important because they may act as a reservoir of mycorrhizal fungi after a forest disturbance (Torres et al., 1995). To properly maintain these areas it is necessary to carry out management techniques in order to increase economic benefits and to avoid the high risk of fire associated to senescent pyrophytic shrublands of *C. ladanifer*.

1.2. Fungal succession after fire

Wildfire events are the major disturbance in Mediterranean ecosystems, having a large impact on the physical, chemical and biochemical soil properties as well as on the above-ground vegetation. Intense heating of soil and ash deposition deteriorate soil structure, alter soil moisture and pH, decreases organic matter and nutrient availability (Grogan et al., 2000; Peay et al., 2009). These effects are more or less pronounced depending on soil characteristics or fire regime (size, intensity, duration and frequency) (Buscardo et al., 2015), being greater closer to the soil surface (Rincón and Pueyo, 2010). Fire produces direct effects on fungal communities by destroying belowground organisms (Buscardo et al., 2011) and indirect effects due to the changes in soil properties and vegetation composition. Thus, fires can affect the subsequent structure of fungal communities following succession patterns mainly driven by the

dynamics of post-fire plant communities (Cairney and Bastias, 2007). After a forest fire, pre-fire fungal communities are largely eradicated and secondary succession begins with the first significant rain (Claridge et al., 2009). Then, early-stage fungi or pioneer species are established in the burned area and their source is usually the spore bank present in the pre-fire soil which largely remains intact even after a high-intensity fire (Glassman et al., 2015). During succession, these fungi are joined but not necessarily replaced by late-stage species whose reproduction is primarily by clonal expansion (Iordache et al., 2009). Both early and late stage fungi are able to establish mycorrhizal association with plant roots although their nutrient requirements are different (Savoie and Largeteau, 2011) . Late-stage fungi require components released from mature plant roots, or the stimulating effect from early-stage mycelium for developing mycorrhizae (Savoie and Largeteau, 2011).

Fungal succession has been previously studied (Frankland, 1998; Savoie and Largeteau, 2011). However, most of the studies are focused only on ectomycorrhizal species growing in forest tree stands. Kipfer et al. (2011) studied the ectomycorrhizal succession in *Pinus sylvestris* stands after fire, and found a rapid resilience in terms of species number but not in species composition. Clark and St. Clair (2011) studied mycorrhizal secondary succession in a mixed aspen-conifer stand and found out that light limitation caused a decrease in ectomycorrhizal root infection in the later stages of the succession.

In Mediterranean ecosystems, Fernández-Toirán et al. (2006) and Gassibe et al. (2011) studied fungal mycorrhizal and saprotrophic sporocarps succession in *Pinus pinaster* stands. Fernández-Toirán et al. (2006) reported an increase of fungal richness with stand age, related to the higher number in the humicolous saprotrophic and mycorrhizal species. On the other hand, Gassibe et al. (2011) found that fungal species production followed different trends according with their trophic group: mycorrhizal decreased 6-fold, while saprotrophic increased 4-fold.

The short life cycle of *C. ladanifer* and its lack of post-fire resprouting capacity make it easier to compare early and late stages of succession. At the end of their life cycle (18-20 years), stands of this species wither and die, creating a very high risk of forest fires (Oria-de-Rueda et al., 2008). Understanding the ecology of the fruiting

pattern of different fungal species and the effect of natural disturbances on them, may be the means to improve natural mushroom richness and production.

1.3. Mushroom harvesting and mycosilviculture

Harvesting of wild mushrooms is becoming a very important socioeconomic resource in depressed rural areas (de Frutos Madrazo et al., 2012). Nowadays, mushroom picking involves 54% of the rural population of Castilla y León region and edible mushroom production can reach 65 million euros in potential income (Martínez-Peña et al., 2007). Although the most important use of harvested mushrooms is related to self-consumption (Voces et al., 2012), the impact of this non-timber forest product can be local and, in some cases, regional (Bonet et al., 2008), since a high proportion of the rural population is involved in their commercial harvesting and processing (de Román and Boa 2004). Increasing interest of mushroom harvesting encourages the installation of companies that transform and commercialize edible wild mushrooms in the producing areas. According to Palahí et al. (2009), the soil expected value generated by both, timber and mushrooms products in some forests is between four and ten times higher than the forests where only timber production is considered.

Traditionally forest planning and management has paid little attention to mushroom harvesting. However, the growing interest in this non-wood forest product makes the management of forest stands for mushroom production (mycosilviculture) increasingly more important (Savoie and Largeteau, 2011). This forest management planning, such as clear-cutting, timber harvesting (Dahlberg et al., 2010), and other land use practices (Azul et al., 2009) have significant effects on nutrient cycles, and may strongly affect higher fungi communities (Azul et al., 2014). This has led scientists to study the state of conservation of diversity and production of wild mushroom communities and the effect of silvicultural treatments on them (Martínez-Peña et al., 2011). Most of these studies are focused on the effect of thinning on fungal communities in tree stands. Shaw et al. (2003) found little effect of fungal productivity five years after thinning under *Pinus sylvestris*. Pilz et al. (2006) reported that sporocarp number and weight production of *Cantharellus formosus*, decreased significantly the

first year after thinning, but no differences were found during the following six years. Temporal relationship between tree growth reaction after thinning and the production of associated fungal community was observed in a mixed old-growth forest in Switzerland (Egli et al., 2010). These authors found low production during the first 3 years after thinning and a recovery during the fourth year. On the other hand, Bonet et al. (2012) found an immediate positive effect of thinning on *Lactarius deliciosus* production in a *Pinus pinaster* plantation. Regarding *B. edulis* production, Salerni and Perini (2004) studied the effect of tree canopy in order to increase the productivity of this species in different forest stands in Italy, finding a major number of *B. edulis* carpophores counted in medium thinning stations.

The effects of forest management on understory vegetation have been studied in various regions in Europe. Azul et al. (2011) tested the influence of land use practices used to control shrub density on macromycetes fruiting in *Quercus suber* woodlands. These authors found that current practices used to control shrub density explain considerable variation in mushroom fruiting. The effects of permanent grazing or soil tillage were more severe than cutting practices without soil tillage. Focusing on *C. ladanifer* shrublands, few previous works have studied the use of different management methods, most of them aimed at increasing plant diversity associated with these ecosystems (Mendes et al., 2015; Tárrega et al., 1995).

From an economic point of view, studies on wild edible mushrooms are limited. Constraints such as the absence of data of the mushrooms picked, or the lack of transparency in value chain traceability (Cai et al., 2011; Voces et al., 2012) could explain this scarcity of studies on some economic aspects of wild edible fungi.

1.4. Empirical models as a useful tool for forest managers

Including of mushroom production as an explicit objective in forest management requires models for assessing, quantitatively, the mushroom yields in different forest stands and management scenarios (Calama et al., 2010). These models can be developed with statistical methods, using empirical data on mushroom production and forest stand characteristics (Bonet et al., 2010, 2008). Empirical models explain

sporocarp production from other parameters more easily measurable such as climate, vegetation and soil characteristics and are a very useful tool to integrate mushroom production and diversity in the management of forest ecosystems (Bonet et al., 2008; de-Miguel et al., 2014). The inclusion of these models in forest simulators may provide quantitative information on mushroom production and its economic effects in alternative forest management schedules (Palahí et al., 2009).

The high inter-annual and spatial variability of fungal fruiting require the systematic collection of long-term annual data series in order to obtain sufficiently representative data (Calama et al., 2010). In addition, these models need to consider the wide range of factors that influence the development of fruiting bodies. Climate and weather are key drivers of mushroom production. Positive correlation between mushroom production and climatic variables (i.e., precipitation and temperature) has been reported in several studies. Straatsma et al. (2001) found that fungal productivity was related to precipitation from June to October in a mixed forest in Switzerland. Gassibe et al., (2014) reported a significant effect of temperature and precipitation on mushroom production in Northwestern Spain. Martínez-Peña et al. (2012) concluded that temperature seems to be more crucial than rainfall for explaining fungal production in *Pinus sylvestris* forests in Spain. However, the manipulation of the climatic conditions is only realistic in special conditions e.g. through irrigation. Therefore, knowing the effects of biotic factors is even more important (Tahvanainen et al., 2016). Management-oriented models should include predictors related to the vegetation structure (e.g., mean height, canopy cover or age), which can be modified by means of silvicultural techniques.

The use of statistical models to study fungal communities is relatively new and only a few models for predicting mushroom yield and diversity have been published (Bonet et al., 2012, 2010, 2008; Dahl et al., 2008; de-Miguel et al., 2014; Gassibe et al., 2014; Martínez-Peña et al., 2012; Tahvanainen et al., 2016)

1.5. Soil fungal communities

Traditionally, studies of fungal communities were based only on epigeous sporocarp inventories, assuming that sporocarp production reflects the relative abundance and importance of the species on the root system (Vogt and Bloomfield, 1992). These studies provide important information on the investment of the fungal species in reproduction, but data about total fungal diversity of the ecosystem are not accurate (Baptista et al., 2015). Some fungal species do not produce noticeable sporocarps (Bidartondo and Gardes, 2005). Thus, the difference between fruiting patterns and vegetative patterns of fungal communities is important (Horton and Bruns, 2001).

The development of molecular tools for biodiversity characterization based on DNA extraction provides new opportunities to study fungal communities (Orgiazzi et al., 2015). Furthermore, the recent emergence of next-generation sequencing (NGS) methods has allowed to obtain millions of DNA sequences in a relatively short period of time (Glenn, 2011). These methods were firstly applied to mycorrhizal root tips, but current application of NGS to DNA extractions from the soil matrix allow to access a great proportion of the soil fungal diversity including saprotrophic and pathogenic fungi (Baptista et al., 2015). The internal transcribed rDNA spacer (ITS) region is the most frequently sequenced genetic marker of fungi (Begerow et al., 2010).

Soil biodiversity studies have two main objectives: firstly, to obtain knowledge about soil biodiversity structure and functions. Secondly, to assess the level and fluctuations of soil biodiversity in different environmental conditions, which can be very useful for decision makers in order to establish the optimal measures for the preservation of soil biodiversity (Orgiazzi et al., 2015). Thus, the knowledge of the effect of management methods on soil fungal communities associated with *C. ladanifer* would provide the necessary information for managers of these ecosystems in order to make the most suitable decisions for the conservation of these areas.

2. Objectives of the thesis

As noted in the introduction, *C. ladanifer* is associated with numerous fungal species. Some of these species reach a high economic value, such as *Boletus* group. Fungal diversity associated with these ecosystems and the production of these high-value species are threatened by the frequent occurrence of wildfires that put an important source of income for the rural population at risk.

To improve this situation, the main objective of this work is to find the best management practices in order to increase mushroom production and prevent forest fires in Mediterranean pyrophytic ecosystems dominated by *C. ladanifer*. The specific objectives of the thesis are:

1. To analyze the fungal community succession following wildfire in a Mediterranean vegetation type dominated by *C. ladanifer* in order to know the behavior of these communities in the absence of specific management **(I)**.

2. To study the effects of different fuel reduction treatments in *C. ladanifer* scrublands on the production and diversity of fruiting bodies of associated fungal communities **(II)**.

3. To develop predictive climate-sensitive models for mushroom production and diversity in *C. ladanifer* scrublands, with special attention to *B. edulis* sporocarp production **(III)**.

4. To determine the management practices which are more economically profitable considering mushroom production and biomass in these ecosystems **(IV)**.

5. To study the effect that of the fuel reduction treatments on the diversity of fungi in the soil (mycelium) **(V)**.

3. Material and methods

3.1. Study site

The study area was located in North-western Spain (0730462–0731929 Longitude-UTM, 4619644–4621757 Latitude-UTM 29T Grid) (Fig. 3.1)
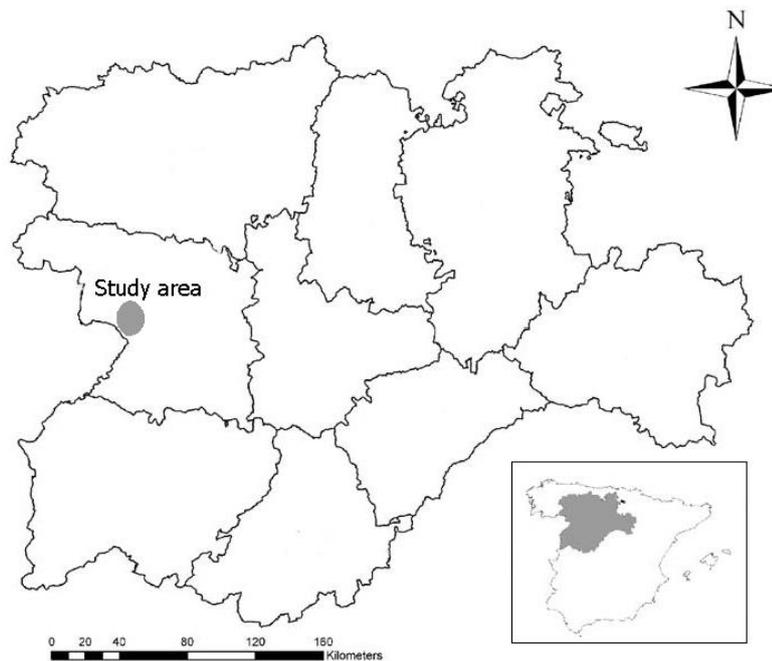
Fig. 3.1: Location of the study area

- Mediterranean ecosystem dominated by *C. ladanifer*
- 750-780 m above the sea level.
- The soil is classified as Inceptisol suborder Xerept (Soil Survey Staff, 2010).
- The area is characterized by a sub-Mediterranean climate with a dry season of three months in the summer and a mean annual precipitation of 450-700 mm.
- Mean temperatures range from 14.5 to 15.8 ºC.

3.2. Experimental design

- All the sampling plots consisted on lineal transects of 2m x 50m **(I, II, III, IV, V)**.

- Post-fire fungal succession **(I, III)**:

  - Six "early stage" plots on areas burnt in August 2002

  - Six "late stage" plots on 15 year-old monospecific stands of *C. ladanifer*.

- Influence of management techniques **(II, III, IV, V)**:

  - Three areas where *C. ladanifer* age and stand origin were different

    a) Middle-age stand (8 years old) whose origin was a forest fire.

    b) Middle-age stand (8 years old) whose origin was the clearing of the previous stand.

    c) Senescent stand (20 years old) whose origin was a forest fire.

  - Treatments were chosen based on their applicability in accordance with the age of the stands and vegetation characteristics (Figs. 2.3 and 2.4):

    a) Both middle-age stands treatments:

      - Control

      - 50% manual clearing

      - Total clearing

    b) Senescent stand treatments:

      - Control

      - Total clearing

      - Controlled burning

Fig. 3.3. Preparation of the security perimeter for controlled burning (left) and controlled burning (right)

Fig. 3.4. Implementation of total clearing (top) and manual clearing (bottom)

3.3. Sampling

Sporocarp sampling, identification and classification (I, II, III, IV).

- Sporocarp sampling was performed on a weekly basis from late October through late December from 2003 to 2006 (I) and from 2010 to 2013 **(II, III, IV).**

- Fungal sporocarps were harvested, transported to the laboratory and stored at 4°C.

- The sporocarps were identified at species level whenever possible.

- Fresh weight and number of sporocarps were measured.

- The sporocarps were dried in air-vented ovens at 35°C and dry weighed.

- Shannon's H' diversity index (Shannon and Weaver, 1949) based on dry weight of the fruiting bodies (Dahlberg, 1991) was calculated **(I, II, III)**. This variable was calculated using the following formula where

coefficient *pi* indicates the proportion of the total biomass of each fungal species: $H' = - \Sigma \, p_i \cdot (\ln p_i)$

- Species richness (S) refers to the total number of taxa found each sampling year (Straatsma and Krisai-Greilhuber, 2003) **(I, II)**.

Soil sampling and molecular work (V)

- Five soil cores per plot were extracted along the transencts' centerline and 5 m from each other (cylindrical 2 cm radius, 20cm deep, 250 cm3 soil borer) in April 2014.
- Samples were frozen immediately on the sampling date upon return to the laboratory and kept at -20ºC until DNA extraction.
- Samples were thawed, let dry at room temperature to constant weight and then sieved with a mesh size of 1 mm.
- The five cores of each plot were pooled resulting in a composite soil sample for each plot.
- DNA extraction with the PowerSoil™ DNA Isolation Kit from 0.25 g of soil per sample.
- Polymerase Chain Reaction (PCR) amplification.
    - Amplification of the ITS1 region using the forward primer ITS1F and barcoded reverse primer ITS2
- Purification of PCR amplicons with Zymo Clean and Concentrate Kit™.
- Normalization and pooling of PCR products.
- Paired-end sequencing (2x150bp) on Illumina MiSeq sequencer.

3.4. Climatic data

Climatic data were provided by the closest meteorological station of the Spanish Meteorological Agency with available data for each period of study.

- 2003-2006 data from Villardeciervos station (41º56'27''N, 06º17'05''W and 850m above sea level) **(I)**.

- 2010-2013 data from Alcañices station (41º41′03′′N, 06º18′04′′W and 806m above sea level) **(II, III)**.
- Mean monthly potential evapotranspiration (ETP) was calculated by the empirical method of Thornthwaite and Mather (1955) for the latitude of Villardeciervos meteorological station **(I)**.

2.5. Data analysis

- Sporocarp production and diversity

    - Repeated measures ANOVA **(I)**
    - Fisher Least Significant Difference (LSD) test **(I, II)**
    - Detrended Correspondence Analysis (DCA) **(I)**
    - Canonical Correspondence Analisis (CCA) **(I)**
    - Monte Carlo Permutation Test **(I)**
    - Linear mixed model of analysis of variance with repeated measurements **(II)**
    - Permutational multivariate analysis of variance (perManova) **(II)**
    - Non-metric multidimensional scaling (NMDS) **(II)**

- Modelling

    - Nonlinear regression analysis **(III)**

- Economic study

    - Optimal rotation analysis **(IV)**
    - Dynamic programming **(IV)**

- Bioinformatic and data analysis of soil fungal diversity **(V)**

    - Forward and reverse reads joined. Trimming off primer sequences (MOTHUR v. 1.35)
    - Sequences filtered using MOTHUR:
        - No ambiguous bases
        - Homopolymers no longer than 10 nucleotides
        - Length range from 150 bp to 400 bp

- Sequences collapsed into unique sequence types, preserving their original read counts and global singletons
- Putative chimeric sequences removed with USEARCH v.8.0
- Reference dataset: UNITE dataset of fungal ITS sequences
- Sequences grouped into operational taxonomic units (OTUs) at 97% sequence similarity using USEARCH.
- Exclusion of OTUs with < 70% similarity or < 150 bp pairwise alignment length to a fungal sequence.
- Non-metric multidimensional scaling (NMDS) on a primary presence/absence matrix of sites by OTUs and a secondary matrix of sites by treatment types (PC-ORD v. 6.0).
- 'Presence' defined as ≥5 sequences on a per sample basis to minimize false positives.
- Statistical differences of fungal communities across shrubland origin and treatment types tested using two different methods:
    - Multiresponse permutation procedure (MRPP)
    - Permutation-based nonparametric MANOVA (PerMANOVA) (Anderson, 2001).
- Indicator species analyses (PC-ORD).

4. Results

4.1. Fungal succession after fire in *C. ladanifer* ecosystems (I)

In the study of the natural postfire succession we verified the high number of taxa that are able to produce sporocarps in *C. ladanifer* Mediterranean ecosystems, finding a total of 146 taxa (Table 2, study **I**). Total richness (number of taxa) and diversity (Shannon diversity Index) were only significantly different the first year after the fire, showing similar values among the early and late stages for the rest of sampled years. However, although quantitatively the richness and diversity recover quickly after a fire, the specific composition of fungal communities associated with early and late stages are very different. Twenty-three taxa were found exclusively in the early stage plots and 56 taxa exclusively in the late stage plots. Sporocarp biomass production showed a growing pattern during the early stage, with mean total values ranging from 4.27 kg ha^{-1} in the first sampling year to 47.43 kg ha^{-1} in the fourth sampling year. For the late stage, biomass remained constant and lower (between 1.43 and 9.33 kg ha^{-1}) (Table 3, article **I**).

Classifying fungi in function of their trophic strategy, both early stage and late stage had a greater number of saprophytic species. However, in the early stage, the number of mycorrhizal taxa presented an increasing tendency becoming greater than the number of the saprotrophic taxa the last sampling year (Fig. 1 & 2, article **I**).

Concerning fungal composition, the results obtained in this study showed an important shift from the early to the late stage of *C. ladanifer* scrubland. According to CCAs, there was a clear effect of *C. ladanifer* age on dry weight of the different fungal taxa in both functional groups. Significant climatic variables were the minimum annual temperature and annual precipitation in the case of mycorrhizal taxa and annual precipitation in the case of saprotrophic taxa (Fig. 3, article **I**).

4.2. Effects of management treatments fungal community (II & V)

Effect of treatments in sporocarp production, richness and diversity (II)

In the study of the effect of the different fuel reduction treatments on fungal communities, we also find a very high fungal species richness (157 taxa). The total taxa found during the four years of sampling revealed a predominance of saprotrophic taxa (95 taxa, 61%), while 39% (62 taxa) were identified as mycorrhizal.

Total average fresh weight production found in this study was 157.45 kg fw ha^{-1} yr^{-1}. Mycorrhizal taxa showed lower production in those treatments where vegetation was completely removed, higher values being presented in the control plots and 50% clearing treatments. In the case of saprotrophic taxa, the only significant difference was the controlled burning in the senescent stand, which presented the lowest saprotrophic production (Fig. 1, article **II**).

Mycorrhizal species richness and diversity was significantly higher in all the treatments where vegetation was not completely removed (control plots and 50% clearing). The highest value of saprotrophic taxa richness was found in the control plots in the senescent stand compared to the control plots in the mid-rotation ones. Controlled burning negatively affected the richness and diversity of saprotrophic taxa, presenting significantly lower values compared to control (Figs. 2 & 3, article **II**).

Treatment similarity and species composition (II & V)

The effect of treatment, type of stand and their interaction significantly affected sporocarp composition (article **II**). However, in the study of soil fungal communities, there are only significant differences between the different origins of the shrublands and not between the different treatments (article **V**). The NMDS plots revealed a strong structuring of fungal communities according to the origin of the shrublands in both studies of sporocarp and soil fungal communities. In addition, both plots show that the source with greater variability in their specific composition is the senescent shrubland

(Fig. 4, article **II**; Fig. 2, article **V**). Sporocarp production was affected by the different fuel reduction treatments. Only the two 50% clearing treatments are very similar to the control treatments located in the same stand according to their specific composition (Fig. 4, article **II**).

4.3. Production and diversity models (III)

C. ladanifer growth models

According to our results, *C. ladanifer* growth in height and coverage evolves over time according to the following equations:

$$H = \exp\left(5.07553 - 3.33490 \cdot \exp\left(-0.34492Y\right)\right)$$

$$CC = 79.7675 \cdot \left[1 - \left(1 + \frac{2Y}{2.7982 - 1.7610 \cdot TR} + \frac{2Y^2}{\left(2.7982 - 1.7610 \cdot TR\right)^2}\right) \cdot \exp\left(-2Y \Big/ \left(2.7982 - 1.7610 \cdot TR\right)\right)\right]$$

where H is the mean height of *C. ladanifer* (centimeters), and Y is the time after treatment (years), CC is the canopy cover of the scrubland (%),and TR is a dummy variable (i.e., equal to 1 if the scrubland was established after fire and equal to 0 if the scrubland was established after total clearing).

The simulation of these models is shown in Fig. 1, Article **III**. Mean height growth for *C. ladanifer* did not show any significant difference between the two studied treatments. However, canopy cover development was significantly different for the two studied treatments. After fire, canopy cover is greater at younger ages than after total clearing during the early years. Fifteen years after the treatments, the canopy cover of *C. ladanifer* becomes similar.

Models for mushroom production

Table 1 shows all the models adjusted for production and diversity of fungi, taking into account both the time after treatment and the characteristics of the vegetation.

Table 1: Empirical models of mushroom yields and diversity as a function of time after treatment and vegetation characteristics. Different models are presented for the two main trophic groups of fungi (mycorrhizal and saprotrophic).

Production	$MycoP = \exp(-4.7588 + 2.4323 \cdot \ln(Tm\min_O) + 4.1331 \cdot \sqrt{Y} - 0.7497Y)$
	$MycoP = \exp(-9.44295 + 1.73855 \cdot Tm\min_O + 2.43656 \cdot \sqrt{H} - 0.12501 \cdot H)$
	$SaproP = \exp(-4.90905 + 3.91042 \cdot \ln(Tm\min_{O+N}) - 0.08398 \cdot Y)$
	$SaproP = \exp(-34.074016 + 12.503545 \cdot \ln(Tm\min_{S+O+N+D}) - 0.008145 \cdot H)$
	$B.edulis = \exp(-17.09504 + 0.21615 \cdot T_{S+O} + 8.70958 \cdot \log(Y + 0.001) - 0.65478 \cdot Y)$
	$B.edulis = \exp(-1537 + 0.3669 \cdot Tm\min_{S+O} + 20.85 \cdot \sqrt{H} - 0.8591 \cdot H - 130.4 \cdot \sqrt{CC} + 587.5 \cdot \ln(CC + 0.001))$
Diversity	$MycoD = \exp(-2.360987 + 0.048370 \cdot T\min_{S+O} + 0.004765 \cdot P_S - 0.185691 \cdot T + 1.383084 \cdot \sqrt{Y})$
	$MycoD = \exp(-6.937640 + 0.117106 \cdot Tm\min_{S+O} + 0.004609 \cdot P_{A+S} - 0.041030 \cdot H + 0.922719\sqrt{H})$
	$SaproD = \exp(-0.15733 + 0.03557 \cdot T\min_{O+N+D} - 0.06509Y + 0.4442\sqrt{Y}$
	$SaproD = \exp(-0.025681 + 0.041956 \cdot T\min_{O+N} - 0.006450 \cdot CC + 0.199355 \cdot \ln(CC + 0.001))$

MycoP: annual mushroom yield from all mycorrhizal taxa (kg ha^{-1}yr^{-1}); *SaproP:* annual mushroom yield of saprotrophic taxa (kg ha^{-1}yr^{-1}); *B. edulis*: annual yield of *Boletus edulis* sporocarps (kg ha^{-1}yr^{-1}); *MycoD*: Shannon diversity index of mycorrhizal taxa; *SaproD*: Shannon diversity index of saprotrophic taxa; *Y*: time after treatment (years); *H*: mean height of the scrubland (cm); *CC*: canopy cover of the scrubland (%). *T*: mean temperature (ºC); $Tm\min$: mean minimum temperature; $T\min$: minimum absolute temperature (ºC); *P:* precipitation (mm); A: August; S: September; O: October; N: November; D: December.

The simulation of all the models presented in Table 1, are shown in Figs. 2, 3 & 4 of the article **III.**

4.4. Economic study (IV)

The results of the optimal rotation showed that, when mushroom picking is not included in the economic analysis (business as usual, BAU), the clearing treatment should be done every six years and the income that can be obtained from *C. ladanifer* biomass reaches 182.8€. If *Boletus edulis* harvesting is included, the net present value (NPV) rises notably and the rotation is situated between 19 years (when the clearing is done) and 21 years (when controlled burning is carried out). When there is a combination between a partial and a total clearing or a partial clearing and burning, the rotation extends to 25 years (Table 1, article **IV**).

According to the results of the dynamic analysis, the maximum land expectation value (LEV) (815.4€ ha^{-1}) was produced when the first (and only) intervention occurred at 24 years and was a total clearing (state 627 in Fig. 1, article **IV**). This optimal solution was clearly superior to that of the BAU (Table 3, article **IV**) and also to the alternative of not performing any silvicultural intervention to reduce the biomass (785.7€ ha^{-1}).

5. Discussion

C. ladanifer ecosystems are very common in large areas in the Iberian Peninsula and North Africa, and other Mediterranean regions. Due to the pyrophytic characteristics of this species, forest fires are the main threat to these ecosystems. Adequate management of these ecosystems could result in a decrease in the effect of fire on forest systems and an increase in the production and diversity of fungal communities adding economic and ecological value to these areas. This thesis addresses different ecological and economic aspects in order to acquire an in-depth understanding about fungal communities associated with *C. ladanifer* shrublands and provide guidelines for proper and sustainable management of these areas.

There is a real interest on the part of its managers in the integration of mycological resources into forest planning in the study area. Thanks to the results of this thesis, forest managers will include shrublands in mycological planning. They are beginning to implement regulation in the areas with the highest mushroom production, in which collectors pay to acquire a picking permit. This will provide new economic benefits in the future. In addition, these ecosystems produce boletes at a much earlier age than the trees (Oria-de-Rueda et al., 2008), so income from them would be received earlier than in a forested area. Therefore, the results obtained in this thesis can be applied to the current management of this type of forest systems. It should be noted that the experimental design was not replicated at different sites, and the results cannot be extrapolated to those in other areas. Thus, further research is necessary in other areas to provide general *C. ladanifer* management guidelines. However, we believe that this thesis provides a first approach that can be useful in the management of these ecosystems.

We found a very high number of fungal taxa associated with *C. ladanifer* shrublands: considering just the species that are able to produce conspicuous sporocarps, 146 taxa were found in the four-year study of the natural fungal succession after fire (**I**) and 157 taxa were found in the four-year study of the effect of management treatments (**II**). Contrary to expectations, fungal richness found in these

ecosystems was similar to or even higher than the number of taxa found in some studies of mature stands of different forest tree species. Gassibe et al. (2011) reported 115 fungal taxa in a four-year study in a nearby *Pinus pinaster* mature stand. Furthermore, Martínez-Peña et al. (2012) found 119 species in a ten-year study in *Pinus sylvestris* stands of different age classes in Spain, and Baptista et al. (2010) Baptista et al., (2010) recorded 73 different species in a four-year study in *Castanea sativa* in Portugal. In addition, the study of soil fungal communities (**V**) reveals a high presence of soil fungal species associated with the rhizosphere of *C. ladanifer* that were not previously identified in the sporocarp sampling

As a pyrophitic species, C. *ladanifer* is adapted to recurrent wildfires. The characteristics of senescent *C. ladanifer* scrublands facilitate fire ignition and spread and seed germination is triggered-off by the fire-induced increase in the temperature of the top layers of the soil (Bastida and Talavera, 2002). This feature is reflected in the growth models presented in the study **III**, which show significant differences in the canopy cover development of *C. ladanifer* scrublands after fire and after total clearing. This result also agrees with Tárrega et al. (2001) Tárrega et al. (2001), who studied the recovery dynamics after controlled burning and cutting during 11 years. They found that *C. ladanifer* recovery is faster after burning than after cutting during the early development stages of the shrubland.

Obviously, fungal communities associated with *C. ladanifer* are also affected by these recurrent wildfires. According with the results of this thesis, we found a decrease in fungal sporocarp richness and diversity the first year after wildfire (**I**) or controlled burning (**II**). In the study of the natural succession after fire, the number of taxa showed quite similar values in both successional stages two years after fire (**I**). This could indicate that the colonization process after a wildfire disturbance, regarding richness and diversity, takes two years. Also in Spain, Rincón and Pueyo (2010) observed that seedling mycorrhizal colonization significantly increased from the first to the second autumn after fire in a fungal community associated with *Pinus pinaster.* The presence of mycorrhizal taxa in the early stage of succession can be explained by their survival strategy in which these taxa remain alive in the form of different resistance structures such as infected root tips, sclerotia or resistant spores (Cairney

and Bastias, 2007). Thus, the ectomycorrhizal fungal community established after a fire is a reflection of the soil resistance structure bank that existed before the disturbance (Baar et al., 1999). Kipfer et al., (2011) also found rapid resilience in fungal species number on burnt *Pinus sylvestris* stands in Central Alps. According to these authors, pioneer tree species and shrubs may facilitate the establishment of a ectomycorrhizal network after a stand-replacing fire.

In order to reduce the incidence and negative effects of wildfires while facilitating the sustainable harvesting of marketable mushrooms, management of these areas should take into account measures to reduce fuel continuity. Due to the traditional belief that monospecific shrublands of *C. ladanifer* do not provide any economic benefit, the few studies that exist on finding the best management practices of these ecosystems are aimed at changing the plant species or favoring the establishment of tree stands (Mendes et al., 2015; Pérez-Devesa et al., 2008).

Temporary site disturbance caused by silvicultural treatments may produce a decrease in fungal production during the first years (Egli et al., 2010). In this thesis we studied the effect of different fuel reduction treatments on sporocarp production of and soil fungal mycelia, in shrublands with different origins. The specific composition of the three different origins of the shrublands (middle age from fire, middle age from total clearing and senescent) is significantly different from each other considering both sporocarp production and soil fungal communities (**II** & **V**). In addition, higher mushroom production was observed in the two middle-aged shrublands compared to the senescent stand (**II**). This result agrees with previous studies (Bonet et al., 2004; Senn-Irlet and Bieri, 1999) and may be explained by the lower photosynthetic activity of the senescent scrubland and its decreased growth. Therefore, to enhance mushroom production, management should be aimed at rejuvenating senescent *C. ladanifer* scrublands.

Although the treatments did not significantly affect soil fungal composition within a single origin (**V**), considering mushroom production we found a significant effect between treatments performed in shrublands of the same origin (**II**). Therefore, although the new conditions created by eliminating the vegetation alter fungal fruiting, the mycelium of the different species is able to remain in the soil. This is consistent

with the findings of Jennings et al. (2012), which concluded that soil fungal communities are resilient to mechanical treatments.

Mycorrhizal sporocarp production, richness and diversity were significantly higher in the treatments where vegetation was not completely removed (**II**). This is an expected result considering the characteristics of mycorrhizal species that require the presence of a host plant species for their survival (Dahlberg, 2002). In contrast to the results found in mycorrhizal species, no differences were found in the species richness of saprotrophic species within the two 8-year-old scrublands (**II**). After a stand clearing disturbance, new soil and light conditions may facilitate the establishment of early succession species (Clark and St. Clair, 2011). In the case of this study, pioneer species are dominated by saprotrophic taxa. In addition, a large number of saprotrophic species were found exclusively in the control treatment of the senescent stand, which is likely due to the large amount of organic matter present in these plots.

As mentioned above, controlled burning decreased considerably mushroom production (**II**). However, it had no significant effect on the presence of soil fungal mycelium (**V**). Prescribed fires are usually conducted under conditions that result in lower fire intensity and severity, and generally outside of the high risk conditions that are most conducive to the high intensity wildfires (Oliver et al., 2015). Thus, soil temperatures reached during a controlled burning are lower than during wildfire and therefore, the impact on the mycelium is lower.

Partial 50% clearing was the treatment with the least impact on the fungal community (production and diversity), and it was associated with a significant production of edible fungi (**II**). Besides, with this silvicultural treatment, there is an increase in total *B. edulis* production across the planning horizon. Oria-de-Rueda et al. (2011) recommended performing partial clearings every seven years, and using mechanized total clearings only to rejuvenate senescent shrublands. This treatment can reduce the amount of fuel, thus decreasing forest fire intensity and facilitating its extinction. In addition, 50% clearing may also enable accessibility and mushroom harvesting by decreasing scrublands density. However, when studying the profitability of the management alternatives that include partial clearing, taking into account the production of mushroom and biomass, the benefits are much lower in a

longer optimal rotation than the alternatives including only total clearings or burning, due to the high cost of this treatment (**IV**).

Considering total clearing or controlled burning as the two economically feasible management options, the results of the study **III** suggest an opposite trend in fungal diversity as compared to mushroom yield. The Shannon diversity index for both mycorrhizal and saprotrophic species was higher after burning than after clearing, whereas mushroom production was higher after total clearing. These results can be of interest from the management point of view. If the management objective is economic (i.e., to increase edible mushroom yield), total clearings may be a better treatment alternative. On the contrary, if the aim is more ecologically oriented (i.e., high diversity), burning may be better than clearing.

According to the economic analysis, *B. edulis* harvesting can increase considerably the economic benefits in these ecosystems (**IV**). This is in accordance with previous studies on tree stands which have shown that including mushroom harvesting in the management can increase the profits, providing an even higher value than timber (Palahí et al., 2009). In addition, integration of fungal resources into forest management plans would ensure their conservation over time and stop depletion (Aldea et al., 2012)

The silvicultural treatment that produced the highest profit was total clearing. When considering only the 25 years of the planning horizon, the ideal age for performing this treatment is 24 years. In the case of considering clearing the shrubland indefinitely at the same age, the most appropriate time is 19 years. This time is determined mainly by the production curve of *B. edulis,* which reaches a maximum at 13 years, decreasing towards the end of the planning horizon (**III**). On the other hand, *C. ladanifer* development is fast at early ages and biomass remains practically unchanged from 10-11 years old until the end of the planning horizon, so the associated benefits remain constant after this age (**III & IV**). The optimal age to perform the total clearing is in the final stage of *C. ladanifer* life cycle. Therefore, this management would ensure that all stages of the shrub are present in the area. Thus, the optimal solution from the economic point of view would also be suitable from an ecological point of view.

6. Conclusions

1. High fungal richness found in the early and late stage of *Cistus ladanifer* scrublands supports the interest of conservation of these habitats, especially in extremely stony and poor soils. There is a shift in taxa composition during the post-fire fungal succession from the early to the late stage of *Cistus ladanifer* life cycle.

2. Mosaic management of these areas may help to keep a high fungal richness associated with the different stages of *Cistus ladanifer* succession in different patches, at the same time that forest fires are prevented resulting from the interruption of fuel continuity. This mosaic management integrating the different treatments must be well planned so that senescent stands can act as a source of propagules for adjacent areas.

3. Rejuvenation of most old senescent scrublands may increase fungal production while decreasing the high risk of fire associated to these areas, the characteristics of which are optimal for fire ignition and spread. However, maintaining some patches of these senescent scrublands would contribute to keeping high fungal diversity and richness in the area by preserving fungal species that are exclusively adapted to senescent stands.

4. 50% clearing of middle-aged scrublands produces high yields of some valuable species such as *Boletus edulis*, *Leccinum corsicum* or *Lyophillum decastes*. This treatment may facilitate sporocarp collection while decreasing the amount of fuel and preventing the propagation of forest fires. However, the high implementation cost of this treatment makes it difficult to apply.

5. The diversity for both mycorrhizal and saprotrophic species is higher after burning than after clearing, whereas mushroom production was higher after total clearing. These results can be of interest from the management point of view. If the management objective is economic (i.e. to increase edible mushroom yield), total

clearings may be a better treatment alternative. On the contrary, if the aim is more ecologically oriented (i.e. high diversity), burning may be better than clearing.

6. The management practice that provides the greatest economic benefit considering biomass and *Boletus edulis* production, is performing one total clearing close to the end of the planning horizon. In addition, total removal of vegetation by mechanical clearing in may favor the appearance of new edible heliophilous species, while breaking fuel continuity completely. Since, the optimal age to perform the total clearing is in the final stage of *Cistus ladanifer* life cycle, this management would ensure that all stages of the shrub are present in the area. Thus, the optimal solution from the economic point of view would also be suitable from an ecological point of view.

7. Soil fungal communities found in these shrublands are primarily shaped by history of major disturbance, whereas current fire fuel reduction treatments do not have a significant impact on them. Therefore, although these treatments create new conditions that alter fungal fruiting, they do not produce a permanent negative impact on fungal communities because the mycelium of the different species is able to remain in the soil after the treatments.

8. Adequate management of these areas would derive important economic benefits from mushroom harvesting in ecosystems, traditionally considered unproductive, and generally located in poor and economically disadvantaged rural areas.

7. Conclusiones

1. La elevada riqueza fúngica encontrada, tanto en estadios tempranos como tardíos de la sucesión fúngica en matorrales de *Cistus ladanifer,* respaldan el interés de conservación de estos hábitats, especialmente en suelos extremadamente pedregosos y pobres. Existe un cambio en la composición específica durante la sucesión fúngica tras el fuego, desde el estadio temprano de la sucesión al tardío.

2. Un manejo en mosaico de estas áreas puede ayudar a mantener la elevada riqueza fúngica asociada a los diferentes estadios sucesionales de *Cistus ladanifer*, al mantener al mismo tiempo parches de diferentes edades. Además, gracias a este manejo, se previenen los incendios forestales debido a la ruptura de la continuidad de combustible. Este manejo en mosaico integrando diferentes tratamientos debe estar bien planificado de manera que las masas senescentes actúen como una fuente de propágulos para áreas adyacentes.

3. El rejuvenecimiento de gran parte de los matorrales senescentes produciría un aumento en la producción fúngica a la vez que disminuye el elevado riesgo de incendio asociado a estas zonas, de características óptimas para la ignición y propagación del fuego. Sin embargo, mantener algunos parches de arbustos senescentes contribuiría a conservar la elevada diversidad y riqueza fúngica al preservar las especies fúngicas asociadas únicamente a matorrales de elevada edad.

4. El desbroce al 50% de los jarales de mediana edad produce elevadas cantidades de especies comestibles de interés como *Boletus edulis, Leccinum corsicum* o *Lyophillum decastes*. Este tratamiento facilitaría la recolección de carpóforos, a la vez que disminuye la cantidad de combustible evitando la propagación del fuego. Sin embargo, el alto coste de implementación de este tratamiento dificulta su aplicación.

5. La diversidad de especies, tanto micorrícicas como saprófitas, es mayor después del fuego que después del desbroce, mientras la producción sigue la tendencia contraria siendo mayor tras el desbroce. Este resultado puede tener gran interés

desde el punto de vista de la gestión. De esta forma, si el objetivo del manejo es económico (aumentar la producción de especies comestibles), los desbroces pueden ser la mejor alternativa. Por otro lado, si el objetivo tiene una orientación más ecológica (aumentar la diversidad), las quemas controladas serían una mejor opción.

6. El manejo óptimo desde el punto de vista económico y considerando la producción de biomasa y de *Boletus edulis*, consiste en realizar un único desbroce en el horizonte de planificación, cercano al final del mismo. Además, la eliminación total de la vegetación mediante este tratamiento favorecería la aparición de nuevas especies heliófilas comestibles, al mismo tiempo que se rompe completamente la continuidad de combustible. Dado que el desbroce se produciría al final del ciclo de vida de *Cistus ladanifer*, este manejo aseguraría la existencia de matorrales de todos los estadios sucesionales en la zona gestionada. Por lo tanto, la solución óptima desde el punto de vista económico sería también adecuada desde un punto de vista ecológico.

7. Las comunidades de hongos presentes en el suelo están principalmente condicionadas por las perturbaciones mayores que determinan el origen del jaral, mientras que los tratamientos habituales de reducción de combustible no tienen un impacto significativo sobre ellos. Por lo tanto, aunque los tratamientos crean nuevas condiciones que afectan a la fructificación de las especies, éstos no producen un efecto negativo permanente sobre las comunidades fúngicas, ya que el micelio de las diferentes especies es capaz de permanecer en el suelo tras los tratamientos.

8. El manejo adecuado de estas áreas puede derivar en importantes beneficios económicos por el aprovechamiento micológico de dichos ecosistemas, tradicionalmente considerados improductivos y generalmente localizados en zonas rurales económicamente desfavorecidas.

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Original Article I

Post-fire fungal succession in a Mediterranean ecosystem dominated by *Cistus ladanifer* L.

María Hernández-Rodríguez, Juan Andrés Oria-de-Rueda & Pablo Martín-Pinto

Resumen

Sucesión fúngica post-fuego en un ecosistema Mediterráneo dominado por *Cistus ladanifer* L.

Los incendios forestales son la principal perturbación en ecosistemas Mediterráneos, afectando tanto a las comunidades vegetales como a las fúngicas. Tras el fuego, las comunidades fúngicas siguen unos patrones de sucesión motivados principalmente por las dinámicas de las comunidades vegetales establecidas tras el fuego. El objetivo de este estudio es analizar la sucesión post fuego en un ecosistema Mediterráneo dominado por *Cistus ladanifer* en la zona noroeste de España. Se recogieron semanalmente todos los cuerpos de fructificación durante las temporadas de otoño desde 2003 a 2006, en parcelas de 100 m2 localizadas en zonas recientemente quemadas (estadio inicial de la sucesión) y masas maduras (estadio tardío de la sucesión). Se encontraron 146 taxones fúngicos durante los 4 años de muestreo (56 únicamente en masas maduras y 23 únicamente en zonas quemadas). Se constató un cambio en la composición específica de las comunidades fúngicas durante la sucesión de *C. ladanifer*. Varios taxones como *Coprinus* spp. y *Pholiota carbonaria* aparecieron en los estadios iniciales de la sucesión, mientras otros taxones propios de estadios tardíos de la sucesión como *Lepista* spp. o *Conocybe* spp. fructificaron en las masas maduras. Los taxones específicos del género *Cistus* como *Entoloma cistophilum, Hebeloma cistophilum* y *Lactarios cistophilus* aparecieron tanto en los estadios iniciales como en los finales. Además, varios taxones micorrícicos, generalmente asociados a masas arbóreas maduras, fueron capaces de fructificar mucho antes en zonas dominadas por *C. ladanifer*. De acuerdo a los resultados de este estudio, estos ecosistemas tradicionalmente considerados improductivos tanto ecológicamente como económicamente, demostraron estar asociados a una riqueza fúngica muy elevada, pudiendo jugar un papel importante en la conservación de la diversidad a la vez que pueden actuar como puente para el inóculo micorrícico en la recuperación de una masa boscosa después de un fuego.

Post-fire fungal succession in a Mediterranean ecosystem dominated by *Cistus ladanifer* L.

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ABSTRACT

Wildfires are the major disturbance in Mediterranean ecosystems affecting both vegetation and fungal communities. After fire, fungal communities follow succession patterns mainly driven by the dynamics of post-fire plant communities. The aim of this study is to analyze post-fire fungal succession in a Mediterranean ecosystem dominated by *Cistus ladanifer* in northwestern Spain. Sporocarps were collected and identified on a weekly basis during the autumn season from 2003 to 2006 in 100 m^2 plots located in recently burned plots (early stage) and mature stands (late stage). 146 fungal taxa were found during the 4-year sampling (56 late stage only and 23 early stage only). There was a shift in the taxa composition of fungal community during *C. ladanifer* succession. Several taxa such as *Coprinus* spp. and *Pholiota carbonaria* were found in the early stage of the succession, whereas late stage taxa like *Lepista* spp. or *Conocybe* spp. fruited in the last years of succession. *Cistus*-specific taxa such as *Entoloma cistophilum*, *Hebeloma cistophilum* and *Lactarius cistophilus* were classified as multi-stage taxa as they were able to fruit in both early and late stages. Furthermore, several mycorrhizal taxa, usually associated with mature forest tree stands, were able to fruit much earlier in *C. ladanifer* scrublands. According to the results of this study, these ecosystems, traditionally considered ecologically and economically unproductive, seem to exhibit significant levels of fungal richness and can play an important role in diversity conservation as well as acting as a bridge for mycorrhizal inoculum in the recovery of forest stands after fire.

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1. Introduction

The genus *Cistus* comprises obligate seeder pyrophytic shrubs, which constitute early successional stages in Mediterranean ecosystems (Agueda et al., 2008). This genus is mainly distributed around the Mediterranean basin and it is represented in the Iberian Peninsula by 12 shrub species (Agueda et al., 2008). They frequently colonize highly degraded areas after fire, as high temperatures generated by fire in the top soil layers trigger seed germination (Bastida and Talavera, 2002). *Cistus* species may form both ectomycorrhizae and vesicular arbuscular mycorrhizae and they are associated with more than 200 ectomycorrhizal fungal species of 40 different genera (Comandini et al., 2006). *Cistus ladanifer* L. is, within this genus, the most abundant species, forming vast scrublands in the Iberian Peninsula.

Stand-replacing wildfires are the major disturbance in Mediterranean ecosystems and can alter the physical, chemical and biochemical soil properties mainly due through intense heating and ash deposition (Peay et al., 2009). In this sense, fire destroys soil organic matter, and alters soil pH and nutrient availability (Grogan et al., 2000). These effects are greater closer to the soil surface and their duration differs among sites depending on soil characteristics or fire severity (Rincón and Pueyo, 2010). Changes in soil properties and vegetation composition may significantly impact on fungal communities. Furthermore, fire produces direct effects on fungal communities by destroying belowground organisms (Buscardo et al., 2012). Differences in the return interval of fires can also modify the structure, composition and diversity of fungal communities (Buscardo et al., 2010). Thus, fires can affect the subsequent structure of fungal communities following succession patterns mainly driven by the dynamics of post-fire plant communities (Cairney and Bastias, 2007). As plant species grow, changes in succession of the associated fungi occur (Gassibe et al., 2011). After a forest fire, pre-fire fungal communities are largely eradicated and secondary succession begins with the first significant rain (Claridge et al., 2009). Early-stage fungi or pioneer species

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are defined as those that develop just after fire and their source is usually the spore bank present in the pre-fire soil (Claridge et al., 2009). Post-fire fungi are included within that group of species whose fruiting is enhanced by the new conditions created by fire. During succession, these fungi are joined but not necessarily replaced by late-stage species whose reproduction is primarily by clonal expansion (Iordache et al., 2009). Both early and late stage fungi are able to establish mycorrhizal association with plant roots although their nutrient requirements are different (Savoie and Largeteau, 2011). However late-stage fungi require components released from mature plant roots, or the stimulating effect from early-stage mycelium for developing mycorrhizae (Savoie and Largeteau, 2011).

Fungal succession has been previously studied (Frankland, 1998; Savoie and Largeteau, 2011). However, most of the recent studies are focused only on ectomycorrhizal species growing in forest tree stands. Kipfer et al. (2011) studied the ectomycorrhizal succession in *Pinus sylvestris* stands after fire, and found a rapid resilience in terms of species number but not in species composition. Clark et al. (2011) studied mycorrhizal secondary succession in a mixed aspen-conifer stand and found out that light limitation caused a decrease in ectomycorrhizal root infection in the later stages of the succession.

In Mediterranean ecosystems, Fernández-Toirán et al. (2006) and Gassibe et al. (2011) studied fungal mycorrhizal and saprotrophic sporocarps succession in *Pinus pinaster* stands. Fernández-Toirán et al. (2006) reported an increase of fungal richness with stand age, related to the higher number in the humicolous saprotrophic and mycorrhizal species. On the other hand, Gassibe et al. (2011) found that fungal species production followed different trends according with their trophic group: mycorrhizal decreased 6-fold, while saprotrophic increased 4-fold.

The short life cycle of *C. ladanifer* and its lack of post-fire resprouting capacity make it easier to compare early and late stages of succession with other longer-lived species. At the end of their life cycle (18–20 years), stands of this species wither and die, creating a very high risk of forest fires (Oria-de-Rueda et al., 2008). In the absence of fire, the area will be progressively occupied by other species, such as *Crataegus monogyna* Jacq. shrubs or of more advanced successional stages like *Quercus ilex* L. or *Quercus pyrenaica* Willd. Therefore, managers may decide to favor the dominant plant species depending on their interests. In northwestern Spain, cropland abandoned during the depopulation of rural areas in the last century has been progressively colonized by scrubs and nowadays remains economically unproductive.

Sporocarps of both mycorrhizal and saprotrophic species can be edible, representing an important non-timber forest economic resource in rural areas. Wild edible mushroom harvesting is becoming a widespread activity which may generate even higher economic benefit than timber production (Martín-Pinto et al., 2006). Indeed, *C. ladanifer* ecosystems have been proved to provide a high production of these edible fungi, being some of these species marketed in local industries (Martín-Pinto et al., 2006).

Understanding the ecology of the fruiting pattern of different fungal species and the effect of disturbances on them, may be the means to improve natural mushroom richness. Therefore, the aim of this study was to analyze fungal community succession following wildfire in a Mediterranean vegetation type dominated by *C. ladanifer* in Northwest Spain. Our specific objectives are to: (1) assess changes in species richness (number of species), diversity and biomass along *C. ladanifer* succession; (2) detect changes in the specific composition of the fungi associated with *C. ladanifer* during early and late age stages; (3) relate sporocarp abundance patterns to climatic variables and *C. ladanifer* age.

2. Materials and methods

2.1. Study site

The study area was located in North-western Spain. It is a Mediterranean ecosystem dominated by *C. ladanifer* situated at 750–780 m above the sea level. The soil of this area is constituted by Paleozoic metamorphic rocks, mainly Ordovician and Silurian shales (García Rodríguez et al., 1964). The soil is classified as Inceptisol suborder Xerept (Soil Survey Staff, 2010) and its characteristics are stoniness, acidity (pH 5.0–5.5), and shortage of calcium and phosphorous. Nitrogen and potassium availability is variable and the humification degree is generally good (García Rodríguez et al., 1964). The area is characterized by a sub-Mediterranean climate with a dry season of three months in the summer and a mean annual precipitation of 450–700 mm. Mean temperatures range from 14.5 to 15.8 °C.

Twelve early and late stage *C. ladanifer* plots were randomly selected in 2003 in order to assess the fungal succession after fire. Six "Early stage" plots were located on areas burnt in August 2002, while six "late stage" plots were established on 15 year-old monospecific stands of *C. ladanifer* (Table 1). Before the fire, the characteristics of the early stage stands were similar to the "late stage" stands. As the lifespan of this species ranges between 15 and 20 years (Oria-de-Rueda et al., 2008), our "late stage" stands can be considered mature, being very dense and approximately 2 m in height. These stands were also established after a forest fire.

2.2. Sampling

The sampling plots consisted in transects of 2 m × 50 m, established in accordance with previous studies (Ohenoja, 1989; Luoma et al., 1991; Smith et al., 2002). Sampling was performed on a weekly basis as it was in previous studies (Ohenoja, 1984; Dahlberg, 1991). The period of study comprised the autumn mushroom season, from late October to late December in the years 2003, 2004, 2005 and 2006. Thus, 8 years of *C. ladanifer* succession are studied. Given that this species life cycle is usually 18–20 years (Oria-de-Rueda et al., 2008), our results show reliable data about fungal community behavior over *C. ladanifer* cycle.

The spring fruiting season was ignored because in this area it is characteristically very short and almost insignificant. Sporocarps were fully harvested, in order to facilitate identification. All the sporocarps were carried to the laboratory, stored at 4 °C and processed within 24 h after collection.

2.3. Identification and classification

Sporocarps were identified at the species level whenever possible following several keys (Moser et al., 1983; Breitenbach, 1984,

Table 1
Location of study plots.

Plot number	Stage	X – UTM	Y – UTM	Altitude (m a.s.l.)
1	Early	0731929	4619795	847
2	Early	0730523	4621683	828
3	Early	0731916	4619807	846
4	Early	0730508	4621690	830
5	Early	0731901	4619814	846
6	Early	0730549	4621757	839
7	Late	0731690	4619644	867
8	Late	0730468	4621573	815
9	Late	0731659	4619699	866
10	Late	0730462	4621585	817
11	Late	0731664	4619702	865
12	Late	0730466	4621592	818

1986, 1991, 1995, 2000, 2005; Bon, 1987; Andrés-Rodríguez et al., 1999; Knudsen and Vesterholt, 2008; Antonín and Noordeloos, 2010). However, there were some samples that could only be identified to the genus level and were grouped into genus taxa as described in previous studies (Bonet et al., 2004; Oria-de-Rueda et al., 2010). Data were grouped into the two different life strategies (saprotrophic/mycorrhizal) for further statistical analysis.

2.4. Climatic data

Annual rainfall, mean annual temperature, mean annual soil temperature, minimum mean temperature and maximum mean temperature from the period 2003–2006 were provided by the closest meteorological station (Villardeciervos, 0725092 Longitude-UTM, 4646789 Latitude-UTM, 29T Grid and 850 m above sea level, Spanish Meteorological Agency). Mean monthly potential evapotranspiration (ETP), was calculated by the empirical method of Thornthwaite and Mather (1955) for the latitude of Villardeciervos meteorological station. These data were included as environmental factors in the CCA analysis.

2.5. Data analysis

Shannon's H' diversity index (Shannon and Weaver, 1949), based on dry weight of the fruiting bodies (Dahlberg, 1991) was calculated. This variable was calculated using the following formula where coefficient p_i indicates the relative abundance of each fungal species:

$$H' = -\Sigma p_i \cdot (\ln p_i)$$

Richness (number of taxa) and the Shannon Index were analyzed statistically for both life strategies (saprotrophic/mycorrhizal) in earlier stages (1–4 years after fire) and later stages (15–18 years after fire). Data were subjected to a Repeated Measures ANOVA analysis and means were compared by Least Significance Difference (LSD) Fisher Tests ($P < 0.05$). STATISTICA '08 Edition software (StatSoft Inc., 1984–2008) was used for the analysis.

Species composition was analyzed using ordination techniques on fungal dry weight data. Firstly, data of dry weight of mycorrhizal and saprotrophic taxa were subjected to a detrended correspondence analysis (DCA) (Ter Braak and Prentice, 1988). Since the length of the extracted gradient was bigger than 3 SD units in both analyses (3.751 and 3.813 respectively), canonical correspondence analysis (CCA) (Ter Braak, 1986) was used to assess the effect of environmental variables in fungal dry weight. Two CCA tests were conducted, separately with mycorrhizal and saprotrophic taxa, in order to analyze whether there were differences in the ecological behavior of these two groups. Forward selection was used to select significant explanatory variables and only those significant at the $P < 0.05$ level were included in the models. Monte Carlo permutation tests (499 permutations) were performed to study the signification of the models. These analyses were conducted using CANOCO for Windows version 4.5 software (Ter Braak and Šmilauer, 2002). CCA results were displayed by ordination diagrams drawn with Cano Draw 4.1. software.

3. Results

3.1. Richness, diversity and biomass

A total of 146 fungal taxa were found on *C. ladanifer* plots during the 4-year sampling (Table 2). Ninety taxa were harvested in the early stage plots, 23 of which were exclusive to this stage. In the late stage plots we found a total of 122 taxa, 56 of which were found exclusively in this stage (Table 2). Sixty seven taxa were

found in common for both early and late stage treatments. Considering the number of taxa and the Shannon Index across the 4-year sampling, differences among early and late stage were only present in the first year of sampling ($P < 0.001$; $P < 0.023$, respectively) (Figs. 1a and 2a). Regarding the functional group classification, 65 mycorrhizal taxa and 81 saprotrophic taxa were found. In the early stage 42 taxa were mycorrhizal and 48 saprotrophic. On the other hand, in the late stage plots, 58 of the taxa were mycorrhizal and 64 saprotrophic (Table 2).

The number of mycorrhizal taxa showed an increasing trend during the early stage. The first year of sampling (1 versus 15 years old), there were significantly less mycorrhizal taxa fruiting in the early stage ($P < 0.001$) but at the fourth sampling year (4 versus 18 years old), the pattern was reversed (Fig. 1b) ($P < 0.007$). However, Shannon Index was significantly lower during the first and the second sampling year ($P < 0.003$ and $P < 0.011$, respectively) and very similar during the third and the fourth (Fig. 2b). On the other hand, the number of saprotrophic taxa remained lower in the early stage during the four sampling years, although significant differences were found only in the first ($P < 0.008$) and fourth ($P < 0.022$) years (Fig. 1c). Nevertheless, Shannon Index does not show this tendency and the only differences between early and late stages are found in the last sampling year (Fig. 2c).

Biomass (dry weight) showed a growing pattern during the early stage, with mean total values ranging from 4.27 kg ha^{-1} in the first sampling year to 47.43 kg ha^{-1} in the fourth sampling year. For the late stage, biomass remained constant and lower (between 1.43 and 9.33 kg ha^{-1}). Comparing early and late stage, differences were found in the third and fourth sampling year ($P < 0.05$ and $P < 0.001$, respectively) (Table 3).

3.2. Taxa composition

Fungal community assemblies along *C. ladanifer* succession can be analyzed from the results obtained in the two canonical correspondence analyses (CCAs) (Table 4). The results of both CCAs are displayed in two ordination biplots (Fig. 3). The projection of the taxa points onto any axis indicates the position of taxa presence along an environmental factor (Ter Braak, 1986). Minimum annual temperature ($P = 0.002$), annual precipitation ($P = 0.014$) and *C. ladanifer* age ($P = 0.038$) were significant in the ordination of mycorrhizal taxa dry weight according to the forward selection process (Table 5). A Monte Carlo permutation test was significant for the first axis ($P = 0.022$) and for all canonical axes ($P = 0.002$). In this case, axis 1 is negatively correlated with the three environmental variables selected, whereas axis 2 is negatively correlated with the two weather variables and positively correlated with *C. ladanifer* age. In the case of saprotrophic species, only *C. ladanifer* age and precipitation were significant ($P = 0.02$ and $P = 0.018$, respectively) (Table 5, Fig. 3b). The model is significant according to Monte Carlo permutation test for the first axis ($P = 0.024$) and for all canonical axes ($P = 0.002$). Axis 1 is negatively correlated with precipitation and positively correlated with host age, whereas axis 2 is negatively correlated with the two environmental variables.

4. Discussion

4.1. Richness, diversity and biomass

Regarding the number of taxa, very high fungal richness (146 taxa) was found associated to the early and late stage of *C. ladanifer* located in degraded and stony soils. Contrary to expectations, fungal richness found in these ecosystems was similar or even higher than that found in mature stands of different forest tree species.

Table 2
Total taxa collected from *Cistus ladanifer* L. plots.

Taxa	Code	Early stage 1	2	3	4	Late stage 15	16	17	18	Group
Agaricus sp. L.	Agsp	x	x			x	x			S
Amanita mairei Foley	Amma				x				x	MY
Amanita muscaria (L.) Lam.	Ammu			x	x	x	x			MY
Amanita pantherina (DC.) Krombh.	Ampa				x	x			x	MY
Amanita phalloides (Vaill.ex Fr.) Link	Amph					x				MY
Amanita rubescens Pers.	Amru								x	MY
Amanita vaginata (Bull.) Lam.	Amva			x		x				MY
Bjerkdera adusta (Willd. ex Fr.) Karst.	Bjad			x				x		S
Boletus edulis Bull.	Boed					x	x		x	MY
Bovista plumbea Pers.	Bovpl				x					S
Chalciporus piperatus (Bull.) Bataille	Chpi					x	x			MY
Clitocybe angustissima (Lasch) P. Kumm.	Clan							x		S
Clitocybe diatreta (Fr.) P. Kumm.	Cldi				x				x	S
Clitocybe fragrans (With.) P. Kumm.	Clfr				x				x	S
Clitocybe inornata (Sowerby) Gillet.	Clin					x				S
Clitocybe sect. *Fragantes*	ClFr								x	S
Clitocybe sect. *Pseudolyophyllum*	ClPs								x	S
Clitocybe sp. (Fr.) Staude	Clsp	x	x	x	x	x	x	x	x	S
Clitocybe vibecina (Fr.) Quél.	Clvi					x				S
Collybia butyracea (Bull.) P. Kumm.	Cobu		x	x		x	x	x	x	S
Collybia dryophila (Bull.) P. Kumm.	Codr				x			x		S
Collybia erythropus (Pers.) P. Kumm.	Coer				x					S
Collybia maculata (Alb. & Schwein.) P. Kumm.	Coma				x					S
Collybia sp. (Fr.) Staude	Cosp			x		x				S
Conocybe brunneola Kühner ex Kühner & Watling	Cobr							x		S
Conocybe tenera (Schaeff.) Fayod.	Cote							x		S
Coprinus angulatus Peck	Cpan			x						S
Coprinus atramentarius (Bull.) Fr.	Cpat		x							S
Coprinus domesticus (Bolton) Gray	Cpdo				x					S
Coprinus micaceus (Bull.) Fr.	Cpmi	x	x				x			S
Coprinus plicatilis (Curtis) Fr.	Cppl			x	x					S
Coprinus sp. Pers.	Cpsp		x		x					S
Cortinarius arvinaceus Fr.	Crar					x				MY
Cortinarius castaneus (Bull.) Fr.	Crca					x				MY
Cortinarius cinnamomeobadius Rob. Henry	Crci				x				x	MY
Cortinarius glaucopus (Schaeff.) Fr.	Crgl		x				x			MY
Cortinarius mucosus (Bull.) J. Kickx f.	Crmu		x				x			MY
Cortinarius sect. *Cyanophylli*	CrCy				x				x	MY
Cortinarius sect. *Firmiores*	CrFi								x	MY
Cortinarius sect. *Fulvi*	CrFu				x					MY
Cortinarius sect. *Variicolores*	CrVa				x					MY
Cortinariu sp. (Pers.) Gray	Crsp	x	x	x	x	x	x	x	x	MY
Cortinarius sulphureus (Kauffman) J.E. Lange	Crsu	x				x	x			MY
Cortinarius violaceus (L.) Gray	Crvi					x				MY
Craterellus tubaeformis (Schaeff.) Quél.	Crtu								x	MY
Crucibulum laeve (Huds.) Kambly	Crla				x					S
Cystoderma amianthinum (Scop.) Fayod	Cyam					x	x	x	x	S
Cystoderma cinnabarinum (Alb. & Schwein.) Fayod	Cyci						x		x	S
Cystoderma superbum Huijsman	Cysu								x	S
Cystoderma terrei (Berk. & Broome) Harmaja	Cyte					x				S
Dacrymyces chrysospermus Berk. & M.A. Curtis	Dach				x					S
Dermocybe cinnamomeolutea (P.D. Orton) M.M. Moser	Deci			x		x	x	x		MY
Entoloma cistophilum Trimbach	Enci	x			x	x	x	x		S
Entoloma hirtipes (Schumach.) M.M. Moser	Enhi								x	S
Entoloma icterinum (Fr.) M.M. Moser	Enic								x	S
Entoloma juncinum (Kühner&Romagn.) Noordel.	Enju		x				x			S
Entoloma sericeum (Bull.) Quél.	Ense				x				x	S
Entoloma sp. (Fr.:Fr.) Kummer	Ensp			x	x		x	x	x	S
Galerina sp. Earle	Gasp				x				x	S
Galerina uncialis (Britzelm.) Kühner	Gaun								x	S
Galerina vittiformis (Fr.) Earle	Gavit				x				x	S
Hebeloma anthracophilum Maire	Hean				x					MY
Hebeloma cistophilum Maire	Heci	x	x	x	x	x	x	x	x	MY
Hebeloma mesophaeum (Pers.) Quél.	Heme	x				x				MY
Hebeloma sp. (Fr.) P. Kumm.	Hesp		x	x	x	x		x		MY
Hohenbuehelia geogenia (DC.) Singer.	Hoge					x				S
Hygrocybe sp.	Hgsp				x					S
Hygrophorus chrysodon (Batsch) Fr.	Hych	x		x	x	x	x	x	x	MY
Hygrophorus eburneus (Bull.) Fr.	Hyeb					x				MY
Hygrophorus pseudodiscoideus var.*cistophilus* Bon & G. Riousset	Hyps				x				x	MY
Hygrophorus sp. Fr.	Hysp		x	x			x	x		MY
Hypholoma fasciculare (Huds.) P. Kumm.	Hyfa				x				x	S
Inocybe fuscidula Velen.	Infu					x				MY

Table 2 (continued)

Taxa	Code	Early stage				Late stage				Group
		1	2	3	4	15	16	17	18	
Inocybe sp. (Fr.) Fr	*Insp*	x			x	x	x		x	MY
Laccaria amethystina Cooke	*Laam*					x				MY
Laccaria bicolor (Maire) P.D. Orton	*Labi*			x					x	MY
Laccaria laccata (Scop.) Cooke	*Lala*	x	x	x	x	x	x	x	x	MY
Lactarius chrysorrheus Fr.	*Lach*					x			x	MY
Lactarius cistophilus Bon & Trimbach	*Laci*				x				x	MY
Lactarius mitissimus (Fr.) Fr.	*Lami*					x				MY
Lactarius pubescens (Fr.) Fr.	*Lapu*						x			MY
Lactarius sp. Pers.	*Lasp*								x	MY
Lactarius tesquorum Malençon	*Late*		x	x		x		x	x	MY
Lactarius violascens (J. Otto) Fr.	*Lavi*	x				x				MY
Leccinum corsicum (Rolland) Singer	*Leco*	x	x		x	x			x	MY
Lepiota castanea Quél.	*Leca*					x				S
Lepiota sp. (Pers.) Gray	*Lesp*					x				S
Lepista inversa (Scop.) Pat.	*Lein*								x	S
Lepista nuda (Bull.) Cooke	*Lenu*					x	x		x	S
Lycoperdon foetidum Bonord.	*Lyfo*								x	S
Lycoperdon molle Pers.	*Lymo*								x	S
Lycoperdon perlatum Pers.	*Lype*		x		x	x	x	x	x	S
Lyophyllum decastes (Fr.) Singer	*Lyde*				x		x		x	S
Macrolepiota mastoidea (Fr.) Singer	*Mama*							x		S
Marasmius anomalus Lasch ex Rabenh.	*Maan*				x			x		S
Marasmius cohaerens (Alb. &Schwein.) Cooke&Quél.	*Maco*							x		S
Marasmius curreyi Berk. & Broome	*Macu*		x	x				x		S
Marasmius scorodonius (Fr.) Fr.	*Masc*							x		S
Marasmius sp. Fr.	*Masp*		x	x				x		S
Micromphale foetidum (Sowerby) Singer	*Mifo*							x		S
Mycena alcalina (Fr.) P. Kumm.	*Myal*							x		S
Mycena epipterygia (Scop.) Gray	*Myep*		x	x		x	x		x	S
Mycena flavoalba (Fr.) Quél.	*Myfl*								x	S
Mycena polygramma (Bull.) Gray	*Mypo*				x					S
Mycena pura (Pers.) P. Kumm.	*Mypu*		x	x			x	x	x	S
Mycena rosea (Schumach.) Gramberg	*Myro*					x				S
Mycena sp. (Pers.) Roussel	*Mysp*	x	x		x	x	x	x	x	S
Omphalina sp. Quél.	*Omsp*				x					S
Panaeolus sphinctrinus (Fr.) Quél.	*Pasp*	x			x					S
Paxillus atrotomentosus (Batsch) Fr.	*Paat*								x	S
Paxillus involutus (Batsch.) Fr.	*Pain*	x	x		x		x			MY
Peziza sp. Fr.	*Pesp*					x				S
Phaeomarasmius erinaceus (Fr.) Scherff. ex Romagn.	*Pher*			x				x	x	S
Pholiota carbonaria (Fr.) Sing.	*Phca*	x	x	x	x		x	x		S
Pisolithus tinctorius (Mont.) E. Fisch.	*Piti*				x					MY
Psathyrella chondroderma (Berk. & Broome) A.H. Sm.	*Psch*			x				x		S
Psathyrella sp. (Fr.) Quél.	*Pssp*								x	S
Ramaria flavoides Schild (1981)	*Rafl*								x	MY
Ramaria sp. (Fr.) Bonord.	*Rasp*				x	x	x		x	MY
Rhodocybe sp. Maire	*Rhsp*							x		MY
Rickenella fibula (Bull.) Raithelh.	*Rifi*		x			x	x		x	S
Russula cutefracta Cooke	*Rucu*					x				MY
Russula pectinatoides Peck	*Rupe*				x					MY
Russula puellaris Fr.	*Rupu*					x				MY
Russula sect. *Lilaceae* Konrad & Joss.	*RuLi*				x					MY
Russula sp. Pers.	*Rusp*	x	x		x	x	x		x	MY
Russula subsect *atropurpurinae* (Romagn.) Bon	*Ruat*				x				x	MY
Schizophyllum commune Fr.	*Scco*			x	x					S
Stereum hirsutum (Willd.) Pers.	*Sthi*		x	x	x		x	x	x	S
Stereum rameale (Schwein.) Burt	*Stra*				x					S
Stropharia aeruginosa (Curtis) Quél.	*Stae*					x				S
Suillus bellini (Inzenga) Watling	*Sube*		x				x			MY
Thelephora terrestris Ehrh.	*Thte*				x				x	MY
Trametes versicolor (L.) Lloyd	*Trve*				x		x			S
Tremella foliacea Pers.	*Trfo*	x			x					S
Tremella mesenterica Schaeff.	*Trme*	x	x	x	x	x		x	x	S
Tricholoma auratum (Paulet) Gillet	*Trau*		x		x	x	x			MY
Tricholoma fracticum (Britzelm.) Kreisel	*Trfr*		x			x	x			MY
Tricholoma portentosum (Fr.) Quél.	*Trpo*					x				MY
Tricholoma saponaceum (Fr.) P. Kumm.	*Trsa*			x					x	MY
Tricholoma sp. (Fr.) Staude	*Trsp*	x	x			x				MY
Tricholoma terreum (Schaeff.) P. Kumm.	*Trte*						x			MY
Tricholoma ustale (Fr.) P. Kumm.	*True*				x				x	MY
Tricholoma ustaloides Romagn.	*Trus*			x						MY
Tubaria furfuracea (Pers.) Gillet	*Tufu*								x	S
Xerocomus ferrugineus (Boud.) Bon	*Xefe*					x				MY
Annual precipitation (mm)		962	490	449	1032	962	490	449	1032	

Table 2 (*continued*)

Taxa	Code	Earlystage				Late stage				Group
		1	2	3	4	15	16	17	18	
Mean temperature (°C)		11.95	11.18	11.23	12.01	11.95	11.18	11.23	12.01	
Mean minimum temperature (°C)		6.65	5.76	5.37	6.63	6.65	5.76	5.37	6.63	
Mean maximum temperature (°C)		16.17	15.56	16.07	16.35	16.17	15.56	16.07	16.35	
Soil temperature (°C)		14.70	13.71	14.34	14.9	14.70	13.71	14.34	14.9	
ETP		701.6	673.1	684.2	704.2	701.6	673.1	684.2	704.2	

MY: mycorrhizal; S: saprotrophic.
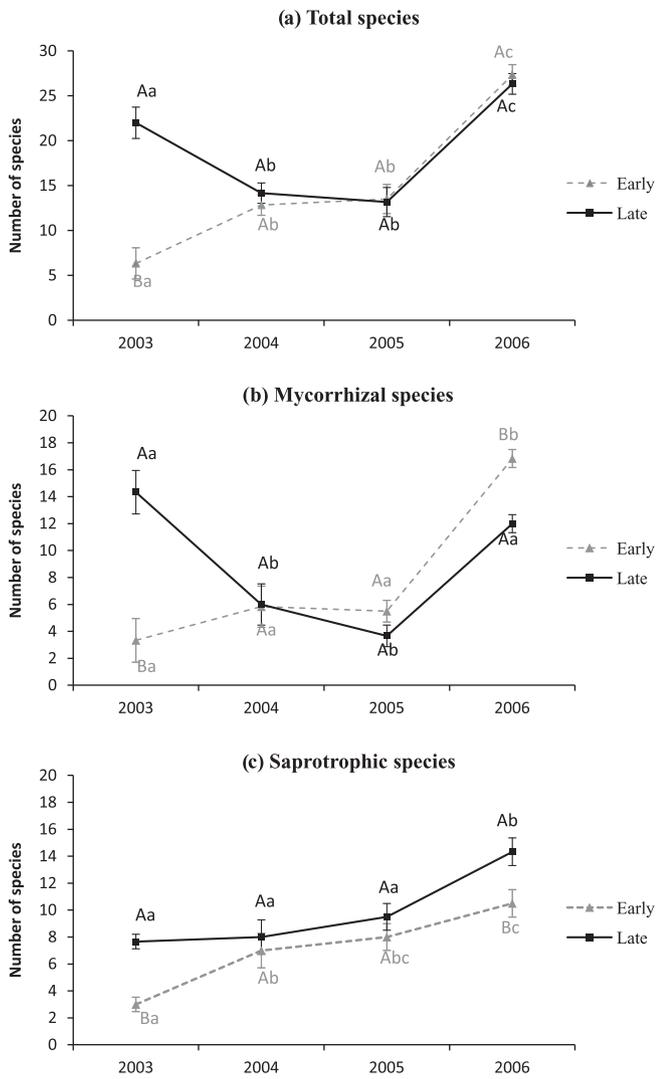
Fig. 1. Number of fungal taxa in the early and late succession stages of *Cistus ladanifer* for each sampling year: (a) number of total taxa; (b) number of mycorrhizal taxa; (c) number of saprotrophic taxa. The data are plot means ± standard error. Upper case letters indicate differences between early and late stage within a sampling year. Lower case letters indicate differences among the different sampling years within each stage. Values with the same letter are not significantly different.

Fig. 2. Shannon diversity index in the early and late succession stages of *Cistus ladanifer* for each sampling year: (a) total diversity; (b) mycorrhizal diversity and (c) saprotrophic diversity. The data are plot means ± standard error. Upper case letters indicate differences between early and late stage within a sampling year. Lower case letters indicate differences among the different sampling years within each stage. Values with the same letter are not significantly different.

During the same period of time, 115 fungal taxa were collected in a nearby *P. pinaster* Ait. mature stand (Gassibe et al., 2011). Furthermore, Martínez-Peña et al. (2012) found 119 species in a 10-year study in *P. sylvestris* L. stands of different age classes in Spain, and Baptista et al. (2010) recorded 73 different species in a 4 year study in *Castanea sativa* Mill. in Portugal.

A decrease in fungal number of taxa and the Shannon diversity index was found in the early stage after fire, but only in the first

year after fire. In the following years, the number of taxa showed quite similar values in both successional stages. This could indicate that the colonization process after disturbance, regarding richness and diversity, takes 2 years. Also in Spain, Rincón and Pueyo (2010) observed that seedling mycorrhizal colonization significantly increased from the first to the second autumn after fire in a fungal

Table 3
Fungal biomass production (dry weight, kg ha^{-1}) in the early and late stages for each sampling year.

Sampling year	Early stage	Late stage
2003	4.27 ± 2.04 Aa	9.33 ± 2.04 Aa
2004	19.46 ± 5.85 Ab	2.61 ± 5.85 Aa
2005	21.43 ± 6.51 Ab	1.42 ± 6.51 Ba
2006	47.44 ± 5.63 Ac	7.28 ± 5.63 Ba

The data are plot means ± standard error. Upper case letters indicate differences between early and late stage within a sampling year. Lower case letters indicate differences among the different sampling years within each stage. Values with the same letter are not significantly different.

Table 4
Summary of canonical correspondence analysis of fungal taxa presence and environmental factors according to functional groups in *Cistus ladanifer* plots.

Functional group	Mycorrhizal		Saprotrophic	
Axes	1	2	1	2
Eigenvalues:	0.718	0.526	0.761	0.454
Taxa-environment correlations:	0.984	0.994	0.965	0.955
Cumulative percentage variance				
Of taxa data	27.7	47.9	27.2	43.4
Of taxa-environment relation	42.9	74.3	62.6	100

community associated with *P. pinaster*. Moreover, Kipfer et al. (2011) found rapid resilience in fungal species number on burnt *P. sylvestris* stands in Central Alps. According to these authors, pioneer tree species and shrubs may facilitate the establishment of a ectomycorrhizal network after a stand-replacing fire. Buscardo et al. (2012) found about one third of the ectomycorrhizal species, present in both pine and shrubs. Thus, these authors concluded that shrubs may play an important role in post-fire recovery of tree stands, acting as a bridge between the pre-fire fungal communities and the emerging seedlings.

There were some differences in the behavior of mycorrhizal and saprotrophic taxa over the sampling years. Mycorrhizal number of taxa was highly affected by fire, diminishing drastically their fruiting the first year following fire. Shannon index was also significantly lower just after fire comparing to the late stage stand, and the differences remained the first two sampling years. Given that the presence of a plant host is necessary for the establishment of the mycorrhizal fungi, it can be expected that the destruction of the host species by fire will cause a decrease in the presence of mycorrhizal species (Dahlberg, 2002). The presence of mycorrhizal taxa in the early stage of succession can be explained by their survival strategy in which these taxa remain alive in the form of different resistance structures such as infected root tips, sclerotia or resistant spores (Cairney and Bastias, 2007). The ectomycorrhizal fungal community established after a fire is a reflection of the soil resistance structure bank that existed before the disturbance (Baar et al., 1999).

The mean number of mycorrhizal taxa per plot increased rapidly. Thus, the mean number of mycorrhizal taxa found in the 4 year-old stand was higher than those found in the 18 year-old stand. This may be explained by the higher photosynthetic activity of the young scrubland and its increased growth compared to the late stage stand. As host species require more nutrients in their early development, mycorrhizal fungal species associated to their roots are able to use more nutrients, enhancing the fruiting (Ortega-Martínez et al., 2011). In the case of the Shannon diversity index, there were no differences between early and late stage in the fourth sampling year. This may indicate that although the number of species had increased markedly in the early stage, these new species did not fruit uniformly. Low uniformity values resulted in a low Shannon diversity index despite the high richness observed.

In the case of the saprotrophic fungi, the number of taxa remained lower in the early stage than in the late stage during the four sampling years. This could be due to the characteristics of the mature scrubland: very dense and with abundant organic matter in soil which may enhance the development of saprotrophic fruiting bodies. However, the Shannon diversity index did not show the same tendency, and it was very variable depending on the weather conditions.

Concerning biomass data, the rapid increase in dry weight production during the early stage of the succession may be due to the high vigor of *C. ladanifer* emerging plants after fire. The higher nutrient availability and insolation enhance the biological activity of the plants and, consequently, the fruiting of associated fungal communities. Nevertheless, fungal biomass in the late stage stands was lower. These stands are characterized by the presence of senescent plants, a low level of nutrient availability due to the high competition and a lack of insolation which difficulties fungal fruiting. This result is in accordance with previous studies. Thus, Smith et al. (2002) found six times greater sporocarps production in young and rotation-age stands of *Pseudotsuga menziesii* compared with old-growth stands and Bonet et al. (2010) also reported lower sporocarp production in old-growth stands of different pine species.

4.2. Taxa composition

Concerning fungal composition, the results obtained in this study show an important shift from the early to the late stage of *C. ladanifer* scrubland. According to CCAs, there was a clear effect of *C. ladanifer* age on dry weight of the different fungal taxa in both functional groups.

Several studies have reported changes in mycorrhizal fungal community composition associated with different stages of post-fire forest succession, like Gassibe et al. (2011) in *P. pinaster* stands in Spain, who found superpioneer, pioneer and mature fungal species associated with the analyzed forest stands. Kipfer et al. (2010) reported a change in species composition of the ectomycorrhizae associated to *P. sylvestris* roots due to soil heating. In contrast, different results have been reported in a *Picea* forest in Canada, where authors concluded that after fire there is a shift in the relative abundance of each species rather than a change in ectomycorrhizal species composition (Mah et al., 2001). These different results may be due to differences in fire severity. Stand replacing fires like that studied in Gassibe et al. (2011) generally cause drastic changes in fungal community composition whereas moderate intensity fires result in lower impacts on fungal community composition. In our case, several mycorrhizal genera such as *Amanita, Boletus, Cortinarius, Inocybe, Lactarius* and *Tricholoma*, fructified in *C. ladanifer* stands. This is remarkable as these genera are characteristic of late stage pine stands, 30–40 years old (Chu-chou, 1979; Visser, 1995). This earlier fruiting suggests that some species belonging to these genera behave as early-stage mycorrhizal mushroom when associated with Cistaceae (Savoie and Largeteau, 2011). This result agrees with Oria-de-Rueda et al. (2008) who found *Boletus edulis* carpophores in a 3 year-old *C. ladanifer* scrubland.

Regarding saprotrophic taxa, several of them appeared to be associated to the early stage of the *C. ladanifer* succession, fruiting just after fire (pyrophytic taxa). Within this set of taxa, we found taxa belonging to the genus *Coprinus*, whose basidiospore germination is known to be activated by some substances produced in forest fires (e.g. furfural) (Mills and Eilers, 1973). Another example is *Pholiota carbonaria*, also typically considered as a pyrophytic taxa as organic debris burning induces its fruiting (Singer and Moser, 1964). The presence of *P. carbonaria* in the late stage plots is indicative of the origin of these stands, established after a forest fire. On the other hand, some taxa were found to fruit mainly during the
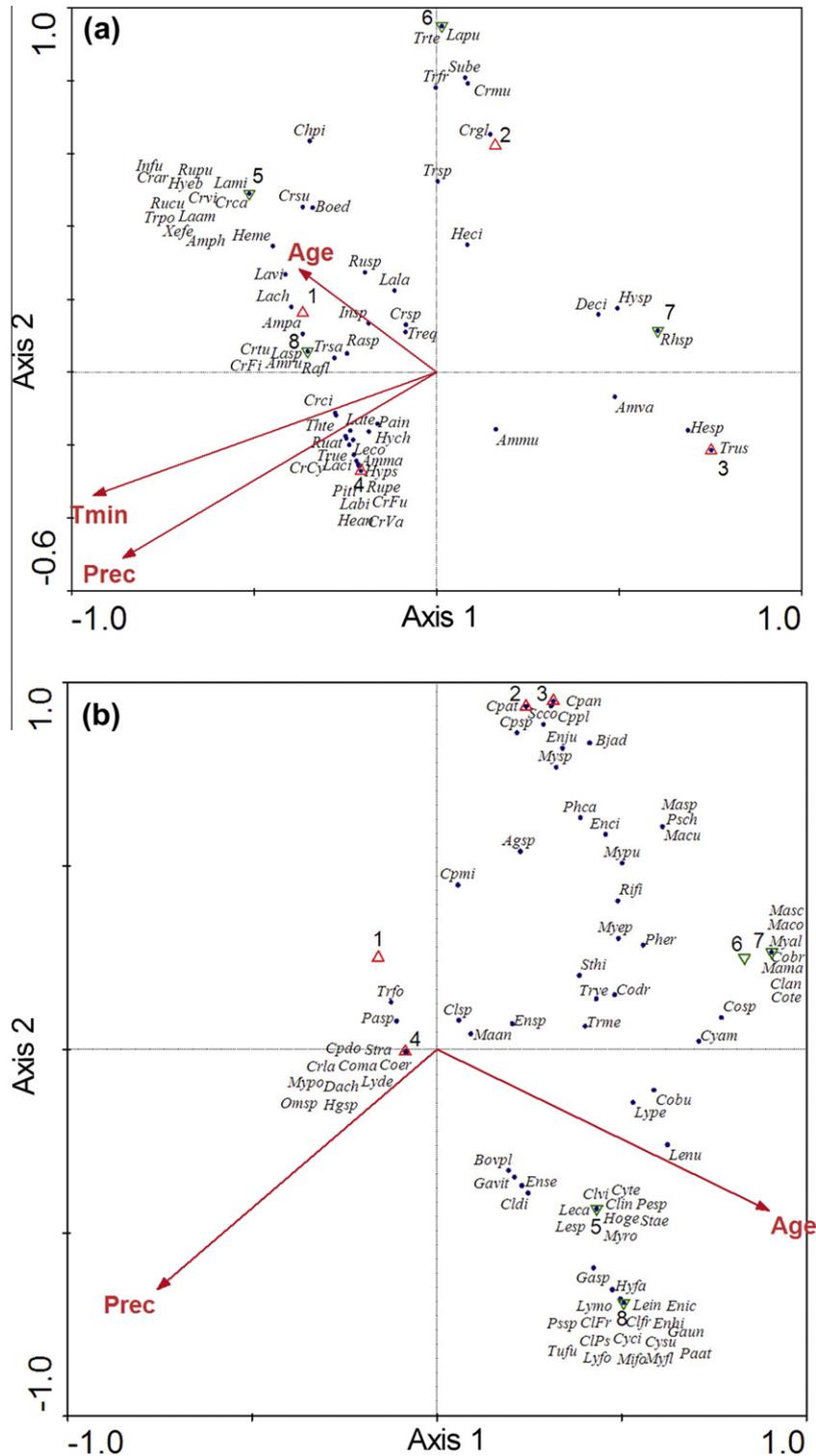
Fig. 3. Canonical correspondence analysis ordination triplots showing fungal: (a) mycorrhizal, (b) saprotrophic taxa (dots), early stage (up-triangles) and late stage (down-triangles) sampling years and environmental factors (arrows). Prec: annual precipitation; *T*: mean annual temperature, Age: *Cistus ladanifer* age. Fungal taxa are identified by the code shown in Table 2.

late stage, such as those belonging to the genera *Cystoderma, Conocybe* and *Lepista*, according to Gassibe et al. (2011) findings which could be explained by the higher availability of substrata in older stands, where conditions of temperature and moisture are more adequate for fungal fruiting (Fernández-Toirán et al., 2006).

Finally, regarding stand age, both mycorrhizal and saprotrophic fungal taxa that are known to be exclusively associated with *C.*

ladanifer (*Hebeloma cistophilum, Entoloma cistophilum, Lactarius cistophilus*) fructified in both, early and late stages. These taxa can thus be considered as multi-stage fungi.

Weather conditions were also significant for fungal fruiting. In the case of saprotrophic species only annual precipitation was significant. For mycorrhizal taxa, mean minimum temperature and precipitation had an effect on abundance, i.e. fruiting of the

Table 5
Results of the forward selection process in mycorrhizal and saprotrophic CCA analyses.

Functional group	Variables	F-ratio	P-value
Mycorrhizal	T_{min}	2.20	0.002
	Prec	1.97	0.014
	Age	1.92	0.038
Saprotrophic	Age	2.01	0.020
	Prec	1.62	0.018

Only significant variables are included. T_{min}: mean minimum temperature (°C); Prec: annual precipitation (mm); Age: *Cistus ladanifer* age (years).

different species. In the case of the genus *Tricholoma*, different taxa fructified depending on precipitation and temperature. *T. ustale* and *T. portentosum* are hygrophilous species that fructified in more humid and warmer years (higher mean minimum temperature), whereas *T. fracticum, T. terreum* and *T. ustaloides* are xerophilous species typical of Mediterranean pine forests, which thrived in drier years. Several species of these genera are marketable edible species, and harvesting them may produce an important economic benefit for the rural population (de Román and Boa, 2004).

Although this study comprises only aboveground fungal community composition data, which prevent us from drawing firm conclusions about the number of entire fungal species associated with *C. ladanifer*, fruiting succession patterns of epigeous fungal communities provide some useful guidelines for the management of these scrublands.

C. ladanifer scrublands have been traditionally regarded as unimportant from both environmental and economic points of view. However, fungal richness and diversity is also associated with a diverse population of animals that feed on them (Fogel and Trappe, 1978). Thus, the conservation of these scrublands may be very important for the maintenance of the high richness and diversity associated with them. Furthermore, these ecosystems can act as a bridge in the recovery of forest stands after fire. Therefore, after fire, managers of these ecosystems can opt to keep this scrublands, in order to maintain fungal richness and diversity associated with *C. ladanifer*, or to use this species as a tool to provide fungal propagules in the establishment of a new forest stand.

The pyrophytic characteristics of *C. ladanifer* scrublands facilitate fire ignition and spread. Thus, forest fires are the main threat for the conservation of these ecosystems.

Therefore, management of these areas should take into account measures to prevent fuel continuity. In this sense, mosaic structure management, including early and late stage areas simultaneously, may help to conserve fungal richness and diversity while preventing the occurrence of large uncontrolled fires. In addition, an adequate management of these scrublands maintaining different age stages can provide production of some appreciated and demanded edible species, even forming part of the understory in young forest stands established after fire, where this production would occur markedly earlier.

5. Conclusions

High fungal richness found in the early and late stage of *C. ladanifer* scrublands supports the interest of conservation of these habitats, especially in extremely stony and poor soils. There was a shift in taxa composition during the post-fire fungal succession from the early to the late stage of *C. ladanifer* life cycle.

To our knowledge, this is the first study of fungal succession associated with a *C. ladanifer* scrubland. According to the results of this study, *C. ladanifer* scrublands have an important role as a bridge in the recovery of forest stands after fire, providing mycorrhizal inoculum available to colonize tree roots as the new stand is developed. Since these *C. ladanifer* stands have been proved to produce important marketable fungal species, it may be also interesting to keep some of these areas as *C. ladanifer* scrublands. Mosaic management of these areas may help to keep a high fungal richness associated with the different stages of *C. ladanifer* succession in different patches at the same time that forest fires are prevented resulting from the interruption of fuel continuity. Furthermore, economic benefits would derive from valuable fungal species marketing in poor and depressed rural areas.

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Original Article II

Impact of fuel reduction treatments on fungal sporocarp production and diversity associated with *Cistus ladanifer* L. ecosystems

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Resumen

Impacto de distintos tratamientos de reducción de combustible sobre la producción y diversidad de carpóforos asociados con ecosistemas de *Cistus ladanifer* L.

Los matorrales mediterráneos dominados por *Cistus ladanifer* pueden proporcionar una importante producción de hongos, a menudo muy demandados. Sin embargo, debido a las características pirófitas de esta especie, los incendios son la principal amenaza de estos ecosistemas. El objetivo de este estudio es analizar el efecto de diferentes tratamientos de reducción de combustible realizados en matorrales de *C. ladanifer* sobre la fructificación de hongos, para así mejorar la producción y diversidad micológica y prevenir los incendios forestales. El muestreo de carpóforos tuvo lugar de manera semanal durante los periodos de producción otoñales entre 2010 y 2013. Se establecieron 27 parcelas (100m²) en matorrales de diferente edad y origen: (a) jaral de mediana edad (8 años) cuyo origen fue un incendio forestal, (b) jaral de mediana edad (8 años) cuyo origen fue un desbroce y (c) jaral senescente (20 años). Los tratamientos considerados fueron desbroce total, desbroce manual al 50% y quema controlada. Todos los carpóforos fueron identificados y pesados en fresco y en seco. Se recolectaron en total 63 436 carpóforos pertenecientes a 157 taxones dentro de 64 géneros durante los cuatro años de muestreo. Se encontró mayor producción en peso fresco en los jarales de mediana edad en comparación con los jarales senescentes. Después del desbroce al 50% la producción, diversidad y composición específica era muy similar a las parcelas control donde no se realizó ningún tratamiento. Este parece ser el tratamiento más adecuado para la producción de especies comestibles, especialmente *Boletus edulis*, a la vez que puede reducir la intensidad y severidad del fuego. Además, los desbroces totales favorecen la fructificación de especies nuevas, especialmente saprófitas. Por tanto, según los resultados de este estudio, el rejuvenecimiento de jarales senescentes y la alternación de diferentes tratamientos de reducción de combustible en jarales de mediana edad parecen ser las mejores pautas para el manejo sostenible de este recurso.

Palabras clave: Manejo sostenible, Prevención de incendios, Setas, Producción, Diversidad

Impact of fuel reduction treatments on fungal sporocarp production and diversity associated with *Cistus ladanifer* L. ecosystems

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ABSTRACT

Mediterranean *Cistus ladanifer* scrublands can provide an important fungal production, often in high demand. However, due to the pyrophytic characteristics of this species, forest fires are the main threat to these ecosystems. The aim of the study is to analyze the effects of different fuel reduction treatments on *C. ladanifer* scrublands on production and diversity of fungal communities in order to enhance mushroom production and diversity and prevent forest fires. Sporocarp sampling was carried out on a weekly basis during autumnal production periods between 2010 and 2013. Twenty-seven plots (100 m^2) were established in scrublands of different age and origin: (a) a middle-age scrubland (8 years old) whose origin was a forest fire, (b) a middle-age scrubland (8 years old) whose origin was the total clearing of the previous stand, and (c) a senescent scrubland (20 years old). Considered fuel reduction treatments were total clearing, 50% clearing and controlled burning. All the sporocarps were identified and fresh and dry weighed. A total of 63,436 sporocarps belonging to 157 taxa within 64 genera were collected during the four years' sampling. Higher total fungal fresh weight production was found in middle-aged compared with senescent scrublands. After the 50% clearing, production, diversity and species composition of fungal communities were very similar to the control plots in which no treatment was performed. It seemed to be the most appropriate treatment for the production of edible species, especially *Boletus edulis* and this treatment may also reduce fire intensity and severity. Furthermore, total clearing favors the fructification of new species, especially saprotrophic ones. Therefore, in this study, the rejuvenation of senescent scrublands and the alternation of different fuel reduction treatments in middle-aged stands seemed to be the best management guidelines for the sustainable management of this resource.

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1. Introduction

Wild mushroom harvesting is becoming a very important socioeconomic resource in economically disadvantaged rural areas (Bonet et al., 2014). The benefit obtained from this resource is not limited to the marketing and processing of the product. It is also necessary to take mushroom picking into account as a recreational activity and environmental service (Martínez de Aragón et al., 2011; Schulp et al., 2014). There is also an important touristic activity associated with this resource that can provide high economic benefits in the production areas (de Frutos Madrazo et al., 2012). In Castilla y León (North-central Spain) mushroom picking involves 54% of the rural population and edible mushroom production can reach 65 million euros in potential income (Martínez-Peña et al., 2007).

Cistus species may form both ectomycorrhizas and vesicular arbuscular mycorrhizas and they are associated with more than 200 ectomycorrhizal fungal species of 40 different genera (Comandini et al., 2006). This genus comprises several obligate seeder shrub species of pyrophytic shrubs (12 in the Iberian Peninsula), which are mainly distributed around the Mediterranean basin and constitute early successional stages in Mediterranean forest ecosystems (Agueda et al., 2008). They can

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colonize highly degraded areas after fire as high temperatures generated by fire in the top layers of the soil trigger seed germination (Bastida and Talavera, 2002). The most abundant *Cistus* species in the Spain is *Cistus ladanifer* L., which has a distribution area of about 3 million hectares and is present in 15% of the forest area of the country (Martín Morgado et al., 2005).

The *C. ladanifer* ecosystem provides high production of edible mushroom species, some of them in great demand due to their gastronomic interest. Species belonging to the genus *Boletus* are the most economically valuable fungal species in these ecosystems (Oria-de-Rueda et al., 2008). *Boletus edulis* is a wild species widely marketed in many countries (Alonso Ponce et al., 2011). It is one of the highest priced species in Spanish markets, reaching 40–50 €/kg and significantly increasing each year. *B. edulis* is usually collected in forest areas dominated by species of *Pinus*, *Quercus* and *Castanea*. However, fruiting of these fungi in forest occurs only in mature stands (30–40 years old) (Oria-de-Rueda et al., 2008). Since fruiting in *C. ladanifer* ecosystems occurs at a much earlier age (3–4 years old), *C. ladanifer* scrubland conservation could be of interest in areas with poor and degraded soils, where the productivity of forested areas is very low and economic benefit scarce. The main problem for mushroom picking in these scrublands is that the high density reached in mature stands hampers harvester accessibility. Therefore, silvicultural management aimed at reducing shrub density could well facilitate mushroom harvesting and increase the economic benefit obtained from this resource.

Although traditionally, forest planning and management has paid little attention to mushroom harvesting, the growing interest in this non-wood forest product has made the management of forest stands for mushroom production (mycosilviculture) increasingly more important (Savoie and Largeteau, 2011). This has led scientists to study the state of conservation of diversity and production of wild mushroom communities and the effect of silvicultural treatments on them (Martínez-Peña et al., 2011). Most of these studies are focused on the effect of thinning on fungal communities in tree stands. Shaw et al. (2003) found little effect of fungal productivity five years after thinning under *Pinus sylvestris*. A temporal relationship between tree growth reaction after thinning and the production of associated fungal community was observed in a mixed old-growth forest in Switzerland (Egli et al., 2010). These authors found low production during the first 3 years after thinning and a recovery during the fourth year. On the contrary, Bonet et al. (2012) found an immediate positive effect of thinning on *Lactarius deliciosus* production in a *Pinus pinaster* plantation, and de-Miguel et al. (2014) found greater mushroom yields in pine stands with more intensive management practices. Regarding *B. edulis* production, Salerni and Perini (2004) studied the effect of tree canopy and presence/absence of litter in order to increase the productivity of this species in different forest stands in Italy. These authors found a positive effect of medium thinning and a negative effect of litter layer removal in *B. edulis* fructification.

Azul et al. (2011) tested the influence of land use practices used to control shrub density on macromycetes fruiting in *Quercus suber* woodlands. These authors found that current practices used to control shrub density account for considerable variation in mushroom fruiting. The effects of permanent grazing or soil tillage were more severe than cutting practices without soil tillage.

However, to our knowledge, this is the first study of the effect of fuel reduction treatments on fungal communities associated with *C. ladanifer* scrublands. Some regions in the northwest of the Iberian Peninsula are characterized by the traditional use of fire to control shrubs. This fact, together with the pyrophytic ecology of *C. ladanifer*, results in a very high incidence of wildfires in these areas. In some cases, the existence of forest fires may be linked to mushroom harvesting either because mushroom pickers try to favor some species fructification or because they seek to improve transit and visibility through the stands, thus facilitating sporocarp collection (Martínez de Azagra et al., 1998). Different fuel reduction treatments can be used in the management of scrublands to prevent wildfires and reduce their negative effects. Studying the effect of these treatments on mushroom production may lead to finding a suitable option for managing *C. ladanifer* scrublands, improving edible mushroom production and preventing fires in adjacent forests. The present study was requested by the forest managers of this area. The aim of this study is to analyze the effects of different fuel reduction treatments in *C. ladanifer* scrublands on associated fungal communities in order to enhance sporocarp production and diversity and prevent forest fires. Our specific objectives are to analyze the influence of these treatments on: (1) sporocarp production; (2) fungal richness and diversity; and (3) the similarity of the specific composition of the fungal communities.

2. Materials and methods

2.1. Study site

The study area is located in Zamora province in North-western Spain (0730462–0731929 Longitude-UTM, 4619644–4621757 Latitude-UTM 29T Grid).

It is a Mediterranean ecosystem dominated by *C. ladanifer* situated at 750–780 m above sea level. The soil in this area is constituted by Paleozoic metamorphic rocks, Ordovician and Silurian shales being predominant (García Rodríguez et al., 1964). The soil is classified as Inceptisol suborder Xerept (Soil Survey Staff, 2010) and is characterized by stoniness, acidity (pH 5.0–5.5), and lack of calcium and phosphorous. Nitrogen and potassium availability is variable and the level of humification is generally good (García Rodríguez et al., 1964). The area is characterized by a sub-Mediterranean climate with a dry season of three months in the summer and a mean annual rainfall of 450–700 mm. Mean temperatures range from 14.5 to 15.8 °C.

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).

2.2. Fuel reduction treatments

Plots were established in three areas where *C. ladanifer* age and stand origin were different: (a) a middle-age stand (8 years old) whose origin was a forest fire, (b) a middle-age stand (8 years old) whose origin was the clearing of the previous stand, and (c) an senescent stand (20 years old) whose origin was a forest fire. Treatments were chosen based on their applicability in accordance with the age of the stands and vegetation characteristics. The study site was not replicated across several sites.

C. ladanifer mean height in the middle-age stands (a and b) was 1.30 m when the treatments were carried out. Mechanized cutting practices (clearing) are used to control shrub density in these stands. In the two middle-age stands the following treatments were carried out: (1) Control; (2) 50% clearing; and (3) Total clearing.

In the senescent stand (c), *C. ladanifer* mean height was 2 m. It is a senescent stand with characteristics (high density, presence of dead plants and branches and lichens – *Evernia prunastri*-covering the stems) that are optimal for the ignition and spread of fire. Destroying dead plants by mechanical tools or by controlled burning can be the means to avoid forest fires and reduce their negative effects in these ecosystems (Oria-de-Rueda et al., 2008). Thus, in the senescent stand the studied fuel reduction treatments were: (1) Control; (2) Total clearing; and (3) Controlled burning.

Total clearing was carried out in spring 2010 with a New Holland TS115 tractor with a brush thrasher mower, whereas

50% clearing was performed by two operators manually removing half of the plants with a brushcutter. Controlled burning was performed with the help of Zamora EPRIF (Integral Fire Prevention Team) (Ministry of Agriculture, Food and Environment) in October 2010 under favorable weather conditions that allowed ignition without the risk of any fire getting out of control. Treatments were performed in strips and boundaries were marked with wooden stakes. Perimeter security areas were established around the controlled burning plots.

Thus, we studied three treatments in each of the three different areas. Three sampling plots were established in each of the treatments. As a result, twenty-seven sampling plots were analyzed. These sampling plots consisted of transects of 2 m × 50 m, established in accordance with previous studies (Luoma et al., 1991; Smith et al., 2002).

2.3. Sampling

All sporocarps were collected on a weekly basis during the autumn mushroom season from late October to late December from 2010 to 2013. Sampling began the first autumn production season after the treatments had been implemented. The spring fruiting season was ignored because in this area it is characteristically very short and almost insignificant. As the average duration of fruiting bodies varies among species from 4 to 20 days (Vogt et al., 1992) it is difficult to choose a sampling frequency that suits all species and does not distort production. Weekly sampling frequency has been used by several authors in previous works (Baptista et al., 2010; Ohenoja and Koistinen, 1984). Fungal sporocarps were harvested, transported to the laboratory and stored at 4 °C. Fresh weight was measured and fresh characters for identification were recorded within 24 h after collection.

2.4. Identification and classification

The sporocarps were identified at species level whenever possible. As in previous works (Bonet et al., 2004; Martín-Pinto et al., 2006) samples that could only be identified to genus level were grouped into genus taxa. Fungal taxa names and authors were obtained from the Index Fungorum database (www.indexfungorum.org). Taxa were classified according to their trophic group (saprotrophic/mycorrhizal) for further statistical analysis according to various bibliographic sources (e.g. Agerer, 2006; Gadd et al., 2007; Hobbie and Agerer, 2010; Taylor et al., 2003; Tedersoo et al., 2010; Trudell et al., 2004). Species traditionally consumed in the study region and those classified as edible in most of the literature consulted were listed as edible (Gassibe et al., 2015; Martínez de Aragón et al., 2007).

After identification, the fresh weight and number of sporocarps were measured. The sporocarps were then dried in air-vented ovens at 35 °C and dry weighed in order to obtain comparable biomass data. Dried sporocarps were stored and used to complete identification from microscopic key characters when necessary.

2.5. Data analysis

Shannon's H' diversity index (Shannon and Weaver, 1949), based on the dry weight of the fruiting bodies (Dahlberg, 1991) was calculated. This variable was calculated using the following formula where coefficient pi indicates the proportion of the total biomass of each fungal species:

$$H' = -\sum pi(\ln pi)$$

Species richness (S) is the total number of taxa found each sampling year (Straatsma and Krisai-Greilhuber, 2003).

Production was determined by measuring sporocarp fresh weight [kg fw ha^{-1} yr^{-1}]. Also, number of fruiting bodies, H', and S were analyzed statistically.

A linear mixed model of analysis of variance with repeated measurements was used for the statistical analysis, considering three between-subjects factors (stand origin with three levels, treatment with nine levels nested within stand type and year with four levels as a random effect) and one within-subjects factor (way of life, with two levels). The mathematical formulation of the model was given by:

$$Y_{ijkt;l} = \mu + \alpha_i + \beta_{j(i)} + \delta_t + \gamma_l + \alpha\gamma_{il} + \beta\gamma_{jl(i)} + \varepsilon_{ijkt;l}$$

with i = 1, 2, 3 for stand type; j = 1, 2, 3 for treatments within each level of stand type, k = 1, 2, 3 for the replicates of plots with the treatments, t = 1, 2, 3, 4 for years and l = 1, 2 for the way of life, where:

$Y_{ijkt;l}$ = observed value of the dependent variable (logarithm of fresh weight production, Shannon diversity index and species richness) for plot k with treatment j nested within stand type i in year t, in way of life l.
μ = general mean effect.
α_i = main effect of stand type i.
$\beta_{j(i)}$ = main effect of treatment j nested within stand type i.
δ_t = random effect of year t.
γ_l = main effect of way of life l.
$\alpha\gamma_{il}$ = interaction effect between stand type i and way of life l.
$\beta\gamma_{jl(i)}$ = interaction effect between treatment j, nested within stand type i, and way of life l.
$\varepsilon_{ijkt;l}$ = random error in the dependent variable for plot k with treatment j nested within stand type i in year t, in way of life l.

Finally, Fisher's LSD test was used for the comparisons of least square means within the same stand type. On the other hand, comparisons between treatments nested within different stand types was performed after removing the difference due to the stand types and using individual contrast tests for $(\overline{Y_{ij...;l}} - \overline{Y_{i'j'...;l}}) - (\overline{Y_{i...;l}} - \overline{Y_{i'...;l}})$. The procedure MIXED in SAS software was used for the statistical analysis of the model. Fresh weight data were log-transformed in order to meet normality of the residuals assumption.

The effect of fuel reduction treatments on community composition was tested by permutational multivariate analysis of variance (perManova). In addition, non-metric multidimensional scaling (NMDS) with the "Jaccard" similarity matrix was used to represent graphically differences in fungal community composition produced by the different treatments. Finally, in order to facilitate interpretation, the standard error ellipses of each silvicultural treatment were used to illustrate the position of each treatment on the biplots. The "adonis", "metaMDS" and "ordiellipse" functions in the vegan package (Oksanen et al., 2015) in R software (R Core Team, 2013), were respectively used for these analyses. Taxa that were recorded only one sampling day and only in one plot were excluded from the analysis.

The significance level was p-value < 0.05 in all the statistical analysis.

3. Results

3.1. General data

A total of 63,436 individual sporocarps were collected from the 27 plots (2700 m^2 total), during the four sampling years. Fungi were classified into 157 different taxa belonging to 64 genera (Table A1). There were several generic level taxa for which further identification was not possible, generally including more than a single species.

Table 1
Number of total taxa collected in the different silvicultural treatments classified into species groups.

	8 yr-clear			8 yr-burn			20 yr			Total
	Control	50% Clearing	Total clearing	Control	50% Clearing	Total clearing	Control	Total clearing	Burn	
Mycorrhizal	35	34	25	37	36	19	35	6	24	62
Saprotrophic	39	42	39	37	32	35	48	36	35	95
Total	74	76	64	74	68	54	83	42	59	157

8 yr-clear: 8-year-old stand whose origin was a mechanical clearing; 8 yr-burn: 8-year-old stand whose origin was a forest fire; 20 yr: 20-year-old stand.
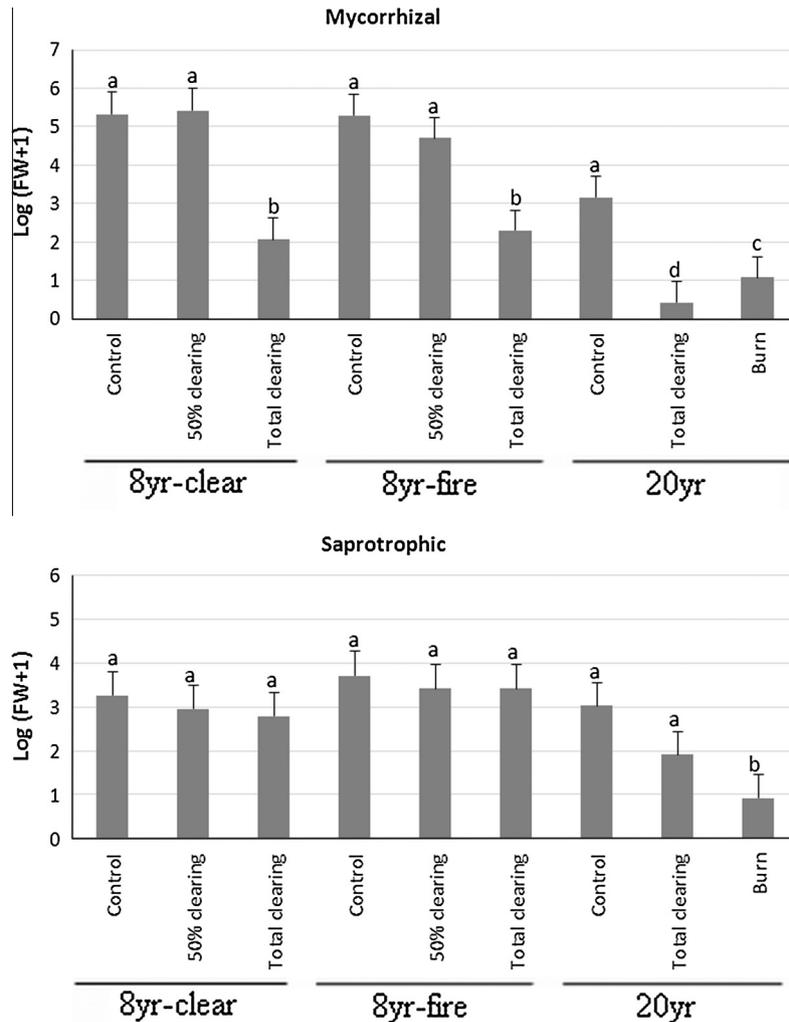
Fig. 1. Fresh weight production in the different silvicultural treatments. The data are plot means ± standard error. Data was log-transformed (Log [Fresh Weight +1]) for statistical analysis. Lower case letters indicate differences between silvicultural treatments (*p*-value < 0.05). Fisher's LSD test was used for the comparisons of least square means within the same stand type. Comparisons between treatments nested within different stand types were performed after removing the difference due to the stand types and using individual contrast tests. 8 yr-clear: 8-year-old stand whose origin was a mechanical clearing; 8 yr-burn: 8-year-old stand whose origin was a forest fire; 20 yr: 20-year-old stand. 50% cl: 50% clearing; Total cl: Total clearing; Burn: Controlled burn.

The total taxa found during the four years of sampling revealed a predominance of saprotrophic taxa (95 taxa, 61%), while 39% (62 taxa) were identified as mycorrhizal (Table 1). However, considering different stands and treatments, this tendency was especially clear in all the treatments where vegetation was completely removed and in the control plots situated in the 20-year-old senescent stand (Table 1).

3.2. Effect of treatments in sporocarp production, richness and diversity

Total average fresh weight production found in this study was 157.45 kg fw ha^{-1} yr^{-1}. Mycorrhizal taxa showed lower production

in those treatments where vegetation was completely removed, higher values being presented in the control plots and 50% clearing treatments (Fig. 1). Although the individual contrast test in the nested model did not show significant differences among control treatments, data tendency suggests that production in senescent stand is lower than in mid-rotation scrublands. In the case of saprotrophic taxa, the only significant difference was the controlled burning in the senescent stand, which presented the lowest saprotrophic production (Fig. 1).

Mycorrhizal species richness was significantly higher in all the treatments where vegetation was not completely removed (control plots and 50% clearing) (Fig. 2). The highest value of saprotrophic taxa richness was found in the control plots in the senescent stand
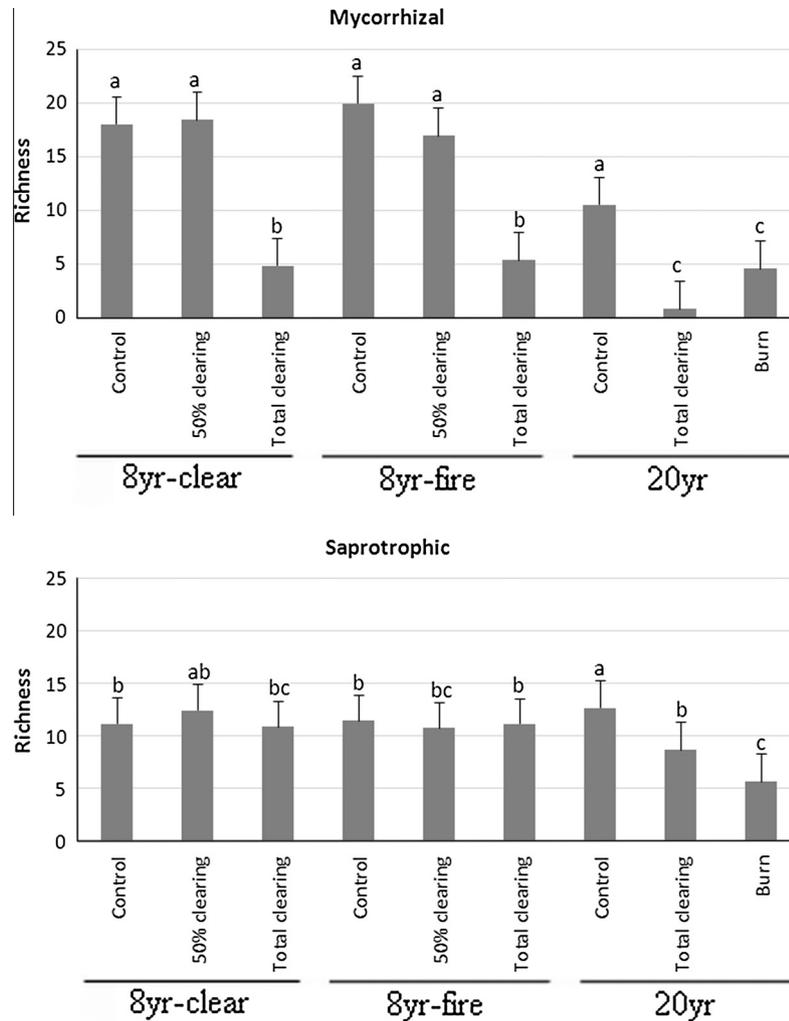
Fig. 2. Species richness (number of species) in the different silvicultural treatments. The data are plot means ± standard error. Lower case letters indicate differences between silvicultural treatments within a sampling year (*p*-value < 0.05). Fisher's LSD test was used for the comparisons of least square means within the same stand type. Comparisons between treatments nested within different stand types were performed after removing the difference due to the stand types and using individual contrast tests.8 yr-clear: 8-year-old stand whose origin was a mechanical clearing; 8 yr-burn: 8-year-old stand whose origin was a forest fire; 20 yr: 20-year-old stand. 50% cl: 50% clearing; Total cl: Total clearing; Burn: Controlled burn.

compared to the control plots in the mid-rotation ones (Fig. 2). Controlled burning negatively affected the richness of saprotrophic taxa, presenting significantly lower values compared to most of the other treatments (Fig. 2).

In regard to the Shannon Diversity Index, the two-level classification according to the presence or absence of vegetation was also found in the mycorrhizal taxa (Fig. 3). Regarding saprotrophic taxa, the only stand that presented significant differences among treatments was the 20-year-old senescent one, in which controlled burning showed a lower Shannon Index than control and total clearing (Fig. 3).

3.3. Treatment similarity and species composition

The effect of treatment, type of stand and their interaction significantly affected species composition (perManova p-values: 0.001, 0.002 and 0.001, respectively).

NMDS ordination biplot (stress: 0.19; Fig. 4) and standard error ellipses showed an ordination of the plots regarding the age of stands along axis 1. All the treatments located in the 8-year-old mid-rotation scrublands are located in the left part of the plot whereas all the treatments located in the 20-year-old senescent stand are located in the right part of the plot. Among the plots located in the 8-year-old mid-rotation stand, there is also an

ordination along axis two, in which the treatments located in the stand that was established after a fire are at the top of the plot whereas the plots located in the scrubland whose origin was the clearing of the previous stand are located at the bottom of the plot. The two 50% clearing treatments are very similar to the control treatments located in the same stand according to their specific composition (Fig. 4).

4. Discussion

4.1. General data

C. ladanifer ecosystems are very common in large areas in the Iberian Peninsula and North Africa, and other Mediterranean regions. Adequate management of these ecosystems could result in a decrease in the effect of fire on forest systems and an increase in the production and diversity of fungal communities adding economic and ecological value to these areas. To our knowledge, this is the first study of the influence of different fuel reduction techniques on fungal communities associated to *C. ladanifer* scrublands. Due to the fact that forest managers in this area requested this study, our results are especially valuable as they will have direct application in these ecosystems, reducing the incidence and
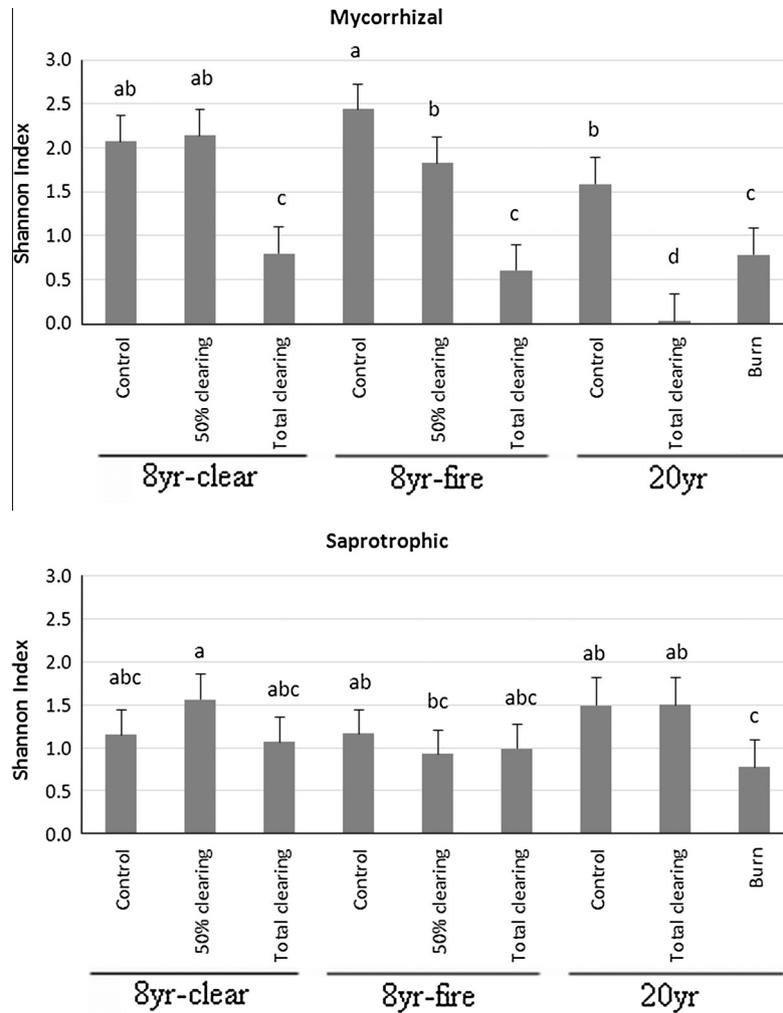
Fig. 3. Shannon diversity index in the different silvicultural treatment. The data are plot means ± standard error. Lower case letters indicate differences between silvicultural treatments (*p*-value < 0.05). Fisher's LSD test was used for the comparisons of least square means within the same stand type. Comparisons between treatments nested within different stand types were performed after removing the difference due to the stand types and using individual contrast tests. 8 yr-clear: 8-year-old stand whose origin was a mechanical clearing; 8 yr-burn: 8-year-old stand whose origin was a forest fire; 20 yr: 20-year-old stand. 50% cl: 50% clearing; Total cl: Total clearing; Burn: Controlled burn.

negative effects of wildfires while facilitating the sustainable harvesting of marketable mushrooms. It should be noted that the experimental design was not replicated at different sites, and the results cannot be extrapolated to those in other areas. Thus, further research is necessary in other areas to provide general *C. ladanifer* management guidelines. However, we believe that this study provides a first approach that can be useful in the management of these ecosystems.

The very high number of total taxa (157) found during the four-year period of sampling *C. ladanifer* plots located in degraded, stony soils, is remarkable. Contrary to expectations, fungal richness found in these ecosystems was similar to or even higher than the number of taxa found in some studies of mature stands of different forest tree species. Gassibe et al. (2011) reported 115 fungal taxa in a four-year study in a nearby *P. pinaster* Ait. mature stand. Furthermore, Martínez-Peña et al. (2012) found 119 species in a ten-year study in *P. sylvestris* L. stands of different age classes in Spain, and Baptista et al. (2010) recorded 73 different species in a four-year study in *Castanea sativa* Mill. in Portugal.

4.2. Effect of treatments on sporocarp production, richness and diversity

Temporary site disturbance caused by silvicultural treatments may produce a decrease in fungal production during the first years

(Egli et al., 2010). This result is in agreement with Pilz et al. (2006), who found a reduction in both the number and weight of *Cantharellus formosus* sporocarps the first year after thinning in a Douglas-fir stand, but found no differences the following six years.

Higher production is generally observed in young forest stands than in mature ones (Bonet et al., 2004; Senn-Irlet and Bieri, 1999). The tendency of lower production in the senescent scrublands compared to the mid-rotation stands may be explained by the lower photosynthetic activity of the senescent scrubland and its decreased growth. Therefore, to enhance this resource, management should be aimed at rejuvenating senescent *C. ladanifer* scrublands.

Mycorrhizal production, richness and diversity were significantly higher in the treatments where vegetation was not completely removed. This is an expected result considering the characteristics of mycorrhizal species that require the presence of a host plant species for their survival (Dahlberg, 2002). 50% clearing treatment in the 8-year-old stands produced as much fresh weight as in the control plots. This treatment can reduce the amount of fuel, thus decreasing forest fire intensity and facilitating its extinction. In addition, 50% clearing may also enable accessibility and mushroom harvesting by decreasing scrublands density.

In contrast to the results found in mycorrhizal species, no differences were found in the species richness of saprotrophic species
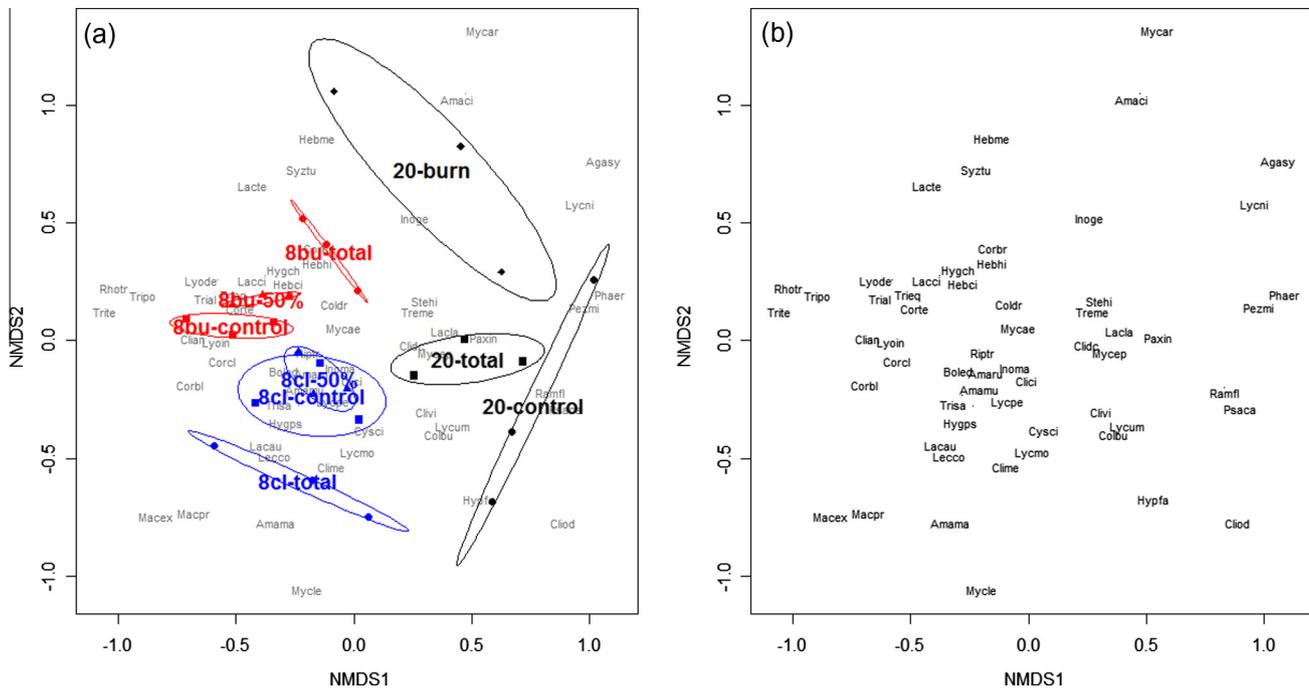
Fig. 4. Non-metric multidimensional scaling (NMDS) ordination of fungal species and plots of different treatment. (a) Plots and distribution of treatment expressed as standard error ellipses (95% confidence intervals) using a species-abundance matrix. (b) Fungal species using a species-abundance matrix. Fungal taxa are identified by the code shown in Table A1. 8 cl: 8-year-old stand whose origin was a mechanical clearing; 8bu: 8-year-old stand whose origin was a forest fire; 20: 20-year-old stand. 50%: 50% clearing; Total: Total clearing; Burn: Controlled burn.

within the two 8-year-old scrublands. After a stand clearing disturbance, new soil and light conditions may facilitate the establishment of early succession species (Clark and St. Clair, 2011). In the case of this study, pioneer species are dominated by saprotrophic taxa. In addition, a large number of saprotrophic species were found exclusively in the control treatment of the senescent stand, which is likely due to the large amount of organic matter present in these plots.

4.3. Treatment similarity and species composition

Differences in community composition are reflected in the NMDS ordination biplot (Fig. 4). Ordination of the treatments along axis 1 shows different specific composition in the different stand ages. After disturbance events, spore bank and other resistant fungal propagules play an essential role in fungal community recovery (Buscardo et al., 2010). Thus, the composition of these resistant propagules may be different for the two different stand ages.

In addition, vertical ordination of the mid-rotation stands along axis 2 depending on the origin of the stands (forest fire or total clearing) may be due to a different fungal succession depending on whether the scrubland was established after a forest fire or after a mechanical clearing. Fire alters soil properties, which can influence the composition of fungal communities (Anderson and Cairney, 2007).

The specific composition of 50%-clearings is similar to the control plots located in the same stand. Furthermore, most of the valuable edible fungal species are associated mainly with these mid-rotation stands, such as *B. edulis*, *Leccinum corsicum*, *Lyophyllum* spp., *Amanita rubescens* or *Lactarius aurantiacus*. This fact may indicate that 50% manual clearing does not have a disturbance effect on fungal species composition. Regarding the total clearing treatments in the middle age stands, several heliophilous species typical of grasslands and open areas, such as *Macrolepiota*

spp., were found. Furthermore, some of these new species that fructified after total clearing are edible, and their harvesting could provide a new economic benefit.

Concerning the plots located in the senescent stands, we found several species that are usually associated with the later stages of the succession of forest such as *Amanita citrina*, *Hypholoma fasciculare* or *Agaricus sylvicola*. This fact, together with higher fungal richness, may indicate that, although fungal production in these plots was lower, conservation of some senescent areas could also enhance fungal richness and diversity.

5. Conclusions

According to the results of this work, rejuvenation of old senescent scrublands may increase fungal production while decreasing the high risk of fire associated to these areas, the characteristics of which are optimal for fire ignition and spread. Furthermore, 50% clearing of middle-aged scrublands produces high yields of some valuable species such as *B. edulis*, *L. corsicum* or *Lyophyllum decastes*. This treatment may facilitate sporocarp collection while decreasing the amount of fuel and preventing the propagation of forest fires. Moreover, total removal of vegetation by mechanical clearing in some areas may favor the appearance of new edible heliophilous species, while breaking fuel continuity completely.

Finally, maintaining some patches of senescent scrublands would contribute to keeping high fungal diversity and richness in the area by preserving fungal species that are exclusively adapted to senescent stands. This mosaic management integrating the different treatments must be well planned so that senescent stands can act as a source of propagules for adjacent areas. Adequate management of these areas would derive important economic benefits from mushroom harvesting in ecosystems that are traditionally considered unproductive, generally located in poor and economically disadvantaged rural areas.

Table A1
Total taxa collected in the different silvicultural treatments during the four sampling years.

Taxa	Code	Trophic group	Edibility	8 yr-clear			8 yr-burn			20 yr		
				Control	50% Clearing	Total clearing	Control	50% Clearing	Total clearing	Control	Total clearing	Burn
Agaricus cupreobrunneus (Jul. Schäff. & Steer) Pilát	Agacu	S	E			+				+		
Agaricus sylvicola (Vittad.) Peck	Agasy	S	E			+				+	+	+
Agrocybe sp.		S					+			+		+
Amanita citrina (Schaeff.) Pers.	Amaci	M								+		+
Amanita mairei Foley	Amama	M	E	+	+	+						
Amanita muscaria (L.) Lam.	Amamu	M		+	+	+	+	+	+	+	+	+
Amanita pantherina (DC.) Krombh.	Amapa	M			+			+	+	+		
Amanita rubescens Pers.	Amaru	M	E	+		+	+	+		+		
Astraeus hygrometricus (Pers.) Morgan	Asthy	M				+						
Boletus aestivalis (Paulet) Fr.	Bolae	M	E		+							
Boletus edulis Bull.	Boled	M	E	+	+	+	+	+		+		
Boletus erythropus Pers.	Boler	M	E					+				
Cantharellula umbonata (J.F. Gmel.) Singer	Canum	S										+
Chalciporus piperatus (Bull.) Bataille	Chapi	M		+	+		+	+		+		
Clavaria sp.		S						+				
Clavariadelphus pistillaris (L.) Donk	Clapi	M			+							
Clitocybe angustifolia (Kauffman) H.E. Bigelow	Clian	S		+	+		+	+		+		
Clitocybe cistophila Bon & Contu	Clici	S		+	+	+	+	+	+	+	+	+
Clitocybe dealbata (Sowerby) P. Kumm.	Clide	S		+						+		
Clitocybe decembris Singer	Clidc	S		+	+		+	+	+	+	+	+
Clitocybe diatreta (Fr.) P. Kumm.	Clidi	S					+		+			
Clitocybe fragrans (With.) P. Kumm.	Clifr	S				+					+	
Clitocybe inornata (Sowerby) Gillet	Cliin	S								+		
Clitocybe metachroa (Fr.) P. Kumm.	Clime	S		+	+	+	+			+	+	
Clitocybe obsoleta (Batsch) Quél.	Cliob	S				+			+			
Clitocybe odora (Bull.) P. Kumm.	Cliod	S								+	+	
Clitocybe phaeophthalma (Pers.) Kuyper	Cliph	S										+
Clitocybe vibecina (Fr.) Quél.	Clivi	S		+	+	+	+	+	+	+	+	+
Clitocybe sp.		S			+		+	+	+	+		
Collybia butyracea (Bull.) P. Kumm.	Colbu	S	E	+	+	+	+	+	+	+	+	+
Collybia cirrhata (Schumach.) Quél.	Colci	S	E				+					
Collybia dryophila (Bull.) P. Kumm.	Coldr	S	E	+	+	+	+	+	+	+	+	+
Collybia erythropus (Pers.) P. Kumm.	Coler	S	E	+			+					
Collybia sp.		S			+		+	+				
Conocybe sp.		S			+	+				+	+	
Coprinellus micaceus (Bull.) Vilgalys	Copmi	S									+	
Cortinarius balteatoocumatilis Rob. Henry ex P.D. Orton	Corbl	M		+	+	+	+	+				
Cortinarius balteatus (Fr.) Fr.	Corba	M		+								
Cortinarius brunneus (Pers.) Fr.	Cobr	M		+	+	+	+		+	+		+
Cortinarius cinnamomeoluteus P.D. Orton	Corcl	M		+	+			+				
Cortinarius cinnamomeus (L.) Fr.	Corci	M		+						+		+
Cortinarius eburneus (Velen.) Rob. Henry	Coreb	M								+		
Cortinarius flexipes (Pers.) Fr.	Corfl	M		+								
Cortinarius saturninus (Fr.) Fr.	Corsa	M					+					
Cortinarius sec. *caerulescens*	Corca	M		+	+	+	+	+	+	+		
Cortinarius sub. *telamonia*	Corte	M			+	+	+			+		
Cortinarius trivialis J.E. Lange	Cortr	M						+		+		
Cortinarius sp.		M		+	+	+	+	+	+	+	+	+
Crinipellis scabella (Alb. & Schwein.) Murrill	Crisc	S		+	+	+		+	+	+		+
Cystoderma cinnabarinum (Alb. & Schwein.) Fayod	Cysci	S			+	+				+		
Entoloma hebes (Romagn.) Trimbach	Enthe	S							+			
Entoloma hirtipes (Schumach.) M.M. Moser	Enthi	S		+	+	+				+	+	
Entoloma sp.		S		+	+	+	+	+	+	+	+	+
Fayodia gracilipes (Britzelm.) Bresinsky & Stangl	Faygr	S									+	
Flammulaster sp.		S										+
Galerina uncialis (Britzelm.) Kühner	Galun	S		+		+	+		+			
Galerina sp.		S		+	+	+	+	+	+	+	+	+
Gymnopilus decipiens (Sacc.) P.D. Orton	Gymde	S							+			
Gymnopus foetidus (Sowerby) P.M. Kirk	Gymfo	S										+
Gymnopus ocior (Pers.) Antonín & Noordel.	Gymoc	S				+	+	+	+			
Hebeloma cistophilum Maire	Hebci	M		+	+	+	+	+	+	+	+	+
Hebeloma hiemale Bres.	Hebhi	M		+	+	+	+	+	+	+		+
Hebeloma mesophaeum (Pers.) Quél.	Hebme	M		+			+	+	+	+		+

Table A1 (*continued*)

Taxa	Code	Trophic group	Edibility	8 yr-clear			8 yr-burn			20 yr		
				Control	50% Clearing	Total clearing	Control	50% Clearing	Total clearing	Control	Total clearing	Burn
Hebeloma sp.		M			+		+	+		+		+
Hygrophorus chrysodon (Batsch) Fr.	Hygch	M	E	+	+		+	+	+	+		+
Hygrophorus eburneus (Bull.) Fr.	Hygeb	M	E				+					
Hygrophorus hypothejus (Fr.) Fr.	Hyghy	M	E			+						
Hygrophorus pseudodiscoideus (Maire) Malençon & Bertault	Hygps	M		+	+	+	+	+	+	+	+	+
Hypholoma fasciculare (Huds.) P. Kumm.	Hypfa	S		+	+	+				+	+	
Inocybe geophylla (Bull.) P. Kumm.	Inoge	M		+	+			+		+		+
Inocybe grammata Quél.	Inogr	M								+		+
Inocybe maculata Boud.	Inoma	M		+								+
Inocybe sp.		M		+	+	+	+	+	+	+		+
Laccaria bicolor (Maire) P.D. Orton	Lacbi	M	E		+	+	+	+	+	+		+
Laccaria laccata (Scop.) Cooke	Lacla	M	E	+	+	+	+	+	+	+	+	+
Laccaria proxima (Boud.) Pat.	Lacpr	M	E	+	+	+	+	+	+	+		
Laccaria sp.		M		+		+						
Lactarius aurantiacus (Pers.) Gray	Lacau	M	E	+	+	+	+					
Lactarius chrysorrheus Fr.	Lacch	M										+
Lactarius cistophilus Bon & Trimbach	Lacci	M		+	+	+	+		+	+		+
Lactarius hepaticus Plowr.	Lache	M								+		
Lactarius sp.		M					+					
Lactarius tesquorum Malençon	Lacte	M		+	+		+	+	+	+		+
Leccinellum lepidum (H. Bouchet ex Essette) Bresinsky & Manfr. Binder,	Lecle	M		+								
Leccinum corsicum (Rolland) Singer	Lecco	M	E	+	+	+	+	+				
Lentinellus micheneri (Berk. & M.A. Curtis) Pegler	Lenmi	S		+	+	+	+	+	+	+	+	+
Lentinellus sp.		S		+	+		+					
Lentinus sp.		S		+								
Leocarpus fragilis (Dicks.) Rostaf.	Leofr	S		+						+		+
Lepiota helveola Bres.	Lephe	S									+	
Lepista nuda (Bull.) Cooke	Lepnu	S	E			+						
Limacella illinita (Fr.) Maire	Limil	M										+
Lycoperdon molle Pers.	Lycmo	S	E		+	+	+	+	+	+	+	
Lycoperdon nigrescens Pers.	Lycni	S				+					+	+
Lycoperdon perlatum Pers.	Lycpe	S	E	+	+	+	+	+	+	+	+	+
Lycoperdon pyriforme Schaeff.	Lycpy	S	E	+								
Lycoperdon umbrinum Pers.	Lycum	S		+					+	+	+	
Lycoperdon sp. P. Micheli		S										+
Lyophyllum decastes (Fr.) Singer	Lyode	M	E	+	+		+	+	+			
Lyophyllum fumosun (Pers.) P.D. Orton	Lyofu	M	E					+				
Lyophyllum infumatum (Bres.) Kühner	Lyoin	M	E	+				+		+		+
Lyophyllum loricatum (Fr.) Kühner	Lyolo	M	E					+				
Macrolepiota excoriata (Schaeff.) Wasser	Macex	S	E		+	+						
Macrolepiota mastoidea (Fr.) Singer	Macma	S	E			+						
Macrolepiota procera (Scop.) Singer	Macpr	S	E	+	+	+						
Marasmius androsaceus (L.) Fr.	Maran	S		+						+	+	
Marasmius scorodonius (Fr.) Fr.	Marsc	S		+				+				
Micromphale perforans (Hoffm.) Gray	Micpe	S						+				
Mycena aetites (Fr.) Quél.	Mycae	S		+	+	+	+	+	+	+	+	+
Mycena alcalina (Fr.) P. Kumm.	Mycal	S		+	+	+	+	+	+			
Mycena arcangeliana Bres.	Mycar	S					+					+
Mycena aurantiomarginata (Fr.) Quél.	Mycau	S			+			+		+		
Mycena cinerella (P. Karst.) P. Karst.	Mycci	S		+						+		+
Mycena clavicularis (Fr.) Gillet	Myccl	S		+	+					+		+
Mycena crocata (Schrad.) P. Kumm.	Myccr	S								+		
Mycena epipterygia (Scop.) Gray	Mycep	S		+	+	+			+	+		+
Mycena galopus (Pers.) P. Kumm.	Mycga	S				+						
Mycena leptocephala (Pers.) Gillet	Mycle	S		+		+				+	+	+
Mycena olida Bres.	Mycol	S					+					
Mycena pura (Pers.) P. Kumm.	Mycpu	S		+	+	+	+		+	+	+	+
Mycena rosea Gramberg	Mycro	S								+		
Mycena rosella (Fr.) P. Kumm.	Mycrs	S										+
Mycena vulgaris (Pers.) P. Kumm.	Mycvu	S							+			
Mycena sp.		S		+	+	+	+	+	+	+	+	
Omphalina sp. Quél.		S		+		+	+	+		+		+
Panaeolus rickenii Hora	Panri	S			+							
Paxillus involutus (Batsch) Fr.	Paxin	M		+	+	+		+		+	+	+
Peziza micropus Pers.	Pezmi	S						+		+		
Phaeomarasmius erinaceus (Fr.) Scherff. ex Romagn.	Phaer	S		+						+	+	+
Pholiota higlandensis (Peck) Singer	Phohi	S										+
Pleurotus ostreatus (Jacq.) P. Kumm.	Pleos	S	E							+		
Pluteus sp.		S					+					

Table A1 (*continued*)

Taxa	Code	Trophic group	Edibility	8 yr-clear			8 yr-burn			20 yr		
				Control	50% Clearing	Total clearing	Control	50% Clearing	Total clearing	Control	Total clearing	Burn
Psathyrella candolleana (Fr.) Maire	Psaca	S		+	+	+		+	+	+	+	+
Psathyrella corrugis (Pers.) Konrad & Maubl.	Psaco	S						+				
Psathyrella lacrymabunda (Bull.) M.M. Moser	Psala	S	E							+		
Psathyrella sp. (Fr.) Quél.		S		+	+	+	+	+	+	+	+	+
Ramaria flavoides Schild	Ramfl	M					+			+		
Ramaria sp.		M					+					
Rhodocybe truncata (Schaeff.) Singer	Rhotr	S					+	+		+		
Ripartites tricholoma (Alb. & Schwein.) P. Karst.	Riptr	S			+		+	+	+	+	+	+
Russula sp. Pers.		M		+	+	+	+	+	+	+		
Stereum hirsutum (Willd.) Pers.	Stehi	S		+	+	+	+	+	+	+		+
Stropharia aurantiaca (Cooke) M. Imai	Strau	S							+			
Stropharia sp.		S			+							
Protostropharia semiglobata (Batsch) Redhead	Prose	S		+								
Syzygospora tumefaciens (Ginns & Sunhede) Ginns	Syztu	S		+			+	+	+			
Thelephora caryophyllea (Schaeff.) Pers.	Theca	S			+							
Trametes versicolor (L.) Lloyd,	Trave	S							+			
Tremella mesenterica Retz.	Treme	S			+	+	+		+	+	+	
Tricholoma albobrunneum (Pers.) P. Kumm.	Trial	M					+	+		+		
Tricholoma equestre (L.) P. Kumm.	Trieq	M		+	+		+	+	+	+		
Tricholoma portentosum (Fr.) Quél.	Tripo	M					+	+				
Tricholoma saponaceum (Fr.) P. Kumm	Trisa	M		+	+		+	+		+		
Tricholoma terreum (Schaeff.) P. Kumm.	Trite	M					+					
Tricholoma sp. (Fr.) Quél.		M						+				
Tubaria sp. (Fr.) Staude		S		+	+	+	+	+	+	+	+	+
Vascellum pratense (Pers.) Kreisel	Vaspr	S		+								

M: mycorrhizal; S: saprotrophic; E: edible; 8 yr-clear: 8-year-old stand whose origin was a mechanical clearing; 8 yr-fire: 8-year-old stand whose origin was a forest fire; 20 yr: 20-year-old stand.

Appendix A

See Table A1.

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Original Article III

Climate-sensitive models for mushroom yields and diversity in *Cistus ladanifer* scrublands.

María Hernández-Rodríguez, Sergio de-Miguel, Timo Pukkala, Juan Andrés Oria-de-Rueda & Pablo Martín-Pinto

Resumen

Modelos susceptibles al clima para la producción y diversidad de setas en matorrales de *Cistus ladanifer*.

Las especies del género *Cistus* son matorrales pirófitos ampliamente distribuidos alrededor de la cuenca del Mediterráneo. Estos matorrales proporcionan elevadas producciones de hongos comestibles como *Boletus edulis*. Un manejo óptimo de estas zonas contribuiría a disminuir el riesgo de incendio y a incrementar los beneficios económicos derivados de la producción de setas. El objetivo de este estudio es desarrollar modelos predictivos para producción y diversidad de setas para comprender mejor la ecología de los jarales de *Cistus ladanifer* y proporcionar herramientas para su manejo. Los datos se tomaron en parcelas permanentes que consistían en transectos de 2mx50m. Los modelos para la producción y diversidad (índice de Shannon) anuales se desarrollaron ajustando ecuaciones diferentes para hongos micorrícicos y saprófitos. Además la producción de *B. edulis* se modelizó separadamente debido a su elevada importancia económica. Las variables utilizadas para ajustar los modelos incluyeron el tratamiento (desbroce o quema de la vegetación), tiempo trascurrido tras el tratamiento, características estructurales de la vegetación y variables climáticas. La altura media y la cobertura de matorral de los jarales de *C. ladanifer* también fueron modelizados en función del tratamiento y el tiempo tras el mismo. La temperatura media de las mínimas de otoño fue la variable climática que afectó a la fructificación de los hongos de manera más significativa. En relación a las características de la vegetación, la altura media de los matorrales fue la variable más significativa para la producción y diversidad de hongos. La producción de *B. edulis* se espera que comience 5 años después del tratamiento y su máximo de producción se alcanza a los 14 años. El efecto del tratamiento en la diversidad mostró seguir una tendencia opuesta que la producción. La diversidad fue mayor tras el fuego mientras la producción fue mayor tras el desbroce.
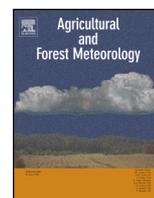
Climate-sensitive models for mushroom yields and diversity in *Cistus ladanifer* scrublands

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A B S T R A C T

Cistus species are pyrophytic scrubs widely distributed around the Mediterranean basin. *Cistus* scrublands provide high yields of edible mushroom species, such as *Boletus edulis*. A proper management of these areas may contribute to decreasing fire risk and increasing economic benefits derived from mushroom production. The aim of this study was to develop predictive models for mushroom yield and diversity in order to better understand the ecology of *Cistus ladanifer* scrublands and provide tools for their management. Measurements were conducted in permanent sample plots which consisted of 2 m × 50 m transects. The models for annual production and species diversity (Shannon index) were developed by fitting separate equations for mycorrhizal and saprotrophic fungi. *B. edulis* production was modelled separately due its high economic importance. Predictors accounting for the treatment (i.e. cutting or burning the scrub vegetation), time after treatment, structural characteristics of the vegetation and climatic variables were considered in modelling. Mean height and canopy cover of *C. ladanifer* scrublands were also modelled as a function of treatment and time after treatment. The mean minimum temperature of the autumn was the most significant climatic variable that affected fungal fructification. Regarding the vegetation characteristics, mean height of the scrub vegetation was the most significant predictor of mushroom production and diversity. *B. edulis* yield is expected to start 5 years after treatment and the peak of production is reached after 14 years. The effect of treatment on fungal diversity showed an opposite trend as compared to the effect on mushroom production. Fungal diversity was higher after burning than after clearing, whereas mushroom production was higher after total clearing.

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1. Introduction

Cistus species are mainly distributed around the Mediterranean basin and are typical of early successional stages in Mediterranean ecosystems (Agueda et al., 2008). This genus comprises several obligate seeder species of pyrophytic scrubs that can colonize highly degraded areas with poor and stony soils. The most abundant *Cistus* species in the Iberian Peninsula is *Cistus ladanifer* L., which has a distribution area of about 3 million hectares and is present in 15% of the forest area of the country (Martín Morgado et al., 2005).

Despite being traditionally considered as worthless unproductive ecosystems, *C. ladanifer* scrublands host a broad diversity of fungal species and provide high production of edible and inedible mushroom species (Oria-de-Rueda et al., 2008). The most valuable fungal species associated with this ecosystems is *Boletus edulis*, which is widely marketed in many countries (Boa, 2004) reaching high prices. In these ecosystems, the production of valuable mushroom species usually occurs sooner after clearing or natural disturbance than in tree stands (Oria-de-Rueda et al., 2008). Furthermore, *Cistus* scrublands play an important role in the recovery of forest stands after fire, providing mycorrhizal inoculum available to colonize tree roots as the new forest stands develop (Hernández-Rodríguez et al., 2013). The pyrophytic ecology of this species makes it especially vulnerable to wildfires, which constitute the main source of disturbance of *Cistus* scrublands. Indeed, periodic burning has been traditionally used by shepherds to maintain pas-

tures and prevent scrub development (Tárrega et al., 2001). Despite the decrease of cattle herding and the increasing land abandonment (Tárrega et al., 2001), burning is still common and it is linked to the occurrence of large wildfires. Proper management of these scrubland areas may contribute to decreasing the risk of forest fires and increasing the economic benefits derived from mushroom production. Total clearing or controlled burning are the most common management practices. Both aim at decreasing the damages caused by devastating uncontrolled wildfires (Oria-de-Rueda et al., 2008).

Although forest management planning has been traditionally focused on timber production, it is increasingly moving towards the integration of non-wood forest products (NWFP) and ecosystem services (Calama et al., 2010; Pilz et al., 1999). This represents an opportunity for many areas that, despite being unsuitable for timber production, can be managed for the provision of valuable NWFPs such as mushrooms. Furthermore, biodiversity conservation is gaining importance in land management and, in this regard, wild fungi constitute a significant diversity component (Van der Heijden et al., 1998). However, there is lack of knowledge for several ecosystems about the best management practices to preserve biodiversity (Bonet et al., 2010).

Empirical models are a useful tool to integrate mushroom production and diversity in the management of forest ecosystems (Bonet et al., 2008; de-Miguel et al., 2014). Such models allow for predicting fungal sporocarp production and diversity in a quantitative way throughout the life cycle of *C. ladanifer*, which is essential for the optimal management of these areas. These models need to consider the wide range of factors that influence the development of fruiting bodies. Climate and weather are key drivers of mushroom production. Positive correlation between mushroom production and climatic variables (especially precipitation and temperature) has been reported in several studies. Straatsma et al. (2001) found that fungal productivity was related to precipitation from June to October in a mixed forest in Switzerland. Gassibe et al. (2014) reported a significant effect of temperature and precipitation on mushroom production in Northwestern Spain. Martínez-Peña et al. (2012) concluded that temperature seems to be more crucial than rainfall for explaining fungal production in *Pinus sylvestris* forests in Spain. In addition, since vegetation structure may also affect mushroom yield (Bonet et al., 2008), management-oriented models should include predictors related to the vegetation structure (e.g. mean height, canopy cover or age), which can be modified by means of silvicultural techniques.

The use of statistical models to study fungal communities is relatively new and only a few models for predicting mushroom yield and diversity have been published (Bonet et al., 2012, 2010, 2008; Dahl et al., 2008; de-Miguel et al., 2014; Gassibe et al., 2014; Martínez-Peña et al., 2012). All these studies are focused on pine forests. No predictive mushroom yield or diversity models have been developed so far for scrublands.

The aim of this study was to develop predictive climate-sensitive models for mushroom production and diversity in *C. ladanifer* scrublands, with special attention to *B. edulis* sporocarp production. The models may be used as management tools in *Cistus* scrubland areas.

2. Materials and methods

2.1. Sample plots

The study area was located in Zamora province in Northwestern Spain (Latitude: 41°41′43″N–41°42′50″N; Longitude: 06°13′50″W–06°12′44″W). It is a Mediterranean ecosystem dominated by *C. ladanifer* situated at 750–780 m above the sea level. The soil is constituted by Paleozoic metamorphic rocks, with domain of Ordovician and Silurian shales (García Rodríguez et al., 1964). The soil is classified as Inceptisol suborder Xerept (Soil Survey Staff, 2010) and its characteristics are stoniness, acidity (pH 5.0–5.5), and shortage of calcium and phosphorous (García Rodríguez et al., 1964). The area is characterized by sub-Mediterranean climate with a dry season of three months in the summer and a mean annual precipitation of 450–700 mm. Mean temperatures range from 14.5 to 15.8 °C.

The modelling plots included two different treatments: total clearing and total burning. Plots representing total clearing were established in four areas: one site where vegetation was totally removed in 2002 and three other sites in which the total clearing was performed in 2010 (i.e. two young 8-year-old scrublands and one over-mature 22-year-old scrubland). Plots representing the burning treatment were established in two sites that had burned in 2002 and 1988, respectively. These plots had been inventoried in a previous study (see Hernández-Rodríguez et al., 2013), and data on mushroom production and diversity from 2003 to 2006 were also available. The sample plots consisted of 2 m×50 m transects, established in accordance with previous studies (Dahlberg and Stenlid, 1994; Ohenoja and Metsänheimo, 1982; Smith et al., 2002) and three replicates were established in each site (Table 1).

2.2. Data collection

All sporocarps were collected weekly during the autumn mushroom season, from late October to late December, during four consecutive years, since 2010 until 2013. The spring fruiting season was ignored since it is very short and insignificant in this area. All fungal sporocarps were harvested, transported to the laboratory, stored at 4 °C, and processed within 24 h after collection for taxonomic identification and fresh weight measurements.

Sporocarps were identified at the species level whenever possible. As in previous works (Bonet et al., 2004; Martín-Pinto et al., 2006), samples that could only be identified to the genus level were grouped into genus taxa. Taxa were also classified according to their trophic strategy (i.e. mycorrhizal and saprotrophic). After identification, fresh and dry weights were measured.

Shannon's H' diversity index (Shannon and Weaver, 1949), based on the dry weight of the fruiting bodies (Dahlberg, 1991) was calculated using the following formula where p_i indicates the relative proportion of each fungal species and n is the number of species.

$$H' = -\sum_{i=1}^{n} p_i \ln(p_i)$$

Climatic data were retrieved from the closest meteorological station (Alcañices, 41°41′03″N, 06°18′04″W and 806 m above sea level, Spanish Meteorological Agency). The weather conditions during the years of study are summarized in Table 2.

Vegetation structure of all the plots was measured in 2013. Scrub canopy cover was estimated using the line-intercept method and the mean height of the scrub layer was calculated as the mean height of the plants measured at one meter intervals (Ruiz-Peinado et al., 2013). Both measures were taken along the transects' centerline.

2.3. Modelling

2.3.1. Vegetation structure models
The measurements of mean height and canopy cover conducted in 2013 in plots with different scrub stand ages were used to model mean height and canopy cover as a function of treatment and time after treatment. Different growth functions such as Hossfeld I (cited by Peschel, 1938), modified Hossfeld I, Smalian (cited by Peschel,

Table 1
Plot characteristics and mushroom production and diversity of each plot. Y: years after treatment; CC: canopy cover; H: mean height; M: mycorrhizal taxa (including *B. edulis*), S: saprotrophic taxa; div: Shannon diversity index.

Plot	Treatment	Y[a]	CC[a] (%)	H[a] (cm)	M yield[b] (kg ha^{-1} year^{-1})	S yield[b] (kg ha^{-1} year^{-1})	M div.[b] (Shannon)	S div.[b] (Shannon)	*B. edulis* yield[b] (kg ha^{-1} year^{-1})
1	Clearing	3	27.62	58.70	41.6 (54.3)	19.1 (20.2)	0.9 (1.1)	0.9 (0.7)	0.0 (0.0)
2	Clearing	11	81	153.48	262.9 (149.5)	32.9 (22.4)	1.8 (0.6)	1.0 (0.4)	47.4 (47.4)
3	Clearing	3	50.4	63.94	3.9 (7.6)	60.5 (73.3)	0.4 (0.5)	0.9 (0.6)	0.0 (0.0)
4	Clearing	11	89.2	191.50	253.8 (129.2)	74.5 (59.9)	1.1 (0.6)	1.0 (0.3)	11.2 (9.1)
5	Clearing	3	75.2	82.60	160.4 (286.2)	44.0 (61.4)	1.2 (0.8)	0.9 (0.6)	0.8 (1.6)
6	Clearing	11	87.8	141.06	203.4 (168.9)	20.7 (27.3)	1.8 (0.2)	0.9 (0.7)	9.2 (14.1)
7	Fire	11	91	110.79	307.1 (259.5)	33.7 (28.2)	1.5 (0.8)	1.1 (0.3)	25.5 (31.4)
8	Clearing	3	27	51.30	88.2 (124.0)	56.4 (48.1)	0.6 (0.7)	0.8 (0.6)	0.0 (0.0)
9	Fire	11	86.02	128.96	240.0 (173.2)	40.2 (54.0)	1.4 (0.8)	1.1 (0.3)	4.4 (7.9)
10	Clearing	3	30.5	60.47	30.1 (43.4)	141.3 (189.2)	0.5 (0.5)	0.4 (0.4)	0.0 (0.0)
11	Fire	11	84.76	148.20	281.5 (214.6)	71.7 (11.3.0)	1.5 (0.7)	1.2 (0.2)	13.2 (21.2)
12	Clearing	3	21.26	68.58	28.0 (49.1)	83.8 (91.7)	0.6 (1.0)	0.7 (0.6)	0.0 (0.0)
13	Fire	25	91.8	187.98	30.6 (34.1)	14.8 (17.2)	1.7 (0.7)	1.3 (0.4)	6.2 (9.0)
14	Fire	25	85.8	185.26	34.2 (34.0)	10.0 (17.4)	1.4 (1.0)	1.5 (0.5)	7.3 (9.2)
15	Fire	25	73.4	141.00	28.3 (31.1)	34.6 (73.0)	1.2 (0.5)	0.9 (0.4)	7.0 (11.5)
16	Clearing	3	8.22	22.39	0.0 (0.0)	3.3 (1.9)	0.0 (0.0)	1.5 (0.6)	0.0 (0.0)
17	Clearing	3	2.9	25.76	1.9 (2.1)	18.2 (12.7)	0.0 (0.0)	1.2 (0.8)	0.0 (0.0)
18	Clearing	3	15.9	30.60	0.6 (0.4)	4.6 (2.2)	0.1 (0.1)	1.0 (0.8)	0.0 (0.0)

[a] Data refer to year 2013 (three years after the beginning of the study).
[b] Mean (Std. deviation) over all years considered in this study.

Table 2
Weather conditions during all sampling years.

		2003	2004	2005	2006	2010	2011	2012	2013
Total precipitation [P] (mm)	August	36.3	81.1	2.9	23.0	0.0	36.6	6.2	0.0
	September	55.6	25.8	7.2	94.1	19.8	7.2	31.6	51.6
	October	213.5	160.8	186.2	278.1	89.0	51.0	63.0	135.2
	November	137.6	31.3	34.3	211.2	60.8	89.0	82.6	6.8
	December	68.1	43.6	62.4	62.8	170.3	31.0	69.6	134.8
	Total	961.7	531.8	487.3	1120.6	827.5	575.2	400.2	783.4
Mean temperature [T] (°C)	August	22.0	17.7	20.3	19.0	21.9	20.4	20.2	21.3
	September	16.2	16.0	15.5	16.6	17.9	18.9	17.8	19.4
	October	9.6	11.0	11.2	12.4	11.6	15.0	11.8	13.3
	November	7.5	3.9	5.8	8.7	6.6	8.6	7.2	6.9
	December	4.0	3.3	3.1	3.3	3.7	4.9	5.5	4.0
	Total	11.5	10.7	10.7	11.5	11.5	12.7	11.7	11.7
Minimum absolute temperature [$Tmin$] (°C)	August	8.0	7.5	7.0	8.0	7.1	6.2	6.5	6.7
	September	5.0	3.0	1.5	4.0	3.9	2.9	3.8	8.5
	October	−1.5	−1.0	−2.0	0.5	−0.9	0.8	−1.8	0.1
	November	−3.0	−5.0	−5.5	−3.0	−5.6	−2.0	−3.5	−3.7
	December	−5.0	−4.0	−8.0	−6.5	−7.9	−3.7	−3.4	−8.6
	Total	−9.0	−8.0	−11.0	−15.0	−7.9	−5.3	−8.2	−8.6
Mean minimum temperature [$Tmmin$] (°C)	August	14.3	11.8	12.3	11.5	13.2	12.5	11.8	12.3
	September	9.4	9.0	7.7	10.0	10.4	11.0	10.3	11.5
	October	5.9	7.1	6.4	8.3	5.5	7.4	6.2	8.5
	November	4.6	0.1	2.3	6.4	1.8	4.2	2.8	1.8
	December	1.2	−0.2	−1.0	−0.6	−0.1	0.2	1.9	−1.0
	Total	6.7	5.8	5.4	6.6	5.5	6.3	5.1	5.4

1938) and Gompertz (cited by Winsor, 1932) were tested for mean height modelling and Hossfeld I (cited by Peschel, 1938), modified Hossfeld I, Richard-Chapman (Pienaar and Turnbull, 1973), Gompertz (cited by Winsor, 1932) and Van der Vliet (cited by Peschel, 1938) were tested for canopy cover modelling. The best equations were selected based on fitting statistics and biological considerations such as height and canopy development pattern at young ages and logical behaviour of the models in extrapolation when compared to the modelling data.

2.3.2. Mushroom production and diversity models

Models for total mushroom production and diversity were developed by fitting separate equations for the two main functional fungal groups (i.e. mycorrhizal and saprotrophic fungi) occurring in *C. ladanifer* ecosystems. A specific model for the production of *B. edulis* was also fitted separately from other mycorrhizal species due to the economic importance of this species. The predicted variable of the mushroom yield models was the annual sporocarp production in terms of fresh biomass (kg ha^{-1} year^{-1}) whereas in the diversity models, the predicted variable was the annual Shannon index.

The predictors included treatment, time after treatment and climatic variables. When significant, treatment was included in the fitted models as a dummy predictor. In order to facilitate the practical implementation of the models by forest managers, alternative models were fitted in which the predictors described the structural characteristics of the vegetation (cover and mean height of the scrub stands). These models can be used when inventory data are available but the time since fire or total clearing is unknown. The above-mentioned models for *C. ladanifer* development were used to recover some missing data for the vegetation cover and mean height in years lacking field measurements. The missing values were much constrained by the previous and subsequent measured development stages of the shrub, so that there was very little room

for producing biologically wrong estimates. In addition, those values were compared with those reported in previous research in order to further check their biological consistency.

All the models were fitted using nonlinear regression analysis in R software (R Core Team, 2013). Models were evaluated based on the following statistical criteria: (a) accordance with current scientific knowledge, (b) logical behaviour of the models in extrapolations, (c) parsimony, and (d) statistical significance (*p*-value <0.05).

To facilitate the understanding and interpretation of the models, a graphic simulation of each model was conducted as well by considering different climatic conditions. In these simulations, the maximum, average and minimum values recorded during the sampling period for each of the climatic variables listed in Table 2 were used to show the sensitivity of mushroom yield and diversity to changes within the range of variation observed for those predictors.

3. Results

3.1. Vegetation structure models

3.1.1. Mean height model

Mean height growth for *C. ladanifer* did not show any significant difference between the two studied treatments. Thus, the equation for predicting scrub mean height over time did not include treatment as a predictor.

The model for mean height development was fitted based on Gompertz growth function, which was the most consistent from the biological and statistical points of view:

$$H = \exp(5.07553 - 3.33490 \times \exp(-0.34492 \times Y)) \quad (1)$$

where H is the mean height of *C. ladanifer* (centimeters), and Y is the time after treatment (years).

According to the model, mean height increases sharply at early ages reaching its maximum approximately 15 years after total burning or clearing (Fig. 1a).

3.1.2. Canopy cover model

The model for the canopy cover evolution over time was based on Van der Vliet growth function, as it was the most consistent biologically and statistically:

$$CC = 79.7675 \times \left[1 - \left(1 + \frac{2Y}{2.7982 - 1.7610 \times TR} + \frac{2Y^2}{(2.7982 - 1.7610 \times TR)^2}\right)\right. \\ \left. \times \exp\left(\frac{-2Y}{(2.7982 - 1.7610 \times TR)}\right)\right] \quad (2)$$

where CC is the canopy cover of the scrubland (%), Y is the time after treatment (years) and TR is a dummy variable (equal to 1 if the scrubland was developed on burned area and equal to 0 if the scrubland was developed on cleared area).

Canopy cover development was significantly different for the two studied treatments. It develops faster in burned areas than after total clearing. However, after 15 years the canopy cover of *C. ladanifer* becomes similar for the two treatments and attains values of approximately 85% (Fig. 1b).

3.2. Models for mushroom production

3.2.1. Mycorrhizal taxa

The models for mushroom production of mycorrhizal fungi when using time after treatment (Eq. (3)) or vegetation characteristics (Eq. (4)) as predictors were as follows:

$$MycoP = \exp(-4.7588 + 2.4323 \times \ln(Tmmin_O) + 4.1331 \\ \times \sqrt{Y} - 0.7497 \times Y) \quad (3)$$

$$MycoP = \exp(-9.44295 + 1.73855 \times Tmmin_O + 2.43656 \\ \times \sqrt{H} - 0.12501 \times H) \quad (4)$$

where MycoP is annual mushroom yield from all mycorrhizal species (kg ha^{-1} year^{-1}), $Tmmin_O$ is the mean minimum temperature of October (°C), Y is time after treatment (years) and H is mean height of the scrubland (cm). The residual standard error was 135.6 kg ha^{-1} year^{-1} for Eq. (3) and 137.7 kg ha^{-1} year^{-1} for Eq. (4).

The production of mycorrhizal species follows an increasing trend during the early development stages of the scrubland, and a decreasing trend at older ages. The maximum mushroom yield from mycorrhizal fungi is reached in 7-year-old scrublands. For an average value of the mean minimum temperature of October during the study years (6.9 °C), the expected mushroom production of mycorrhizal taxa is higher than 100 kg ha^{-1} year^{-1} between 3 and 16 years. In years with higher mean minimum temperatures in October, the production of mycorrhizal fungi can exceed 400 kg ha^{-1} year^{-1} 5–10 years after burning or clearing (Fig. 2a). There is also an increasing–decreasing trend of mushroom yield as a function of mean height. The maximum mushroom yield of mycorrhizal fungi is achieved in scrublands of approximately 1 m in mean height (Fig. 2c). According to the models of vegetation development, such a height is reached 6–7 years after treatment.

The models for *B. edulis* production when using time after treatment or vegetation characteristics as predictors were as follows:

$$B.edulis = \exp(-17.09504 + 0.21615 \times T_{S+O} \\ + 8.70958 \times \log(Y + 0.001) - 0.65478 \times Y) \quad (5)$$

$$B.edulis = \exp(-1537 + 0.3669 \times Tmmin_{S+O} + 20.85 \times \sqrt{H} - 0.8591 \\ \times H - 130.4 \times \sqrt{CC} + 587.5 \times \ln(CC + 0.001)) \quad (6)$$

where *B. edulis* is the annual yield of *B. edulis* sporocarps (kg ha^{-1} year^{-1}), T_{S+O} is the sum of the mean temperatures of September and October, $Tmmin_{S+O}$ is the sum of the mean minimum temperatures of September and October, Y is time after treatment (years), H is mean height of the scrubland (cm) and CC is canopy cover of the scrubland (%). No significant differences were found between the two treatments. The residual standard errors of Eqs. (5) and (6) were 14.22 kg ha^{-1} year^{-1} and 12.48 kg ha^{-1} year^{-1}, respectively.

The production of *B. edulis* sporcarps is expected to start at about 5 years after treatment and the maximum production is reached at 14 years (Fig. 3a). *B. edulis* production starts when the mean height of the scrubland reaches one meter and reaches its maximum at a mean height of 1.5 m. The highest production was associated with a canopy cover of 80% (Fig. 3b), which is reached already at early ages (before 10 years) and maintained during the rest of the life cycle of *C. ladanifer*.

3.2.2. Saprotrophic taxa

The models for mushroom production of saprotrophic fungi were as follows:

$$SaproP = \exp(-4.90905 + 3.91042 \times \ln(Tmmin_{O+N}) - 0.08398 \times Y) \quad (7)$$

$$SaproP = \exp(-34.074016 + 12.503545 \times \ln(Tmmin_{S+O+N+D}) \\ - 0.008145 \times H) \quad (8)$$

where SaproP is the annual mushroom yield of saprotrophic species (kg ha^{-1} year^{-1}), $Tmmin_{O+N}$ is the sum of the mean minimum tem-

a) Gompertz growth function

b) Van der Vliet growth function
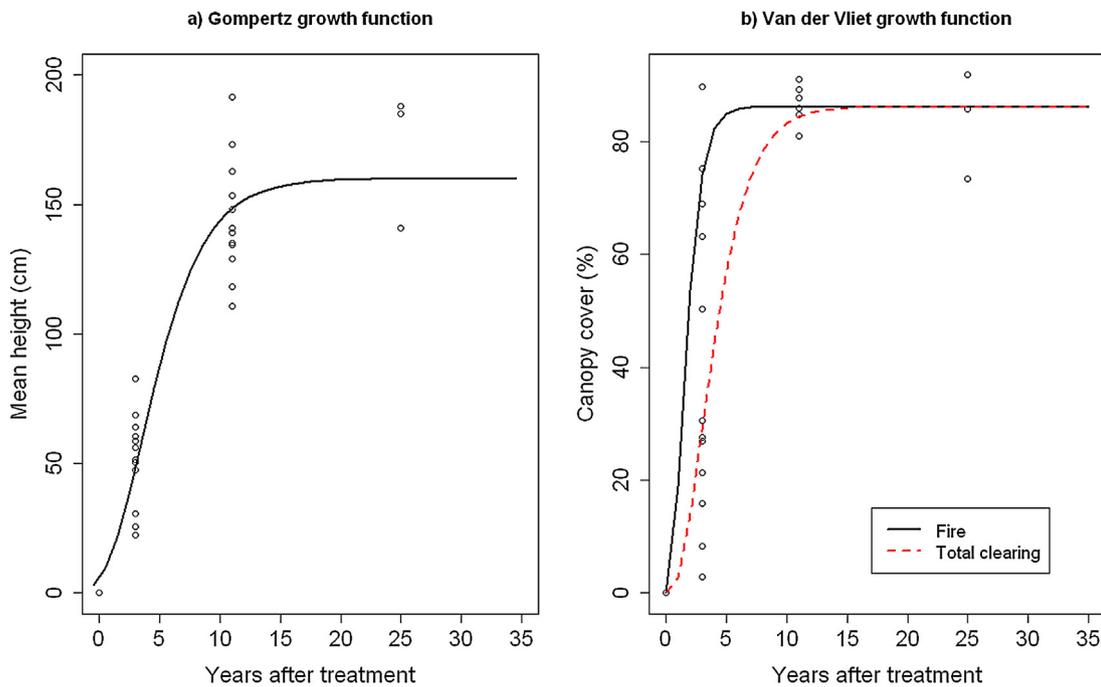
Fig. 1. Mean height (a) and canopy cover (b) development of *C. ladanifer* scrublands over time according to the models.

perature of October and November (°C), Y is time after treatment (year), $Tmmin_{S+O+N+D}$ is the sum of the mean minimum temperature of September, October, November and December (°C) and H is mean height of the scrubland (cm). The residual standard errors of Eqs. (7) and (8) were 57.33 kg ha^{-1} year^{-1} and 55.73 kg ha^{-1} year^{-1}, respectively.

Mushroom yield from saprotrophic fungi was found to decrease as *C. ladanifer* stands develop (Fig. 2b). Furthermore, there are important variations in the production according to weather conditions. Production is very small in years when the mean minimum temperatures of October and November are low (Fig. 2b). The mean height of the scrubland significantly influence the production of saprotrophic taxa and the tendency was similar to that found in the previous model (Fig. 2d).

If mushroom yield is modelled as a function of canopy cover, treatment is a significant predictor for mycorrhizal species and its effect is as shown in Fig. 2e. In the case of saprotrophic fungi, no significant differences were found between the two treatments (Fig. 2f). In these models, the production of mycorrhizal taxa was significantly higher after total clearing than after fire, while the production of saprotrophic taxa showed no significant differences between the treatments.

3.3. Models for mushroom diversity

3.3.1. Mycorrhizal taxa

The models for the Shannon diversity index for mushrooms from mycorrhizal species when using time after treatment or vegetation characteristics as predictors were as follows:

$$MycoD = \exp(-2.360987 + 0.048370 \times Tmin_{S+O} + 0.004765$$
$$\times P_S - 0.185691 \times Y + 1.383084 \times \sqrt{Y}) \quad (9)$$

$$MycoD = \exp(-6.937640 + 0.117106 \times Tmmin_{S+O} + 0.004609$$
$$\times P_{A+S} - 0.041030 \times H + 0.922719 \times \sqrt{H}) \quad (10)$$

where MycoD is the Shannon diversity index of mycorrhizal taxa, $Tmin_{S+O}$ is the sum of the minimum absolute temperature

of September and October (°C), P_s is the total precipitation of September (mm), Y is time after treatment (years), $Tmmin_{S+O}$ is the sum of the mean minimum temperature of September and October, P_{A+S} is the sum of the total precipitation of August and September (mm) and H is mean height of the scrubland (cm). The residual standard errors of Eqs. (9) and (10) were 0.5566 and 0.4641, respectively.

The models for the diversity of mycorrhizal taxa were the only models where precipitation was included as a significant predictor. The diversity of mycorrhizal fungi increased with increasing precipitation of August and September as well as with increasing mean minimum temperature of September and October. The diversity of mycorrhizal fungi increases with time after treatment in young scrublands, starting immediately after the treatment and reaching its maximum approximately 13 years after the treatment (corresponding to a mean height of 130 cm). The Shannon diversity index of mycorrhizal species starts to decrease in senescent stands, approximately 16 years after the treatment (Fig. 4a and c).

3.3.2. Saprotrophic taxa

The models for the Shannon diversity index of mushrooms from saprotrophic species were as follows:

$$SaproD = \exp(-0.15733 + 0.03557 \times Tmin_{O+N+D} - 0.06509 \times Y$$
$$+ 0.4442 \times \sqrt{Y}) \quad (11)$$

$$SaproD = \exp(-0.025681 + 0.041956 \times Tmin_{O+N} - 0.006450$$
$$\times CC + 0.199355 \times \ln(CC + 0.001)) \quad (12)$$

where SaproD is the Shannon diversity index of saprotrophic fungi, $Tmin_{O+N+D}$ is the sum of the minimum absolute temperature of October, November and December (°C), Y is time after treatment (years), $Tmin_{O+N}$ is the sum of the minimum absolute temperature of October and November (°C), and CC is canopy cover of the scrubland (%). The residual standard errors of Eqs. (11) and (12) were 0.4383 and 0.3988, respectively. The diversity of saprotrophic fungi was higher when the minimum absolute temperatures of October and November were higher.
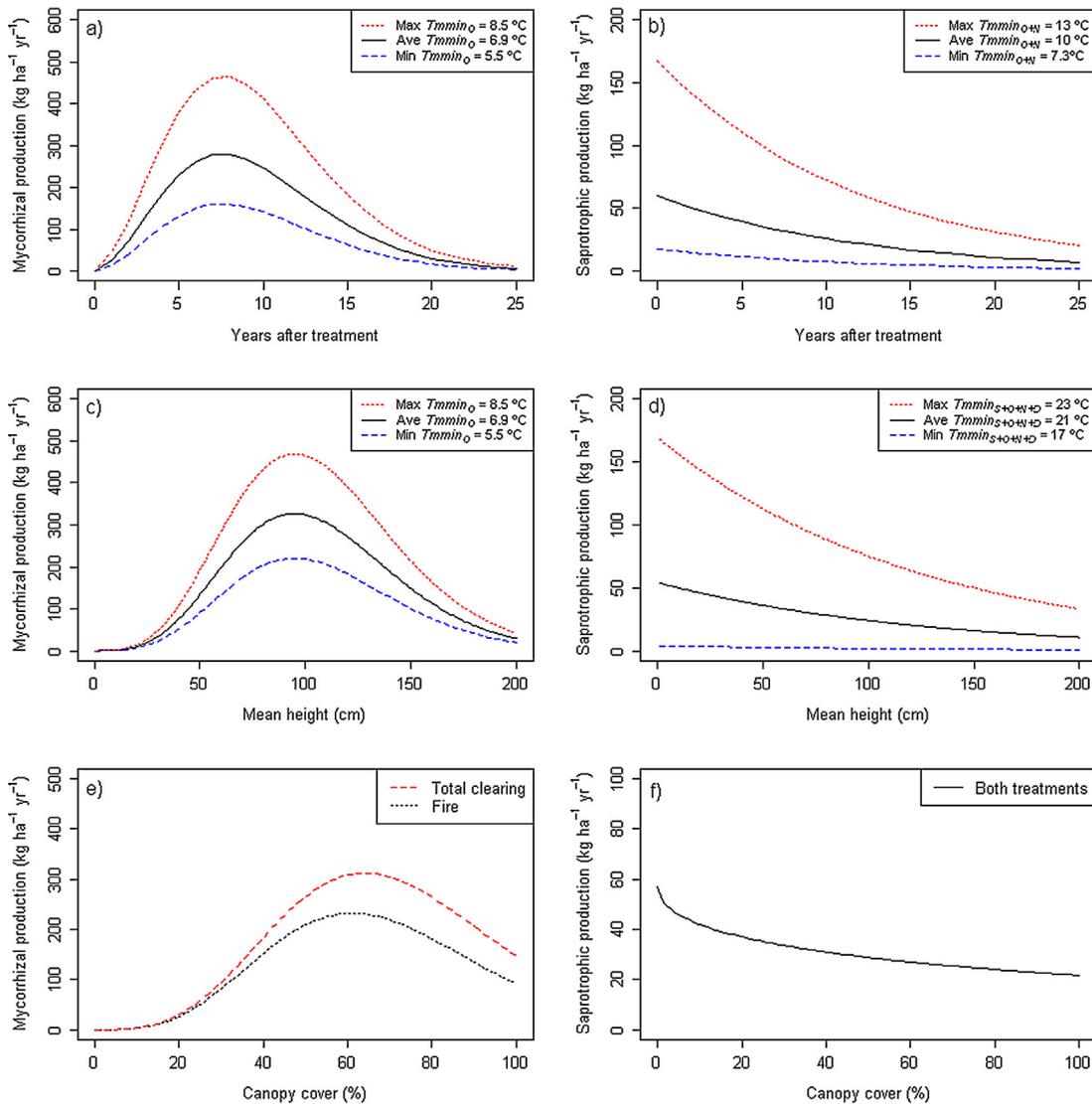
Fig. 2. Effect of climate, treatments, time after treatment and vegetation characteristics and type of treatment on production of mycorrhizal (left) and saprotrophic (right) taxa. $Tmmin_O$: mean minimum temperature of October (°C), $Tmmin_{O+N}$: sum of the mean minimum temperature of October and November (°C), $Tmmin_{S+O+N+D}$: sum of the mean minimum temperature of September, October, November and December (°C). Max: maximum value of a given predictor observed during the sampling period; Ave: average value of a given predictor observed during the sampling period; Min: minimum value of a given predictor observed during the sampling period. Increasing mean minimum temperatures are related to an increase in the production of both mycorrhizal and saprotrophic taxa.

The best predictive model resulted in non-significant differences between the two treatments considered. The model for the diversity of saprotrophic species was the only one that included canopy cover instead of mean height as a predictor accounting for the vegetation characteristics (Fig. 4d). If the effect of treatments is taken into account, the diversity of both mycorrhizal and saprotrophic taxa tends to be significantly higher in stands established after fire than in the cleared scrublands, as shown in Fig. 4 e and f.

4. Discussion

To our knowledge, this work presents the first empirical models for the fungal production and diversity of *C. ladanifer* scrublands. In addition, auxiliary models for predicting the development of *C. ladanifer* scrub stands were also developed (Fig. 1). The combination of these models can be a useful tool for managing these widespread Mediterranean ecosystems.

4.1. Vegetation structure models

Most previous research about *C. ladanifer* development focused on biomass estimation in order to assess carbon storage (Navarro Cerrillo and Blanco Oyonarte, 2006; Ruiz-Peinado et al., 2013). This study developed models for the mean height and canopy cover development of *C. ladanifer* scrublands after the two silvicultural treatments currently used in the management of this ecosystem. To our knowledge, the only model so far for *C. ladanifer* mean height and age was the one presented by Patón et al. (1993). Their observations on *C. ladanifer* height and age are quite similar to the values found in our modeling plots (i.e. similar asymptotic value reached at a similar age) (Fig. 1) despite being located in a different region (Extremadura, southwestern Spain).

Regarding canopy cover, we found significant differences in the development of *C. ladanifer* scrublands after fire and after total clearing (Fig. 1). This result agrees with Tárrega et al. (2001), who studied the recovery dynamics after controlled burning and cutting during 11 years. They found that *C. ladanifer* recovery is faster after burning than after cutting. This can be explained by the pyro-
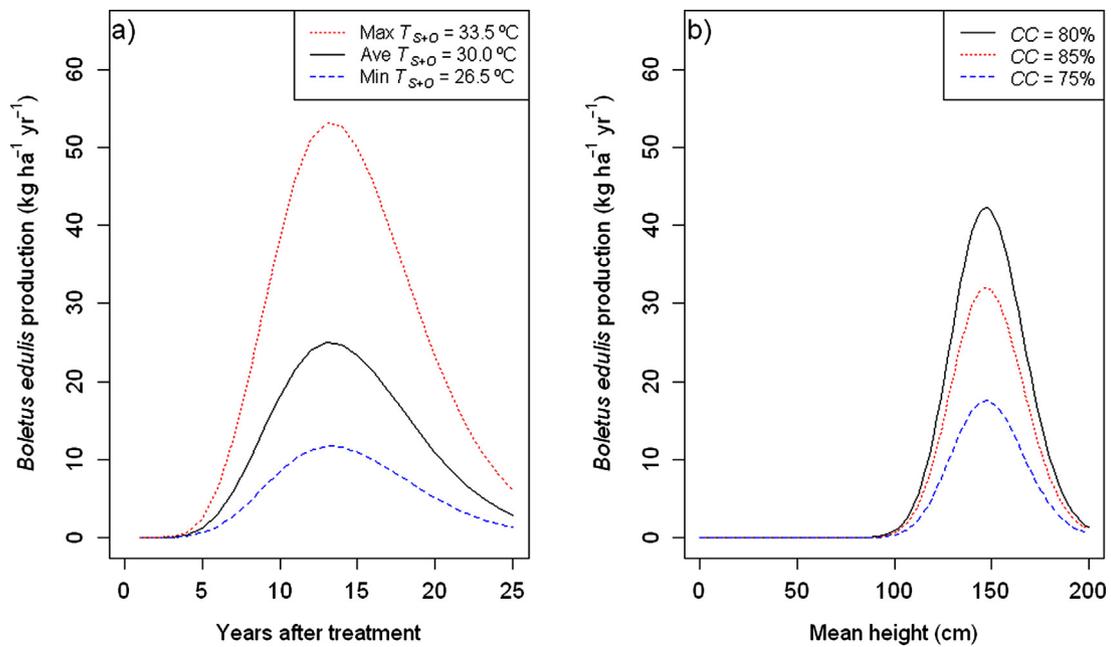
Fig. 3. Effect of climate, time after treatment and vegetation characteristics on *Boletus edulis* production. T_{S+O}: sum of the mean temperature of September and October; Max: maximum value of a given predictor observed during sampling period; Ave: average value of a given predictor observed during the sampling period; Min: minimum value of a given predictor observed during the sampling period; CC: canopy cover. Increasing minimum temperature of September and October produces a high increase in *B. edulis* production.

phytic ecology of *C. ladanifer* since seed germination is triggered-off by the fire-induced increase in the temperature of the top layers of the soil (Bastida and Talavera, 2002). High scrub cover (around 80%) is reached at an early age (6 years after the fire and 9 years after total clearing) and subsequently maintained during the rest of the years of *C. ladanifer* life cycle. This also agrees with Tárrega et al. (2001), who reported 7 or 8 years to reach cover values similar to those before disturbance.

4.2. Models for mushroom production

As expected, weather conditions, especially precipitation and temperature, were found to be important factors affecting mushroom fruiting. Weather may explain as much as 60–80% of the variation in mycorrhizal fungal sporocarp production (Dahlberg, 1991). Although precipitation was not a significant variable in most of the models presented in this study, we cannot affirm that precipitation has no influence on fructification. This is because precipitation was positively correlated with minimum temperature, which was a significant predictor (Spearman correlation test, *p*-value <0.05). Lower mean minimum temperatures are related to clear skies and absence of precipitation.

The most influencing climatic variable for *B. edulis* production was the mean minimum temperature of September and October (Fig. 3). Apparently, precipitation is not as limiting factor in the production of *B. edulis* in *C. ladanifer* scrublands as compared to other ecosystems. According to local expert knowledge, in years of low rainfall *Boletus* yields in these scrublands have been considerably higher than in nearby chestnut, oak and pine forests where the production was very low. This also agrees with the results obtained by Martínez-Peña et al. (2012) who reported that temperature was a more crucial factor than precipitation for the emergence of mushroom sporocarps in Spanish *P. sylvestris* stands affected by a continentalized Mediterranean climate.

The production of mycorrhizal species showed a rapid increase after total clearing or burning and reached its maximum 8 years after treatment, after which it began to decrease reaching values close to zero from 20 years after treatment onwards, moment in which the scrublands are senescent (Fig. 2). This trend may be explained by the higher photosynthetic activity of young scrublands. As host species grow fast and require more nutrients in their early development, mycorrhizal fungal species associated with their roots receive more carbohydrates, which may enhance mushroom fruiting (Ortega-Martínez et al., 2011).

The same increasing-decreasing trend was found for *B. edulis* production (Fig. 3). However, the maximum yield of this species was found in approximately 14 years-old scrublands, whereas it was found about 7 years after treatment in the case of all mycorrhizal species. *B. edulis* is usually associated with late-stage forest stands (Martín-Pinto et al., 2006). However, when associated with *C. ladanifer*, it can fruit very early (Hernández-Rodríguez et al., 2013). If 10 kg ha^{-1} is considered as a high yield in these areas, the scrublands may provide high yields of *B. edulis* from 8 to 20 years after treatment in average climatic conditions. Thus, management aimed at keeping most of *C. ladanifer* scrublands aged between 8 and 20 years may increase the benefit derived from *B. edulis* harvesting.

The expected production of saprotrophic taxa is lower than in the case of mycorrhizal fungi. Saprotrophic production decreased during the development cycle of *C. ladanifer* scrubs (Fig. 2). Treatments increase the amount of dead organic matter in the form of harvesting residues. As a consequence, saprotrophic species can fruit abundantly during the first years after treatment.

The mean height of *C. ladanifer* was the most significant vegetation variable in the models for mycorrhizal and saprotrophic mushroom yield. When this variable was used as a predictor, no significant differences in fungal yield or diversity were found between treatments. This may be explained by similar development of *C. ladanifer* mean height after the two studied treatments (Fig. 1), whereas the development of *C. ladanifer* canopy cover showed some differences between treatments at early ages. This is probably the reason why the effect of treatment became statistically significant when canopy cover was used as a predictor instead of the shrub mean height (Figs. 2 and 4).
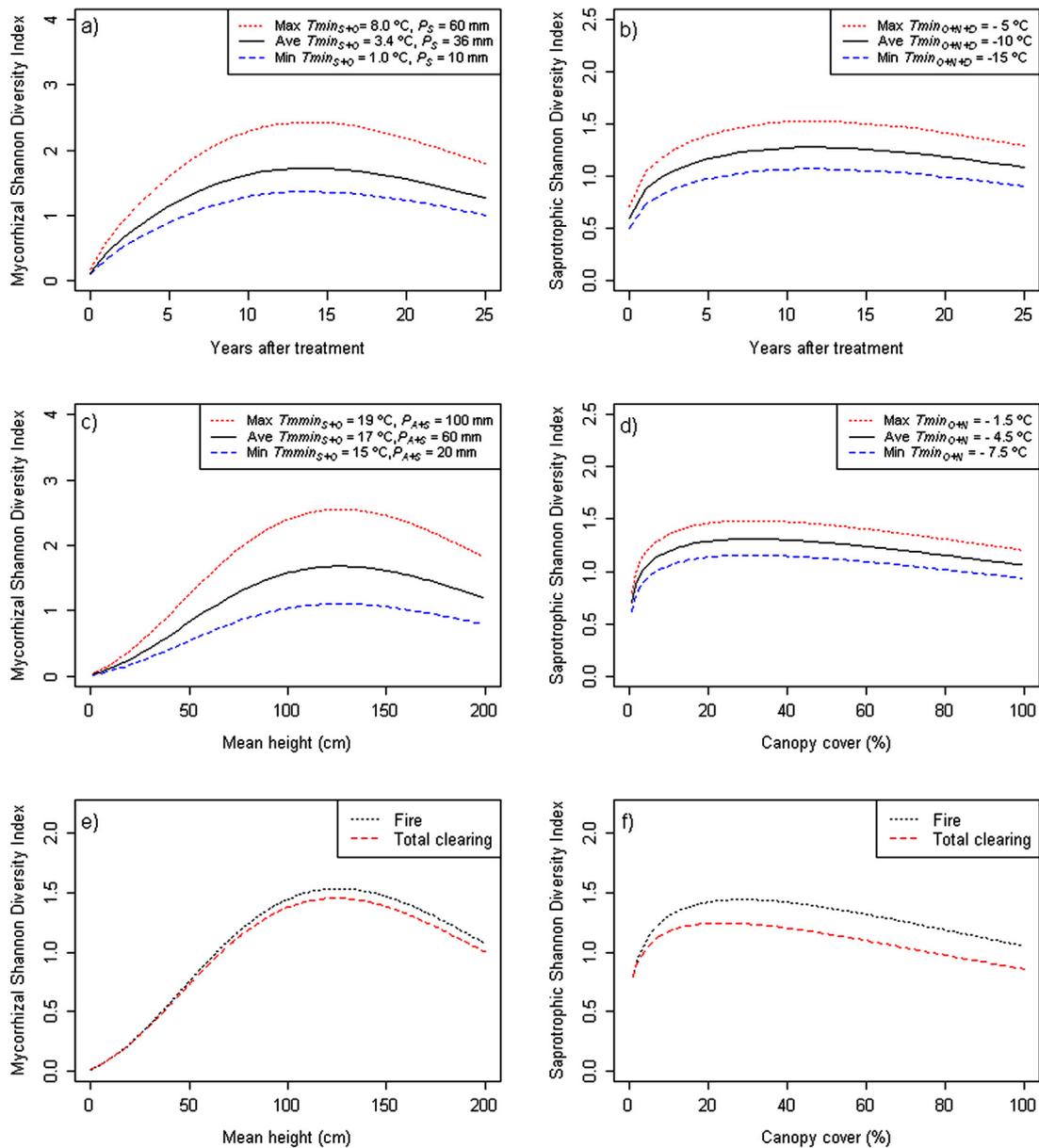
Fig. 4. Effect of climate, treatments, time after treatment and vegetation characteristics and type of treatment on mycorrhizal (left) and saprotrophic (right) taxa diversity. $Tmin_{S+O}$: sum of the minimum absolute temperature of September and October (°C), P_S: total precipitation of September (mm), $Tmmin_{S+O}$: sum of the mean minimum temperature of September and October, P_{A+S}: sum of the total precipitation of August and September (mm), $Tmin_{O+N+D}$: sum of the minimum absolute temperature of October, November and December (°C), $Tmin_{O+N}$: sum of the minimum absolute temperature of October and November (°C), Max: maximum value of a given predictor observed during the sampling period; Ave: average value of a given predictor observed during the sampling period; Min: minimum value of a given predictor observed during the sampling period. Increasing early autumn minimum temperatures and late summer precipitation increase diversity of mycorrhizal fungi. Increasing minimum temperatures of mid and late autumn increase diversity of saprotrophic fungi.

Regarding the model of *B. edulis* production in relation to vegetation characteristics, it is important to note that it responds to the natural evolution of the stand in the absence of any management other than the establishment of the vegetation after the treatments. Thus, mushroom production starts when the mean height attains 1 m, which corresponds to an age of approximately 6 years after treatment. The maximum *B. edulis* production was found when canopy cover was approximately 80%, which is naturally reached very early after treatment and then maintained throughout the life cycle. Furthermore, preliminary analyses of an ongoing study (Hernández-Rodríguez et al., 2015) suggest that the reduction of canopy cover in middle aged scrublands with alternative treatments such as 50% manual clearing may maintain or even enhance *B. edulis* production and facilitate its harvesting. Since this could further assist decision making concerning the management

of scrubland ecosystems, further research should aim at inspecting the impact of such alternative treatments (i.e. partial removal of vegetation) on mushroom yield and diversity. Since this study was carried out in rather homogeneous scrublands in a restricted geographical region, further research on fungal communities associated to *C. ladanifer* scrublands including sample plots with higher variability and from different locations would probably contribute to better understanding of the dynamics of fungal community.

4.3. Models for mushroom diversity

Compared to sporocarp yields, precipitation seems to play a stronger role in determining the diversity of mycorrhizal fungi since these are the only models where precipitation is explicitly included as a significant predictor. Increasing August and September total

precipitation and increasing September and October minimum temperatures were found to increase the diversity of mycorrhizal fungi. This agrees with previous studies that found a delay of one month between the occurrence of precipitation and the influence of temperature on mushroom proliferation, and contributes to the idea that mushroom fruiting needs first a minimum amount of rainfall and, afterwards, warm temperatures (Martínez de Aragón et al., 2007; Martínez-Peña et al., 2012).

Similarly to mushroom production from mycorrhizal fungi, mycorrhizal mushroom diversity showed a large increase during the first years of *C. ladanifer* succession, with a subsequent decrease as scrublands attain the senescence stage. However, maximum diversity occurred several years after the production peak. This may be due to the short life cycle of *Cistus* species, i.e. although the maximum photosynthetic activity is reached quite soon, which favors high mushroom yields, these young scrublands have not yet reached the late successional stage associated with higher diversity. These results differ from those obtained by Bonet et al. (2008) who found highest species diversity of fungal carpophores when the total yield was maximal in *P. sylvestris* forest in the Central Pyrenees.

The diversity of saprotrophic mushrooms was higher than for mycorrhizal taxa during the first years after the execution of the treatments. However, the influence of the stand age was lower than in the mycorrhizal species diversity, and diversity remained more constant throughout the life cycle of these ecosystems (Fig. 4b and d). This is the only model based on vegetation structure characteristics in which mean height was not significant, which suggests that saprotrophic diversity is mainly influenced by canopy cover. This may be explained by the correlation between canopy cover and the amount of organic matter present in the stand.

There is also a difference between mycorrhizal and saprotrophic taxa in the months whose conditions affect fungal diversity. Whereas the diversity of mycorrhizal species increases with increasing temperature of early autumn, the diversity of saprotrophic species is positively influenced by the temperature of the mid and late autumn (October–December) (Fig. 4). This may be due to the vertical pattern of fungal segregation, in which the mycelium of saprotrophic taxa is distributed mainly in the top layers of the soil (McGuire et al., 2013) and may therefore be more sensitive to the effect of frosts in late autumn and winter.

If the effect of the two treatments is considered, the results suggest an opposite trend in fungal diversity as compared to mushroom yield. The Shannon index for both mycorrhizal and saprotrophic species was higher after burning than after clearing, whereas mushroom production was higher after total clearing. These results can be of interest from the management point of view. If the management objective is economic (i.e. to increase edible mushroom yield), total clearings may be a better treatment alternative. On the contrary, if the aim is more ecologically oriented (i.e. high diversity), burning may be better than clearing. The models may also be helpful in prioritizing particular stands for edible mushroom management in a mixed objective landscape. Furthermore, the climate-sensitive models presented in this study may be further used in scenario analysis on the effect of climate change on the fungal productivity and diversity of Mediterranean scrublands.

Acknowledgements

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Original Article IV

Optimal management of *Cistus ladanifer* shrublands for biomass and *Boletus edulis* mushroom production

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Resumen

Manejo óptimo de matorrales de *Cistus ladanifer* para la producción de biomasa y setas de la especie *Boletus edulis*

El manejo de matorrales tradicionalmente no se ha tenido en cuenta en la planificación forestal. Sin embargo, algunos de estos sistemas forestales pueden proporcionar beneficios económicos gracias al aprovechamiento tanto de su biomasa como de la elevada producción de especies de hongos comestibles comercializables asociadas a ellos. Este es el caso de *Cistus ladanifer*, una especie ampliamente distribuida por la región Mediterránea que es capaz de producir grandes cantidades de *Boletus edulis*, especie muy apreciada. El principal objetivo de este estudio es estimar el ciclo óptimo que debería ser adoptado para el manejo de matorrales mediterráneos dominados por *C. ladanifer* considerando dos outputs: la biomasa de *C. ladanifer* y la producción de *B. edulis*, y eligiendo la alternativa que produzca mayores ganancias monetarias. Se han desarrollado dos escenarios diferentes: un escenario estático en el cual se ha calculado la rotación óptima para los jarales de *C. ladanifer* considerando cinco prescripciones que incluyen diferentes operaciones de manejo; y un análisis dinámico en el que se pueden practicar diferentes operaciones de manejo durante los siguientes 25 años. Ambos escenarios consideran tanto la biomasa como el aprovechamiento de setas como outputs. Los resultados de estos análisis muestran que la forma de manejo más apropiada es realizar un desbroce total próximo al final de ese intervalo de tiempo. Estos resultados pueden ser utilizados para justificar la buenas prácticas de gestión en estos sistemas forestales donde el aprovechamiento de hongos centrado en *B. edulis* puede proporcionar beneficios importantes.

Palabras clave: Jarales; *Boletus edulis*; Biomass; Rotación óptima; Programación dinámica.

Optimal management of *Cistus ladanifer* shrublands for biomass and *Boletus edulis* mushroom production.

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Abstract

Shrubland management has not traditionally been considered in forest planning. However, some of these forest systems can provide economic benefits due to both the use of biomass and the high production of marketable edible fungi associated with the shrub species. This is the case for *Cistus ladanifer*, a species widely distributed in the Mediterranean region, which produces high yields of the greatly appreciated mushroom *Boletus edulis*. The main objective of this study is to estimate the optimal cycle that should be adopted for the management of Mediterranean shrublands dominated by *C. ladanifer* in considering two outputs: *C. ladanifer* biomass and *B. edulis* production, and choosing the alternative with the highest associated monetary returns. Two different scenarios have been developed: a static scenario in which the optimal rotation of *C. ladanifer* shrubland considering five prescriptions including different management operations has been calculated; and a dynamic analysis, in which different management operations could be practiced over the next 25 years. Both scenarios consider biomass and mushroom picking as outputs. The results of these analyses show that the most appropriate management option is to perform a total clearing close to the end of that time interval. The results could be used to justify sound

management practices in these forest systems where fungal harvesting focused on *B. edulis* could provide significant incomes.

Key words: Rockroses; *Boletus edulis*; Biomass; Optimal rotation; Dynamic programming

1. Introduction

Cistus ladanifer L. is an obligate-seeder shrub species widely distributed in the Western Mediterranean Region, including the Iberian Peninsula, southern France and North of Morocco and Algeria (Guzmán and Vargas 2009). It is a pyrophytic species that constitutes early successional stages in Mediterranean ecosystems. It can colonize highly degraded areas after fire as high temperatures in the top layers of the soil trigger seed germination (Bastida and Talavera, 2002).

In Spain, *C. ladanifer* has a distribution area of about 3 million hectares. It is present in 15% of the forest area of the country (Martín Morgado et al. 2005). These figures include both monospecific shrublands and stands in which it appears as the understory of other tree species. The presence and maintenance of this species that tends to occupy large areas is due to land abandonment of them during the last few decades and recurrent fire events (Mendes et al. 2015).

Due to the pyrophytic ecology of this species, there is a very high risk of fire in senescent shrublands. In the northwest of the Iberian Peninsula it is very common to use fire to control shrub accumulation (Hernández-Rodríguez et al. 2015b). In addition, some clearings are performed in order to decrease this high fire risk.

C. ladanifer shrublands have traditionally produced very scant economic benefits, derived only from hunting and the use of firewood obtained from fire prevention clearing treatments. Therefore, these areas have not been paid any attention in forest management. However, previous studies have shown potential high profits from a large production of edible wild mushrooms in these ecosystems (Hernández-Rodríguez et al. 2015b). The most valuable fungal species associated with them is *Boletus edulis* Bull., which is a highly prized culinary mushroom in many countries (Boa

2004), and is perceived as a luxury good in the main Spanish markets (Voces et al. 2012). Also, *C. ladanifer* shrublands appear in forest areas characterized by very poor quality soils, in which traditional wood forest productivity is very low (Hernández-Rodríguez et al. 2015b). This could justify maintaining this vegetation in some areas; instead of promoting a change of species towards a tree stand. Thus, Oria-de-Rueda et al. (2008) suggested that promotion and expansion of scrublands for bolete picking could be a valuable stimulant to local rural economies. To properly maintain these areas the carrying out of some management techniques is necessary in order to increase economic benefits.

Few previous works have studied the use of different management methods in *C. ladanifer* shrublands. Most of them aimed at increasing plant diversity associated with these ecosystems (Tárrega et al. 1995; Mendes et al. 2015). In a previous study, we determined the impact of these treatments on diversity and production of fungal communities in these ecosystems (Hernández-Rodríguez et al. 2015b). From an economic point of view, studies on wild edible mushrooms are limited, in spite of favorable circumstances such as the high value of their commercialized products, the economic importance of their consumption, or their profitable potential in Mediterranean countries (Peintner et al. 2013; Zotti et al. 2013; Azul et al. 2014; Alfranca et al. 2015). However, constraints such as the absence of data of the mushrooms picked, or the lack of transparency in value chain traceability (Cai et al. 2011; Voces et al. 2012) could explain this scarcity of studies on some economic aspects of wild edible fungi.

Most of the studies mentioned above have one common characteristic, namely, none of them have determined an optimal management regime when mushroom picking is included in the analysis. However, this is a key question in evaluating their profitability when the two outputs are considered, or in quantifying the opportunity cost of not selecting the best silvicultural option. Previous studies in other forest ecosystems established optimal cycles in which the objective was to maximize the income from different forest outputs including timber (Diaz-Balteiro and Rodriguez 2006) or other non-timber forest products (Klauberg et al. 2014). According to some

authors, this strategy is the optimal policy for any type of natural resource (Romero 2012).

In the case of Spain, wild edible mushrooms are of a moderate importance. Thus, for some authors, their impact is mainly local and, in some cases, regional (Bonet et al. 2008), since a high proportion of the rural population is involved in their commercial harvesting and processing (de Román and Boa 2004). In fact, the most important use of harvested mushrooms is related to self-consumption (Voces et al. 2012).

To the best of our knowledge, no optimal management alternative under different silvicultural options has ever been addressed for these forest systems. Therefore, the main objective of this study was to estimate the optimal cycle that should be adopted for this shrubland in considering two outputs: *C. ladanifer* biomass and *B. edulis* production, and choosing the alternative with the highest associated monetary returns. The results could be used to justify sound management practices in these forest systems.

2. Material and methods

2.1. Study area

The study was carried out in a Mediterranean ecosystem dominated by *C. ladanifer* in North-western Spain Latitude: 41º41′43″N–41º42′50″N; Longitude: 06º13′50″W- 06º12′44″W. The area is located at 750-780 m above sea level and it is characterized by a sub-Mediterranean climate with a dry season of three months in the summer and a mean annual rainfall of 450-700 mm. Mean temperatures range from 14.5 to 15.8ºC. The soil is classified as Inceptisol suborder Xerept (Soil Survey Staff 2010) and is characterized by stoniness, acidity (pH=5.0-5.5), and a lack of any calcium or phosphorous. Nitrogen and potassium availability is variable and the level of humification is generally good (García Rodríguez et al. 1964).

Forest vegetation in the area is dominated by *Quercus pyrenaica* Willd. There is a scant supply of forest products in these areas, and they are mainly devoted to the harvesting of fuelwood, and also used at certain times of the year for livestock shelter.

There are also small areas of pine (*Pinus pinaster* Aiton and *Pinus sylvestris* L.) and chestnut trees (*Castanea sativa* Mill.), usually distributed in small scattered plantations. The understory is dominated by rockroses (*C. ladanifer*), present as a pioneer species in areas whose tilling has been abandoned in recent years and which form large monospecific shrublands, often characterized by poor soil quality. In the traditional management of these areas and in order to decrease their high fire hazard, only some clearings are performed, which can generate a small income from the use of firewood. In this study, this traditional management constitutes our Business As Usual (BAU) scenario.

2.2. Study framework

We established a planning horizon of 25 years. In this number of years the outcomes associated with this study will be evaluated. It corresponds to the end of the short life cycle of *C. ladanifer* (Oria-de-Rueda et al. 2008), and *B. edulis* production is very sparse as from 20 years after treatment (Hernández-Rodríguez et al. 2015a). Therefore, for *C. ladanifer* shrubland management aimed at promoting *B. edulis* production, this was believed by us to be a suitable planning horizon, across which different silvicultural treatments may occur. The latter will be described in detail in the next Section.

The minimum period between two consecutive silvicultural treatments was set at seven years, based on the indications of Oria-de-Rueda et al. (2011). According to the mushroom production models used to conduct this study, a high yield of *B. edulis* begins approximately at this age (Hernández-Rodríguez et al. 2015a), which also coincides with the time when the shrubland reaches a mature stage Oria-de-Rueda et al. (2008). Therefore, it did not appear to make any sense to perform silvicultural treatments with intervals of less than seven years.

With the available data for this study, two different scenarios have been developed across the planning horizon. Firstly, a static scenario is shown in which the optimal rotation of *Cistus* shrubland was calculated by considering 5 prescriptions that included different management operations and the two outputs (biomass and

mushroom picking). The second scenario embraced a dynamic analysis, in which different management operations could be practiced across the planning horizon. Under some hypotheses, we defined different prescriptions, and we sought the most profitable one. Both scenarios were compared with the optimization of the business as usual (BAU) scenario defined above: to implement a clearing in order to reduce the fuel load across the planning horizon, when it is more profitable, but not considering mushroom production.

We have made several assumptions in this study. In short, no cost derived from mushroom collection was assumed. This is usual when mushroom picking objectives are incorporated into the analysis because of a lack of available information (Aldea et al. 2014). The only fungal species included in this study is *B. edulis*, because it is the most economically important mushroom species in the study area as it is a normal practice to focus a study only on the most valued species (Martínez-Peña et al. 2012). Also, the calculations made are based on the total production of *B. edulis* in these forest systems. Finally, we have considered a deterministic environment for this study.

2.3. Baseline data

Total clearing, partial 50% clearing and controlled burning were the management treatments considered in this work. In a first static scenario (optimal rotation analysis), the possibility of a partial clearing eliminating 50% of the canopy cover manually in year 12 of the planning horizon was considered. Due to the high costs of this method and the results of this first analysis, it was decided not to include this treatment in a second scenario built under a dynamic programming framework. The costs of implementing partial clearing per hectare were calculated based on current rates of Tragsa (Grupo Tragsa 2015). These rates are specifically designed to carry out works in the natural environment and are those used in practice by the Regional Forestry Administration of the area. They depend on the canopy cover of the shrubland ranging between 258.96€/ha and 961.83€/ha. The cost of the controlled burning was provided by the Regional Forestry Administration (166.77€/ha).

When studying clearing treatments, one potential receipt is the use of *C. ladanifer* biomass. Several previous studies have shown that pellets with acceptable technical features can be produced from this species (Mustelier et al. 2012). The European Project Enerbioscrub[1] is studying the sustainable management of shrub formations for energy purposes. According to the preliminary results of this project, the cost of transporting *C. ladanifer* packed (300kg bales) with 15% of humidity to a distance of up to 50km is about 60€/t and the receipt derived from this harvesting is about 10% (66€/t) (Rodero, P. personal communication).

The biomass was estimated from the model proposed by Ruiz-Peinado et al. (2013), which calculates *C. ladanifer* biomass from values of mean height and canopy cover of the shrubland, whose evolution, after the implementation of the different management treatments used to calculate the biomass, was estimated from the models presented in Hernández-Rodríguez et al. (2015). Table 1 presents the evolution of the biomass after the different management alternatives and the income and costs associated with it. Controlled burning as an alternative is not included in the table since the biomass is unusable with this treatment.

B. edulis production after total clearing or controlled burning was estimated using the empirical model developed in a previous study in the same study area (see Hernández-Rodríguez et al., 2015). The mean temperatures of September and October used for this work correspond to those found in the study area (T_{S+O} =30ºC). *B. edulis* production after a partial clearing eliminating 50% of the canopy cover was estimated from data collected for a previous study (Hernández-Rodríguez et al. 2015b) (Table 2). The price of *B.edulis* fluctuates greatly from year to year and within the same year (Aldea et al. 2012). Although the market price may exceed 30€/kg (de Román and Boa 2004), the amount paid to collectors by industries is much lower, around 5-6€/kg on average (Oria-de-Rueda et al. 2011). In this case, we used a mean value of 5€/kg for the analysis and evaluated the fluctuations in the price (from 3 to 7€/kg) in a sensitivity analysis included in a next section.

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[1]http://enerbioscrub.ciemat.es/

Table 1. Evolution of *C. ladanifer* biomass after the different management alternatives, including costs and incomes derived by biomass use each year of the planning horizon.

	C. ladanifer biomass					
	Total clearing			**Partial 50% clearing at year 12 + Total clearing**		
Year	**t/ha**	**Income (€/ha)**	**Cost (€/ha)**	**t/ha**	**Income (€/ha)**	**Cost (€/ha)**
0	0.00	0.00	0.00	0.00		
1	0.28	18.56	16.88	0.28		
2	1.26	83.11	75.55	1.26		
3	2.86	188.97	171.79	2.86		
4	4.77	314.74	286.13	4.77		
5	6.64	438.24	398.40	6.64		
6	8.26	545.44	495.85	8.26		
7	9.56	631.13	573.76	9.56		
8	10.54	695.92	632.65	10.54		
9	11.26	743.11	675.55	11.26		
10	11.77	776.67	706.06	11.77		
11	12.12	800.18	727.43	12.12		
12	12.37	816.51	742.28	12.37	81.88	702.95
13	12.54	827.82	752.57	8.28	546.30	496.64
14	12.66	835.65	759.68	9.04	596.81	542.56
15	12.74	841.07	764.61	10.00	660.01	600.01
16	12.80	844.83	768.02	10.87	717.65	652.40
17	12.84	847.44	770.40	11.56	762.70	693.36
18	12.87	849.27	772.06	12.04	794.95	722.68
19	12.89	850.55	773.23	12.38	816.76	742.51
20	12.90	851.44	774.04	12.59	830.92	755.38
21	12.91	852.07	774.61	12.73	839.86	763.51
22	12.92	852.52	775.02	12.81	845.36	768.51
23	12.92	852.83	775.30	12.86	848.70	771.55
24	12.93	853.05	775.50	12.89	850.70	773.36
25	12.93	853.21	775.64	12.91	851.88	774.43

Table 2. Annual production and income of *B. edulis* after the different management alternatives.

	Boletus edulis			
	Total clearing/ Controlled burning		Partial 50% clearing at year 12 + Total clearing/ Controlled burning	
Year	kg/ha	€/ha	kg/ha	€/ha
0	0	0.00	0	0
1	0.00	0.00	0.00	0.00
2	0.00	0.01	0.00	0.01
3	0.05	0.25	0.05	0.25
4	0.32	1.58	0.32	1.58
5	1.14	5.72	1.14	5.72
6	2.91	14.54	2.91	14.54
7	5.79	28.93	5.79	28.93
8	9.62	48.08	9.62	48.08
9	13.93	69.67	13.93	69.67
10	18.12	90.61	18.12	90.61
11	21.59	107.96	21.59	107.96
12	23.93	119.67	9.62	48.08
13	24.97	124.84	13.93	69.67
14	24.74	123.68	18.12	90.61
15	23.44	117.19	21.59	107.96
16	21.36	106.81	23.93	119.67
17	18.82	94.09	24.97	124.84
18	16.08	80.42	24.74	123.68
19	13.38	66.91	23.44	117.19
20	10.87	54.34	21.36	106.81
21	8.64	43.18	18.82	94.09
22	6.73	33.64	16.08	80.42
23	5.15	25.74	13.38	66.91
24	3.88	19.38	10.87	54.34
25	0	0.00	8.64	43.18

The data obtained from this model correspond to total yield and not to the amount collected. Although the harvesting pressure is high in the study area, the amount of collected fruiting bodies is always lesser than their potential yield. There are no studies on the collection rate in these *C. ladanifer* shrublands. However, Ortega-Martínez and Martínez-Peña (2008) found that the percentage of mushrooms collected

in a pine stand also in Spain was 24% on average, varying widely throughout the harvest season and reaching 58%. In order to assess both a decline in production due to natural year-to-year variation and a possible decrease in the harvesting pressure, a drop in *B. edulis* yield to 50% and 25% of production has been added to the sensitivity analysis included in the Results Section.

2.4. Optimal rotation analysis

We calculated the optimal rotation of *Cistus* shrubland by considering 5 prescriptions that include different management operations (controlled burning, total clearing, total clearing + partial clearing, controlled burning + partial clearing and the prescription where these silvicultural practices are not practiced) and the two outputs (biomass and mushroom picking).

In order to compute the optimal production of the two outputs in monetary terms, we calculated the net present value (NPV) associated with biomass and mushrooms, considering the hypothesis of Faustmann in the analysis; in this way, the land expectation value (LEV) was obtained. This approach is widely used in forestry studies, mainly when timber is considered in the analysis (Diaz-Balteiro et al. 2014b), and it can be considered as a net present value maximization logic. However, sometimes variations are introduced in cases of assessment of other non-timber forest products (Klimas et al. 2012; Klauberg et al. 2014). The mathematical expression of the LEV, following (Diaz-Balteiro et al. 2014b), is summarized in the following equation:

$$LEV = MaxNPV(t) = \frac{(P_B - C_B) \cdot QB \cdot e^{-it} + (P_M - C_M) \cdot QM \cdot e^{-it}}{1 - e^{-it}} \tag{1}$$

Expression (1) shows the LEV, and represents the optimal rotation to the one which maximizes its profitability for an infinite number of rotations, where P_B is the biomass price, C_B are the biomass costs, QB is the amount of biomass (t), P_M is the mushroom price, C_M are the mushrooms costs (€), QM is the amount of mushrooms (kg) and i the discount rate. A real discount rate of 4% has been used. This rate is common in studies with fast growing species in Spain (Diaz-Balteiro et al. 2009).

2.5. Dynamic scenario

The above solution makes sense in an ex-ante study, or when it is aimed to compare the profitability of possible land uses in these areas. However, it has been considered as being suitable for including a scenario limited to a time horizon appropriate for these forest systems. Thus, in our case study it was intended to maximize the income obtained in a planning horizon of 25 years, exploring which combinations among the silvicultural practices previously expounded might provide the optimal solution throughout this period.

The basic idea behind this optimization is that each year a choice emerges, independently of the stand age: either to implement a silvicultural practice or to wait another year. And if the decision is to execute a silvicultural treatment, a choice between clearing and controlled burning must be made. Using dynamic programming terminology, stand characteristics (age, previous interventions, etc.) at a certain age constitutes a state. The maximum number of interventions considered to be opportune for this case study fixes the number of stages of the problem. The decision implies moving from one state and stage to another, different state, in the following stage.

According to Hillier and Libiermann (1991), dynamic programming problems can be solved by beginning with the penultimate stage and looking for the best decision, i.e. one that leads to the best value of the objective function in the last stage. Next, and through an iterative procedure, the process could be repeated from the last stage, using a backward recursive method, until arriving at the initial state, obtaining in this way the best solution. Thus, for stage z, the optimal net present value (NPV^*) will be:

$$NPV^* = \max \sum_{\forall z} NPV_z \qquad (2)$$

where NPV_z' is the net present value, corresponding to each stage z, considering returns and costs from biomass and mushrooms. When equation (2) is properly applied backwards all the way to the initial stage, the optimal net present value is obtained. This kind of analysis has been applied frequently in different forest management problems such as adjacency constraints or eucalyptus plantations

management (Borges and Hoganson 1999; Diaz-Balteiro and Rodriguez 2006; Ferreira et al. 2012).

As mentioned above, in our case study it was considered that at least seven years should go by before an intervention took place, but this does not prevent at least one intervention on the planning horizon being made. With regard to the stages, it was decided only to contemplate 3 alternatives: no action, clearing, or controlled burning. On observing the results of the previous analysis, it was opted not to include partial clearing since it was demonstrated that, with the data available, this would never offer the best solution. In all, 630 possible states for this case study have been defined. In Figure 1 a scheme of these states is shown.

Year																										States							
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	....	625	626	627	628	629	630
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24										2	3								2	3						2	3					
25	1											2	3								2	3									2	3

Fig. 1: Scheme of all the possible states considered in the dynamic scenario. 1: No silvicultural practice (only mushroom incomes); 2: Total Clearing; 3: Controlled burning.

3. Results

3.1. Optimal rotation

Table 3 shows the results of the optimal rotation analysis. In relation to the BAU, the clearing treatment should be done every six years and the income reaches 182.8€. This alternative does not carry any possible income associated with mushroom picking. If this harvest is included, it can be seen how, as from the third column in Table 3, the NPV rises notably and the rotation is situated between 19 years (when the clearing is done) and 21 years (when controlled burning is carried out). When there is a combination between a partial and a total clearing or a partial clearing and burning, the rotation extends to 25 years.

Table 3. Results of optimal rotation analysis for the five prescriptions considered.

Year	BAU	Total Clearing	Controlled burning	Total clearing + Partial clearing	Controlled burning + Partial clearing
Year	6	19	21	25	25
LEV (€/ha)	182.80	1403.56	1200.26	801.09	659.01

A sensitivity analysis associated with the principal parameters in equations (1) and (2) was performed. Beginning with the static study, it was noted that the variations in the optimal rotation were highly reduced and never exceeded two years in relation to the initial case. It can also be observed how any alternative was much more profitable than the BAU. That is to say, the incorporation of the mushroom production gave much higher results than those usually achieved in this type of stands (Table 4).

Table 4. Sensitivity analysis of the optimal rotation analysis for variations in discount rate, price of *Boletus*, price of biomass and cost of controlled burning.

Sensitivity analysis	BAU	Total clearing	Controlled burning	Total clearing+ Partial clearing	Controlled burning+ Partial clearing
initial case	6 years/ 182,8€/ha	19 years/ 1430.6€/ha	21 years/ 1200.2€/ha	25 years/ 801.1€/ha	25 years/ 659.0€/ha
i=2%	6 years/ 388.9€/ha	19 years/ 3083.7€/ha	20 years/ 2568.0€/ha	25 years/ 1862.4€/ha	25 years/ 1485.9€/ha
i=6%	6 years/ 114.4€/ha	19 years/ 846.1€/ha	21 years/ 742.8€/ha	25 years/ 455.7€/ha	25 years/ 385.6€/ha
Boletus price=3€/kg	6 years/ 182,8€/ha	19 years/ 869.3€/ha	21 years/ 669.5€/ha	25 years/ 255.5€/ha	25 years/ 113.4€/ha
Boletus price=7€/kg	6 years/ 182,8€/ha	19 years/ 1937.8€/ha	20 years/ 1733.6€/ha	25 years/ 1346.7€/ha	25 years/ 1204.6€/ha
Biomass price=82€/t	6 years/ 670.3€/ha	18 years/ 1592.8€/ha	21 years/ 1200.2€/ha	24 years/ 924.6€/ha	25 years/ 658.9€/ha
Biomass price=50€/t	6 years/*€/ha	20 years/ 1230.2€/ha	21 years/ 1200.2€/ha	25 years/ 680.9€/ha	25 years/ 658.9€/ha
Controlled burning cost=0€/ha	6 years/ 182,8€/ha	19 years/ 1430.6€/ha	19 years/ 1335.6€/ha	25 years/801.1€/ha	25 years/756.0€/ha

*: NPV less than zero

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3.2. Dynamic scenario

Table 5 shows the results of the dynamic analysis, computing the maximum LEV for the different states, whose first intervention was made in the year shown in the first column of the table. Thus, for example, the LEV of 625€/ha means that in all the states whose first intervention is a total clearing at 7 years (170 different states), the maximum LEV reaches that figure. Following this reasoning, it can be noted how the maximum LEV (815.4€/ha) was produced when the first (and only) intervention occurred at 24 years and was a total clearing (state 627 in Figure 1). It should also be pointed out that this optimal solution was clearly superior to that of the BAU (see Table 3) and also to the alternative of not performing any silvicultural intervention to reduce the biomass (785.7€/ha)

Table 5. Results of dynamic programming. Maximum LEV for different states whose first treatment is performed in the year shown in the first column.

Year	LEV*	Year	LEV*
1		14	687.1
2		15	705.4
3		16	723.1
4		17	740.6
5		18	757.7
6		19	755.4
7	625.0	20	773.0
8	613.9	21	788.2
9	625.0	22	800.3
10	619.0	23	809.2
11	632.3	24	815.4
12	649.5	25	814.2
13	668.3	no intervention	785.7

Regarding the sensitivity analysis of the dynamic scenario, it was seen that the results behaved in a highly inelastic manner towards changes in discount rates, in the price of the mushrooms and towards an eventual drop in fungi production. Table 6 shows the results obtained in this sensitivity analysis, comparing them to the no treatment alternative.

Table 6. Sensitivity analysis of the dynamic programming for variations in discount rate, price of *Boletus* and biomass, cost of controlled burning and variations in *Boletus* yield.

Sensitivity analysis	Management treatment(s)	Optimal solution	NPV	NPV No treatment
initial case	clearing	24 years	815.4	785.7
i=2%	clearing	24 years	1084.7	1036.7
i=6%	clearing	24 years	617.8	599.4
Boletus price=3€/kg	clearing	24 years	501.1	471.4
Boletus price=7€/kg	clearing	24 years	1129.7	1100.0
Biomass price=82€/t	2 clearings	18 and 25 years	914.2	785.7
Biomass price=50€/t	clearing	25 years	738.1	785.7
Controlled burning cost=0€/ha	clearing	24 years	815.4	785.7
Controlled burning cost=0€/ha and Biomass price=50€/t	controlled burning	25 years	785.7	785.7
Boletus yield drops by 50%	clearing	24 years	422.5	392.9
Boletus yield drops by 25%	clearing	24 years	619.0	589.3

4. Discussion

Due to the traditional belief that monospecific shrublands of *C. ladanifer* do not provide any economic benefit, the few studies that exist on finding the best management practices of these ecosystems are aimed at changing the plant species or favoring the establishment of tree stands (Pérez-Devesa et al. 2008; Mendes et al. 2015). However, this work demonstrates that proper management of these shrublands can provide economic benefits derived from mushroom production and biomass harvesting. Another advantage of these ecosystems is that these shrublands produce boletes at a much earlier age than the trees (Oria-de-Rueda et al. 2008), so income from them would be received earlier than in a forested area. This issue could be of great interest in the case of Spain, where the economic importance of mushroom picking is mainly local (Bonet et al. 2008), involving a high proportion of the rural population in the commercial harvesting and processing of wild mushrooms (de Román and Boa 2004).

When including *B. edulis* harvesting, the profit can be even seven times higher than in a BAU alternative, when mushroom revenues are not considered, despite having a longer optimal rotation (Table 3). This is in accordance with previous studies on tree stands which have shown that including mushroom harvesting in the

management can increase the profits, providing an even higher value than timber (Palahí et al. 2009). In addition, integration of fungal resources into forest management plans would ensure their conservation over time and stop depletion (Aldea et al. 2012).

The results of the two analyses performed in this study were very consistent and showed that the most appropriate management option was to perform a total clearing close to the end of the planning horizon. The difference between them (i.e. a greater LEV and shorter rotation in static analysis) was that the static analysis considered an infinite number of rotations while the dynamic study only contemplated the 25-year planning horizon.

The optimal time for implementing the total clearing at 19-24 years is determined mainly by the production curve of *B. edulis,* which reaches a maximum at 13 years, decreasing towards the end of the planning horizon (Table 2) (Hernández-Rodríguez et al. 2015a). On the other hand, *C. ladanifer* development is fast at early ages and biomass remains practically unchanged from 10-11 years old until the end of the planning horizon, so the associated benefits remain constant after this age (Table 1).

All cases considered in the sensitivity analysis of the optimal rotation showed that the silvicultural treatment that produced the highest LEV was total clearing. This may indicate that the choice of the economically optimal treatment is mainly determined by the use of biomass. *B. edulis* production over time does not present significant differences depending on whether the treatment is total clearing or controlled burning (Hernández-Rodríguez et al. 2015a). Therefore, what determines which treatment is economically more favorable is the use of biomass made after total clearing even when considering that the cost of controlled burning = 0, a greater economic benefit is obtained by performing a total clearing (Table 4). Furthermore, this result is not altered by any fluctuation in the price of the *B. edulis,* which usually undergoes a great variations (Aldea et al. 2012), again reflecting that the choice of treatment depends more on the use of biomass than on the harvesting of mushrooms.

Previous studies showed that partial clearing was the treatment with the least impact on the fungal community (production and diversity), and it was associated with a significant production of edible fungi (Hernández-Rodríguez et al. 2015b).

Besides, with this silvicultural treatment, there is an increase in total *B. edulis* production across the planning horizon. Oria-de-Rueda et al. (2011) recommended performing partial clearings every seven years, and using mechanized total clearings only to rejuvenate senescent shrublands. However, when studying the profitability of the management alternatives that include partial clearing, taking into account the production of mushroom and biomass, the benefits are much lower in a longer optimal rotation than the alternatives including only total clearings or burning (Table 3).

Although in partial clearing part of the biomass can be used, this treatment is not cost-effective compared to total clearing or controlled burning, due to the high costs of its implementation. Traditionally, these partial clearings have been carried out manually, leading to very high costs (ranging between 461.45 and 1654.88 € ha^{-1},Grupo Tragsa 2015). However, for this study, we considered a mechanized partial clearing, which, despite having reduced costs (of between 258.96 and 961.93 € ha^{-1}), is not yet economically profitable.

According to the dynamic scenario, the optimal option is doing just one total clearing at 24 years (Table 5). This solution is very consistent with changes in the initial parameters, except when the price of biomass increases. If this price reaches 82€, it would be an excellent option to make 2 total clearings: at 18 and 25 years. Burning is only an appropriate solution if clearing is not profitable, but the result obtained is the same as if nothing were done (Table 6). This is due to the function of mushroom production, which tends to 0 in the last years of the considered planning horizon. Even though the production of *B. edulis* or the harvesting pressure decreased to 25%, the most economically profitable solution would still remain the same, providing higher profits than in the case of not implementing any treatment.

Shrubland management has not traditionally been considered in forest planning (San Miguel et al. 2008). Today, however, there is a better understanding among land managers and advisers of the value of scrub communities as a habitat for wildlife and of its importance in ecosystems (Day et al. 2003). Management techniques for each type of shrub depend on the species characteristics, their ecology and the objectives of the management (San Miguel et al. 2008). These techniques frequently involve rotational management and the period of rotation will depend on the lifecycle of the species

involved. Mixed scrub stands begin to develop towards woodland after about 15 years, so a rotation of around that duration will ensure that all stages of shrub are present within a stand or site (Day et al. 2003). The results of this work show that the optimal age to perform the total clearing is in the final stage of *C. ladanifer* life cycle (19-24 years). Therefore, this management would ensure that all stages of the shrub are present in the area. Thus, the optimal solution from the economic point of view would also be suitable from an ecological point of view.

In the study area, there is a real interest on the part of its managers in the integration of mycological resources into forest planning. Thanks to the results of this study, forest managers will include scrublands in mycological planning. They are beginning to implement regulation in the areas with the highest mushroom production, in which collectors pay to acquire a picking permit. This will provide new economic benefits in the future. Therefore, the results obtained in this study can be applied to the current management of this type of forest systems.

This management could also include other criteria (carbon, biodiversity) to integrate them into complex models, which could contain, for example, judicious combinations of multicriteria techniques and of group decision-making with the aim of obtaining the best solution considering diverse opinions and the preferences of a set of stakeholders (Aldea et al. 2014).

One of the initial hypotheses in this work was that a deterministic environment was considered. The absence of any reliable data throughout the time of some of the key variables (mushroom prices, biomass price, yield harvested annually, etc.) meant that a non deterministic environment was not contemplated, following other works associated with non-timber forest products (Aldea et al. 2014; Klauberg et al. 2014). However, possible additional incomes of these ecosystems can be considered in further studies. The use of essential oils from *C. ladanifer* is important especially in the southwestern Iberian Peninsula (Martín Morgado et al. 2005). In addition, hunting, grazing or carbon sequestration can also be considered in future research on the economic benefits from *C. ladanifer* shrublands.

Finally, as wildfires could be an important problem in these shrublands, the risk of fire could be introduced into the analysis. This circumstance could change the prescriptions proposed initially and the results obtained, because, under this new scenario, it may be necessary to modify the frequency of total clearings. The inclusion of this new objective appears as an attractive tool for a further research line applying multi-criteria techniques already used in forest fire problems (Diaz-Balteiro et al. 2014a).

5. Conclusions

In this paper we have determined the optimal guidelines for the management of Mediterranean shrublands dominated by *C. ladanifer*. It has been shown that with proper management, which consists of total clearings, owners could obtain an economic benefit from biomass and mushroom harvesting. According to the results of this work, the management practice that provides the greatest economic benefit is performing only one total clearing in the planning horizon. When considering only the 25 years of the planning horizon, the ideal age for performing this treatment is 24 years. In the case of considering clearing the shrubland indefinitely at the same age, the most appropriate time is 19 years.

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Original Article V

Soil fungal community composition in a Mediterranean shrubland is primarily shaped by history of major disturbance, less so by current fire fuel reduction treatments

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(Manuscript in preparation)

Resumen

Las comunidades fúngicas del suelo en matorrales mediterráneos están principalmente determinadas por la historia de perturbaciones mayores y no tanto por los tratamientos actuales de reducción de combustible

Los matorrales de *Cistus ladanifer* se encuentran ampliamente distribuidos alrededor de la cuenca del Mediterráneo. Constituyen las primeras etapas de la sucesión y normalmente aparecen en suelos pobres y degradados. Estudios previos basados en la producción de carpóforos han demostrado la elevada diversidad de las comunidades fúngicas asociadas a esta especie. Debido a la ecología pirófita de esta especie, es necesario llevar a cabo tratamientos de reducción de combustible para prevenir incendios forestales. El objetivo de este trabajo es estudiar el efecto de estos tratamientos sobre las comunidades fúngicas asociadas a la rizosfera de matorrales de *C. ladanifer*. Para ello se llevó a cabo la secuenciación de la región ITS1 mediante Illumina MiSeq de muestras de suelo de 27 parcelas donde se habían realizado diferentes tratamientos de reducción de combustible. Las comunidades fúngicas asociadas a estos ecosistemas fueron muy diversas y detectamos 1929 unidades taxonómicas operacionales (OTU) con una similitud mayor al 97% con una secuencia fúngica, tras analizar 1 864 114 secuencias filtradas. Las secuencias presentadas en este estudio indican que la composición de la comunidad fúngica está principalmente determinada por el origen de los jarales mientras que los tratamientos de reducción de combustible dentro de un mismo origen no tienen un impacto significativo sobre ellas.

Palabras clave: Illumina MiSeq; Hongos; Biodiversidad; Jaral; OTU

Soil fungal community composition in a Mediterranean shrubland is primarily shaped by history of major disturbance, less so by current fire fuel reduction treatments

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Abstract:

Cistus ladanifer shrublands are widely distributed around the Mediterranean basin. They constitute the early stages of succession and usually appear in degraded and poor soils. Previous studies based on sporocarp production have demonstrated the high diversity of the fungal communities associated with this species. Due to the pyrophytic ecology of this species, it is necessary to carry out fuel reduction treatments in order to prevent forest fires. The aim of this work is to study the effect of different fuel reduction treatments on soil fungal communities associated with the rhizosphere of *C. ladanifer* shrublands. We carried out Illumina MiSeq sequencing of ITS1 region from soil samples taken at 27 plots where different fuel reduction treatments were performed. Soil fungal communities associated with these ecosystems are highly diverse and we detected 1929 non-singleton fungal 97% sequence similarity OTUs after

analyzing 1 864 114 quality-filtered sequences. The deep sequence data presented here indicate that fungal community composition is mainly shaped by origin of the shrublands whereas the fuel reduction treatments within the same origin had no significant impact on them.

Key words: Illumina MiSeq; Fungi; Biodiversity; Shrubland; OTU

1. Introduction

Fungal communities play a key role in forests, contributing to a number of very important ecosystem functions (Courty et al., 2010). Forest practices can have an impact on fungal communities, affecting the occurrence, productivity, and reproduction of mushrooms (Savoie and Largeteau, 2011). Moreover, the impact of these practices on the ground can affect the presence of fungal mycelium. Understanding fungal ecology and the impact of different management techniques could be the way to enhance natural mushroom production (Savoie and Largeteau, 2011).

Fungal communities are very difficult to fully characterize for ecological and biodiversity studies and for conservation purposes (Geml et al., 2014a). Traditionally, studies of fungal communities were based only on epigeous sporocarp inventories, assuming that sporocarp production reflects the relative abundance and importance of the species on the root system (Vogt and Bloomfield, 1992). These studies provide important information on the investment of the fungal species in reproduction, but data about total fungal diversity of the ecosystem are not accurate (Baptista et al., 2015), since some fungal species do not produce noticeable sporocarps (Bidartondo and Gardes, 2005). Thus, the difference between fruiting patterns and vegetative patterns of fungal communities is important (Horton and Bruns, 2001). This fact may even be more important in Mediterranean climates since in arids or semi-arid climates fungal fruiting is even more sporadic than in temperate climates (Gardes and Bruns, 1996)

The development of molecular tools for biodiversity characterization based on DNA extraction provides new opportunities to study fungal communities (Orgiazzi et

al., 2015). Furthermore, the recent emergence of next-generation sequencing (NGS) methods has allowed to obtain millions of DNA sequences in a relatively short period of time (Glenn, 2011). These methods were firstly applied to mycorrhizal root tips, but current application of NGS to DNA extractions from the soil matrix allow to access a great proportion of the soil fungal diversity including saprotrophic and pathogenic fungi (Baptista et al., 2015). The internal transcribed rDNA spacer (ITS) region is the most frequently sequenced genetic marker of fungi (Begerow et al., 2010).

The fungal diversity assessment presented here focuses on *Cistus ladanifer* shrublands. This species is widely distributed around the Mediterranean basin. It constitutes the early stages of succession and usually appears in degraded and poor soils. However, previous studies have demonstrated the high diversity of the fungal communities associated with this species (Comandini et al., 2006; Hernández-Rodríguez et al., 2013). In a previous study, we characterized the early influence of different management alternatives on the fruiting bodies of fungi in *C. ladanifer* shrublands (Hernández-Rodríguez et al., 2015). However, it is unknown whether the impact of treatment affects just the fruiting of fungi or also to the presence of mycelium in the soil.

Soil biodiversity studies have two main objectives: firstly, to obtain knowledge about soil biodiversity structure and functions. Secondly, to assess the level and fluctuations of soil biodiversity in different environmental conditions, which can be very useful for decision makers in order to establish the optimal measures for the preservation of soil biodiversity (Orgiazzi et al., 2015). Thus, the knowledge of the effect of management methods on soil fungal communities associated with *C. ladanifer* would provide the necessary information for managers of these ecosystems in order to make the most suitable decisions for the conservation of these areas.

Our hypothesis is that, although previous studies have shown that fuel reduction treatments affect sporocarp production, fungal communities are able to stay unaltered in the soil after these treatments. Therefore, the aim of this work is to study the effect of different management methods on soil fungal communities associated with *C. ladanifer* shrublands.

2. Materials and methods

2.1. Study site

The study area is located in Zamora province in North-western Spain (0730462–0731929 Longitude-UTM, 4619644–4621757 Latitude-UTM 29T Grid). It is a Mediterranean ecosystem dominated by *C. ladanifer* situated at 750-780 m above sea level. The soil in this area is constituted by Paleozoic metamorphic rocks, Ordovician and Silurian shales being predominant (García Rodríguez et al., 1964). The soil is classified as Inceptisol suborder Xerept (Soil Survey Staff, 2010) and is characterized by stoniness, acidity (pH 5.0-5.5), and lack of calcium and phosphorous. Nitrogen and potassium availability is variable and the level of humification is generally good (García Rodríguez et al., 1964). The area is characterized by a sub-Mediterranean climate with a dry season of three months in the summer and a mean annual rainfall of 450-700 mm. Mean temperatures range from 14.5 to 15.8 °C.

2.2. Fuel reduction treatments

The experimental design for this study is the same as that used for the previous study of fungi sporocarps (Hernández-Rodríguez et al., 2015). Plots were established in three areas where *C. ladanifer* age and stand origin were different: a) a middle-age stand whose origin was a forest fire, b) a middle-age stand whose origin was the clearing of the previous stand, and c) an senescent stand. Treatments were chosen based on their applicability in accordance with the age of the stands and vegetation characteristics. The study site was not replicated across several sites.

In the two middle-age stands the following treatments were carried out: 1) Control; 2) 50% clearing; 3) Total clearing. In the senescent stand (c), the studied fuel reduction treatments were: 1) Control; 2) Total clearing; 3) Controlled burning.

Three sampling plots were established in each of the treatments. As a result, twenty seven sampling plots were analyzed. These sampling plots consisted of transects of 2m x 50m, established in accordance with previous studies (Luoma et al., 1991; Smith et al., 2002).

2.3. Sampling and molecular work

At each plot, five soil cores were taken using a cylindrical (2 cm radius, 20cm deep, 250 cm3) soil borer (De la Varga et al., 2012; Taylor, 2002). Cores were extracted along the plots' centerline and 5 m from each other to collect spatial variability and minimize the probability of sampling the same genet repeatedly. Soil was sampled in April 2014, four years after the implementation of the treatments. Samples were frozen immediately on the sampling date upon return to the laboratory and kept at -20ºC until DNA extraction.

The soil samples were thawed and let dry at room temperature to constant weight and then sieved with a mesh size of 1 mm. The five cores of each plot were pooled resulting in a composite soil sample for each plot. DNA extraction was performed from 0.25 g of soil per sample with the PowerSoil™ DNA Isolation Kit (MoBio laboratories Inc., Carlsbad, CA, USA) according to manufacturer's instructions.

PCR reactions were performed in triplicate for each sample to minimize PCR biases. PCR reactions were performed in 20 µl reaction volumes containing 11.22 µl of MQ water, 1.60 µl of DNA template, 2.00 µl of 10x buffer, 1.40 µl of $MgCl_2$ (50 mM), 1.60 µl dNTPs (10 mM), 0.50 µl BSA (2%), 0.80 µl of reverse and forward primers (10 µM) and 0.08 µl Platinum Taq polymerase (Invitrogen, Carlsbad, CA). We used the following PCR conditions: an initial denaturation step at 94°C for 3 min; then 35 cycles of 94 ºC for 45 s, 50 ºC for 1 min and 72ºC for 1.5 min; and ending with one cycle of 72ºC for 10 min. We amplified the ITS1 region using the forward primer ITS1F and barcoded reverse primer ITS2. A negative control consisting of MQ water instead of DNA was included in each PCR replicate and underwent the PCR under the same experimental conditions and was shown on a gel to be amplicon free.

The three replicates of PCR amplicons per experimental unit were cleaned with Zymo Clean and Concentrate Kit™ to remove short fragments (Zymo Research, Orange, CA). PCR products were normalized and pooled. Normalization was done after quantifying them with a Qubit 2.0 Fluorometer (Invitrogen), and the Qubit dsDNA HS Assay Kit (Invitrogen). Paired-end sequencing (2 x 150 bp) was carried out

on an Illumina MiSeq sequencer at Center for Genome Research and Biocomputing of the Oregon State University, U.S.A.

2.4. Bioinformatic analysis

Raw sequence reads were obtained from the Illumina MiSeq output that comprise demultiplexed sample reads. Forward and reverse reads were joined using the make.contigs command in MOTHUR v. 1.35 (Schloss et al., 2009), simultaneously trimming off primer sequences. Subsequently, sequences were filtered using MOTHUR based on the following settings: no ambiguous bases (maxambig=0), homopolymers no longer than 10 nucleotides (maxhomop=10), and length range from 150 bp to 400 bp (minlength = 150; maxlength = 400), resulting in 3 074 348 quality-filtered sequences with an average read length of 229.6 ± 30.4 (mean ± SD). Sequences were collapsed into unique sequence types, while preserving their original read counts and global singletons (1 189 793) and putative chimeric sequences (6673) were removed with USEARCH v.8.0 (Edgar, 2010). For the latter, the curated UNITE dataset of fungal ITS sequences (Nilsson et al., 2011) was used as reference dataset. The remaining 1 877 876 sequences were grouped into 2674 operational taxonomic units (OTUs) at 97% sequence similarity using USEARCH. We assigned sequences to taxonomic groups based on pairwise similarity searches against the curated UNITE fungal ITS sequence database containing identified fungal sequences with assignments to Species Hypothesis groups (Kõljalg et al., 2013). After excluding OTUs with < 70% similarity or < 150 bp pairwise alignment length to a fungal sequence, 1929 fungal OTUs were retained. The dataset containing only fungal OTUs contained a total of 1 864 114 high-quality sequences with an average of 58 254 ± 16 767 reads per sample.

2.5 Statistical analyses

We used PC-ORD v. 6.0 (McCune and Grace, 2002) to run non-metric multidimensional scaling (NMDS) on a primary presence/absence matrix of sites by OTUs and a secondary matrix of sites by treatment types (Table 2). Given the very high sequencing coverage we achieved, 'presence' was defined as ≥5 sequences on a per

sample basis following the recommendations of Lindahl et al. (2013) to minimize false positives (e.g., OTUs that are common in one sample, but may be low-abundant contaminants in others). The resulting matrix contained 1077 OTUs and was used as input for ordinations. Data were subjected to 500 iterations per run using the Sørensen similarity (Bray-Curtis index) and a random number to start. The solution with the lowest stress was derived from 250 runs using real data and was then subjected to 250 randomized runs using a Monte Carlo test to evaluate the probability of the final NMDS pattern being greater than chance occurrences. The solution with the lowest number of dimensions was selected when the decrease in the final stress was <5 by adding another dimension (McCune and Grace, 2002). We also tested whether fungal communities were statistically different across shrubland origin and treatment types using two different methods: multiresponse permutation procedure (MRPP) and permutation-based nonparametric MANOVA (PerMANOVA) (Anderson, 2001). Finally, we determined any preferences of individual OTUs for specific vegetation or treatment types using indicator species analyses, also in PC-ORD.

3. Results

3.1. OTU diversity

Ascomycota was the dominant phylum and accounted for 49.01% of the OTUs, followed by Basidiomycota (35.60%), Zygomycota (11.20%), Glomeromycota (3.96%), and Chytridiomycota (0.22%), while unidentified fungal OTUs accounted for 15.05% (Fig. 1a). In Ascomycota, the ranking of taxonomic orders based on the number of representative OTUs was as follows: Helotiales (90), Chaetothyriales (57), Pleosporales (51), Pezizales (31), Eurotiales (29), Hypocreales (10), followed by numerous other orders with less than10 OTUs. In addition, there were 17 ascomycete OTUs with incertae sedis classification. In Basidiomycota, Agaricales was by far the most species-rich order in the sample with 127 OTUs, followed by Thelephorales (33), Tremellales (29),Cantharellales (19), Sporidiobolales (19), Russulales (14), Sebacinales (11), Boletales (10) and numerous other orders with less than 10 OTUs each, while there was only 1 basidiomycete OTU with uncertain taxonomic placement (i.e, incertae sedis). The number and proportional distribution of OTUs repre- senting all detected taxonomic phyla and orders are shown in Fig. 1.
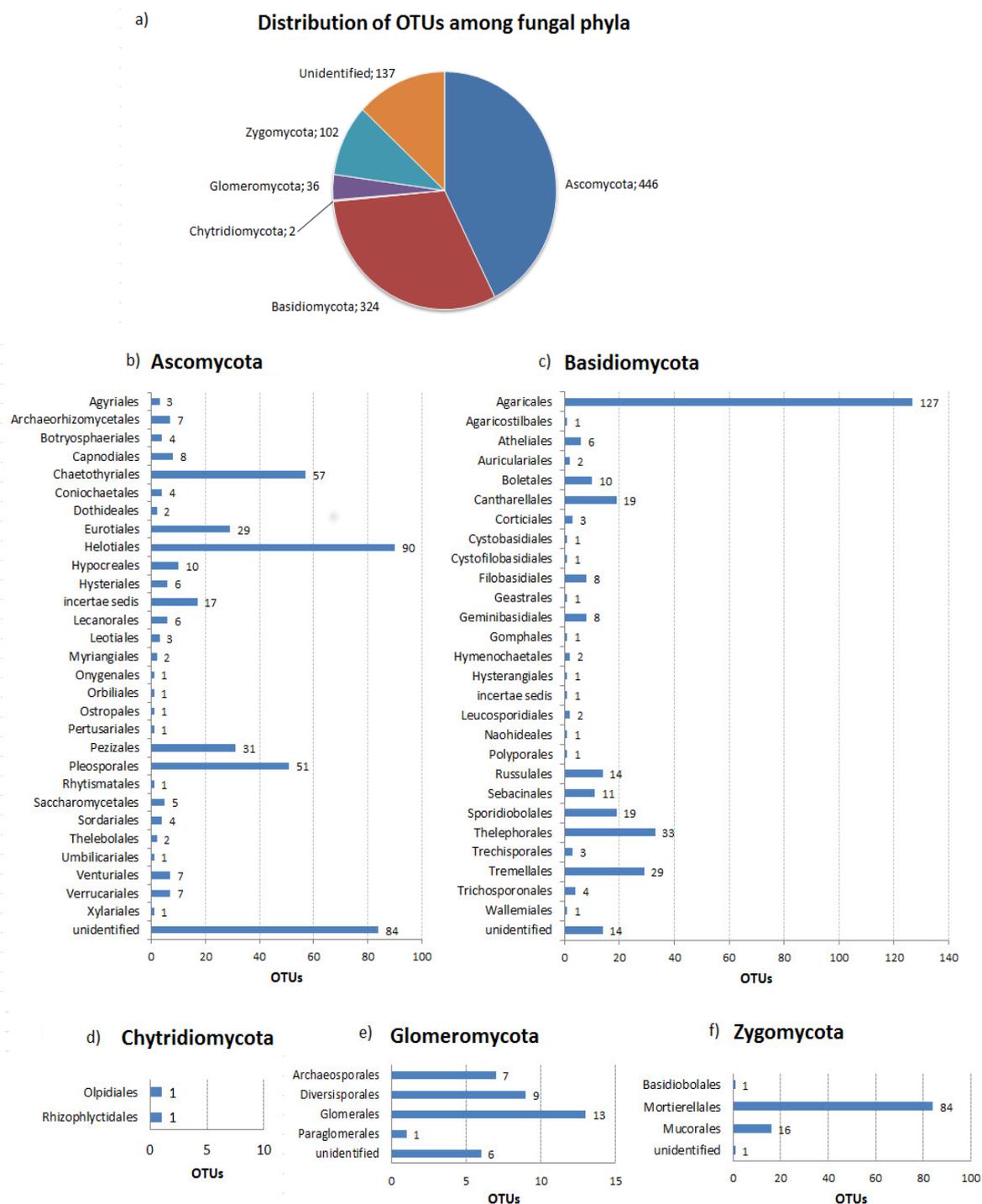
Fig. 1: Taxonomic groupings of fungi retrieved from the samples. Proportional distribution of the OTUs among different phyla (a) and taxonomic orders in Ascomycota (b), Basidiomycota (c), Chytridiomycota (d), Glomeromycota (e) and Zygomycota (f) including all treatments.

3.2. Impact of fuel reduction treatments on soil fungal communities

Four nonmetric multidimensional scaling analyses (NMDS) were performed: one including data about presence/absence of species, another including OTU abundance data and two transformations of abundance (square-root transformed OTU abundance data and relative OUT abundance). All the analyses showed similar trends in the ordination of the plots, and the analysis of species abundance without transformation presented the lowest value of final stress. Thus, in this section we show the results of this analysis (Fig. 2). The results of presence/absence and the two transformations of abundance data can be consulted in the supplementary material (Figures S1, S2, S3).
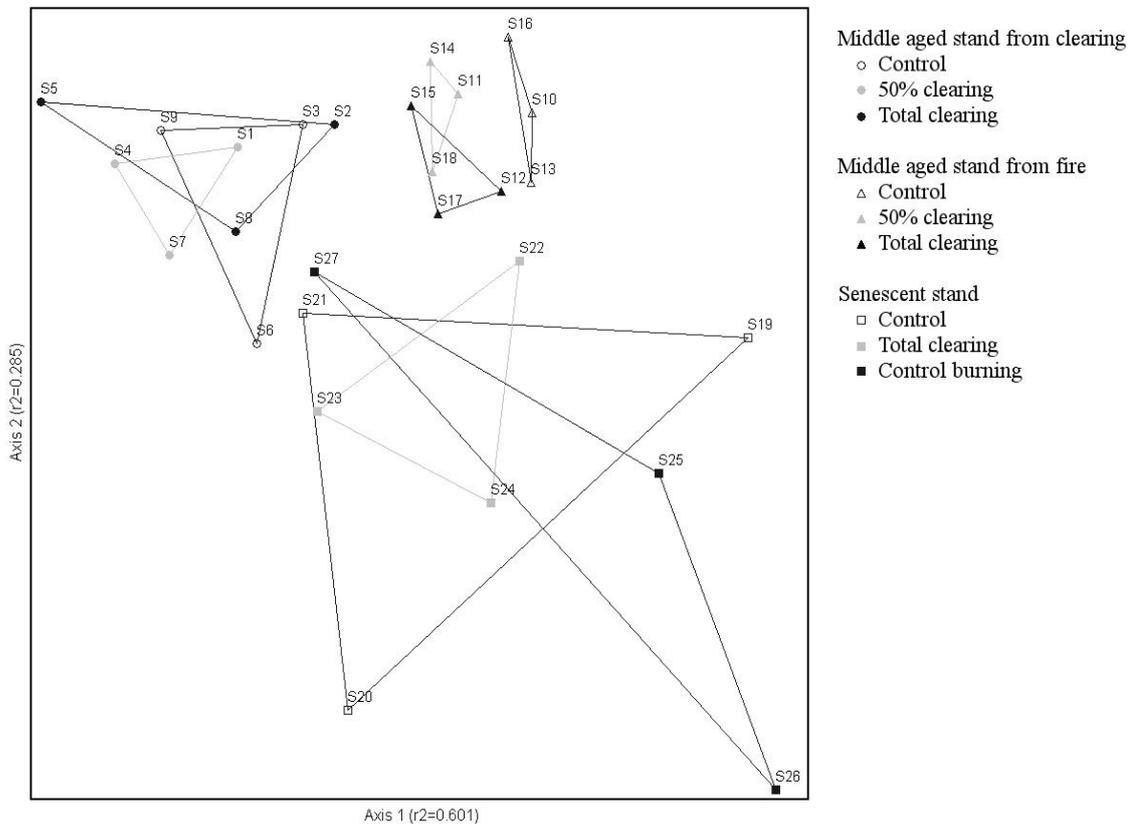
Fig. 2: Non-metric multidimensional scaling (NMDS) ordination plot for fungal communities based on the abundance of OTUs.

NMDS analyses of OTU abundance resulted in a 2-dimensional solution with a final stress of 0.1138 and a final instability < 0.00001. The two axes explained the majority of variability in the sampled fungal communities (axis 1: r^2=0.601; axis 2:

r^2=0.285; total r^2=0.886; orthogonality=86.6%). The NMDS plot revealed a strong structuring of fungal communities according to the origin of the shrubland. Thus, sampling sites representing the same origin of the shrubland grouped together (Fig. 2). MRPP confirmed the importance of the origin of the shrubland in shaping fungal community composition (effect size A = 0.1676, probability p < 0.00001). Similarly, PerMANOVA indicated that fungal community structure differed significantly among the different origins (p = 0.0002). Analyzing OTU abundance, none of these two analyses showed significant differences when comparing the different treatments within each origin of the shrubland (p > 0.05 in all cases). However, in the case of the presence / absence of OTUs, both, MRPP and PerMANOVA analyses showed significant differences among the different treatments in the middle age shrubland originating from a forest fire (MRPP effect size A= 0.03297, p = 0.034486; PerMANOVA p = 0.030800).

We used indicator taxon analyses as a conservative tool to identify OTUs that are associated to each origin of the shrubland. Across the three origins, there were 148 OTUs identified as significant (p <0.05) indicators for a certain origin of the shrubland. Of these, 36 were indicators for the middle aged shrubland whose origin was a forest fire, 78 were indicators for the middle aged shrubland whose origin was the total clearing of the previous stand and 33 were indicators for the senescent shrublands. The full list of indicator OTUs are shown in Table 1.

Table 1: OTUs considered as significant indicators of the different origin of the shrubland with corresponding p-values, and with accession numbers, sequence similarity, pairwise alignment length, name, and taxonomic classification of the most similar sequence in the UNITE+INSD database.

OTUs	Origin	p-value	Accession no.	%	bp	SH	Name	Phyllum	Order
598	middle age-clearing	0.0008	HM239886	98.6	209	SH238581.06FU	*Aspicilia* sp.	Ascomycota	Pertusariales
739	middle age-clearing	0.0022	FJ008616	83.2	131	SH207939.06FU	Acaulosporaceae sp.	Glomeromycota	Diversisporales
144	middle age-fire	0.0128	FJ168588	99	194	SH190780.06FU	Agaricales sp.	Basidiomycota	Agaricales
491	senescent	0.0202	FJ552749	96.6	204	SH214052.06FU	Agaricomycetes sp.	Basidiomycota	unidentified
143	middle age-clearing	0.0102	UDB015169	82.8	250	SH212838.06FU	*Aleuria aurantia*	Ascomycota	Pezizales
85	middle age-clearing	0.0004	UDB011826	85.5	242	SH240736.06FU	*Amanita magnivolvata*	Basidiomycota	Agaricales
169	middle age-clearing	0.0002	EU071906	97.4	233	SH200306.06FU	*Amanita muscaria*	Basidiomycota	Agaricales
294	middle age-fire	0.0128	GQ160037	96.7	152	SH197151.06FU	*Archaeorhizomyces* sp.	Ascomycota	Archaeorhizomycetales
25	middle age-fire	0.0002	JQ618514	100	162	SH006444.06FU	Ascomycota sp.	Ascomycota	unidentified
339	middle age-clearing	0.0002	HM239915	94.6	278	SH190375.06FU	Ascomycota sp.	Ascomycota	unidentified
494	middle age-clearing	0.0036	AY969686	87.4	135	SH223160.06FU	Ascomycota sp.	Ascomycota	unidentified
499	middle age-clearing	0.0038	JQ618514	92.7	165	SH006444.06FU	Ascomycota sp.	Ascomycota	unidentified
436	middle age-clearing	0.017	KC694155	95.8	189	SH208254.06FU	Ascomycota sp.	Ascomycota	unidentified
599	middle age-clearing	0.018	KC007187	88.8	269	SH204723.06FU	Ascomycota sp.	Ascomycota	unidentified
159	middle age-clearing	0.0308	DQ914698	99.5	197	SH208261.06FU	Ascomycota sp.	Ascomycota	unidentified
348	middle age-clearing	0.0006	JN409348	100	196	SH038508.06FU	Ascomycota sp.	Ascomycota	unidentified
611	senescent	0.0254	DQ124138	98.1	157	SH440905.06FU	Ascomycota sp.	Ascomycota	unidentified
41	senescent	0.0006	FJ475711	95.8	166	SH197703.06FU	Ascomycota sp.	Ascomycota	unidentified
349	senescent	0.0066	JQ618514	92.7	165	SH006444.06FU	Ascomycota sp.	Ascomycota	unidentified
678	middle age-fire	0.0394	AY530886	100	17	SH433485.06FU	*Boreoplaca ultrafrigida*	Ascomycota	Umbilicariales
425	middle age-clearing	0.045	GU187874	95.5	177	SH195241.06FU	Capnodiales sp.	Ascomycota	Capnodiales
279	middle age-fire	0.0038	AY559355	100	236	SH014290.06FU	Chaetothyriales sp.	Ascomycota	Chaetothyriales
253	middle age-clearing	0.004	AY559355	95.8	237	SH014290.06FU	Chaetothyriales sp.	Ascomycota	Chaetothyriales
170	senescent	0.0032	AY969568	99	208	SH026895.06FU	Chaetothyriales sp.	Ascomycota	Chaetothyriales
28	middle age-fire	0.0004	FJ611952	88.8	233	SH018590.06FU	Cladophialophora sp.	Ascomycota	Chaetothyriales
399	senescent	0.0272	EU139133	83.9	230	SH017955.06FU	Cladophialophora sp.	Ascomycota	Chaetothyriales
519	middle age-clearing	0.0034	DQ421142	100	236	SH008623.06FU	Clavaria sp.	Basidiomycota	Agaricales
507	middle age-clearing	0.017	DQ182445	97.5	240	SH022838.06FU	Clavaria sp.	Basidiomycota	Agaricales

OTUs	Origin	p-value	Accession no.	%	bp	SH	Name	Phyllum	Order
221	senescent	0.0012	GQ159940	100	181	SH019388.06FU	*Clavaria* sp.	Basidiomycota	Agaricales
514	middle age-clearing	0.0162	JF506746	99.2	241	SH220804.06FU	*Clavulina* sp.	Basidiomycota	Cantharellales
118	middle age-clearing	0.0216	FR682338	77.1	231	SH199924.06FU	*Clavulina* sp.	Basidiomycota	Cantharellales
304	middle age-clearing	0.0212	UDB005698	96.6	208	SH221811.06FU	Cortinariaceae sp.	Basidiomycota	Agaricales
470	middle age-fire	0.0474	FN669189	95.7	207	SH232893.06FU	*Cortinarius* sp.	Basidiomycota	Agaricales
302	middle age-fire	0.003	JF300706	100	195	SH215220.06FU	*Cryptococcus* sp.	Basidiomycota	Tremellales
261	middle age-fire	0.0238	GU328574	96.3	135	SH006005.06FU	*Cryptococcus* sp.	Basidiomycota	Tremellales
327	senescent	0.0242	AY969511	91.7	157	SH024299.06FU	*Cryptococcus* sp.	Basidiomycota	Tremellales
166	middle age-clearing	0.0006	AF145324	100	194	SH215218.06FU	*Cryptococcus aerius*	Basidiomycota	Tremellales
759	middle age-clearing	0.0172	AF472627	70.1	174	SH229459.06FU	*Cryptococcus perniciosus*	Basidiomycota	Tremellales
709	middle age-clearing	0.048	DQ222455	99.5	186	SH383740.06FU	*Cryptococcus stepposus*	Basidiomycota	Filobasidiales
259	middle age-clearing	0.0002	UDB015530	96.3	300	SH207930.06FU	*Delicatula integrella*	Basidiomycota	Agaricales
477	senescent	0.0272	GU237798	96	177	SH100149.06FU	*Didymella applanata*	Ascomycota	Pleosporales
453	middle age-clearing	0.0216	FN393416	99.6	266	SH022498.06FU	*Drechslera* sp.	Ascomycota	Pleosporales
765	middle age-clearing	0.016	JX454812	99.6	260	SH197228.06FU	*Entoloma llimonae*	Basidiomycota	Agaricales
260	middle age-clearing	0.0156	GU477559	94.9	273	SH038256.06FU	*Entoloma rhodopolium*	Basidiomycota	Agaricales
571	middle age-clearing	0.0166	JX042992	97.4	153	SH197095.06FU	*Entrophospora* sp.	Glomeromycota	Diversisporales
224	middle age-fire	0.0312	GQ851861	86.6	224	SH024228.06FU	Eurotiales sp.	Ascomycota	Eurotiales
384	middle age-clearing	0.0218	JQ758592	97	232	SH025793.06FU	Eurotiomycetes sp.	Ascomycota	unidentified
101	middle age-clearing	0.01	FN392313	99	200	SH037222.06FU	Fungal endophyte	unidentified	unidentified
409	middle age-clearing	0.0002	HM036652	100	196	SH018071.06FU	Fungi sp.	unidentified	unidentified
430	middle age-clearing	0.0162	AY843097	75.1	217	SH026642.06FU	Fungi sp.	unidentified	unidentified
595	middle age-clearing	0.0142	JX042997	96.3	164	SH034901.06FU	Fungi sp.	unidentified	unidentified
106	middle age-clearing	0.0146	DQ421188	99.2	245	SH220785.06FU	Fungi sp.	unidentified	unidentified
559	middle age-fire	0.0006	JN889968	99.1	212	SH204173.06FU	*Geminibasidium* sp.	Basidiomycota	Geminibasidiales
153	middle age-fire	0.0008	JN889968	96.3	214	SH204173.06FU	*Geminibasidium* sp.	Basidiomycota	Geminibasidiales
55	middle age-fire	0.0086	JN889968	99.1	212	SH204173.06FU	*Geminibasidium* sp.	Basidiomycota	Geminibasidiales
437	middle age-clearing	0.0006	AY970255	97.7	214	SH010365.06FU	*Geminibasidium* sp.	Basidiomycota	Geminibasidiales
239	senescent	0.016	JX242877	100	214	SH204176.06FU	*Geminibasidium donsium*	Basidiomycota	Geminibasidiales
366	middle age-fire	0.0356	JX242880	99.1	211	SH204178.06FU	*Geminibasidium hirsutum*	Basidiomycota	Geminibasidiales

OTUs	Origin	p-value	Accession no.	%	bp	SH	Name	Phyllum	Order
724	middle age-clearing	0.0002	FJ013091	96.2	236	SH219581.06FU	*Geopora* sp.	Ascomycota	Pezizales
120	middle age-clearing	0.0174	KF206525	100	133	SH235721.06FU	Glomeraceae sp.	Glomeromycota	Glomerales
16	middle age-fire	0.0156	FJ475707	89.5	238	SH196875.06FU	Helotiales sp.	Ascomycota	Helotiales
21	middle age-clearing	0.0086	HQ623451	87.6	185	SH005508.06FU	Helotiales sp.	Ascomycota	Helotiales
305	middle age-clearing	0.0126	HQ445486	99	191	SH209308.06FU	Helotiales sp.	Ascomycota	Helotiales
690	middle age-clearing	0.0154	FJ475759	88.3	239	SH196873.06FU	Helotiales sp.	Ascomycota	Helotiales
150	senescent	0.0004	KF155899	99.5	200	SH209263.06FU	Helotiales sp.	Ascomycota	Helotiales
380	senescent	0.0058	EF434016	88.7	239	SH196870.06FU	Helotiales sp.	Ascomycota	Helotiales
469	senescent	0.0068	HQ157908	98.5	203	SH215668.06FU	Helotiales sp.	Ascomycota	Helotiales
669	middle age-clearing	0.0176	UDB004146	96.2	235	SH225364.06FU	Herpotrichiellaceae sp.	Ascomycota	Chaetothyriales
341	middle age-clearing	0.017	AY843053	94.9	195	SH222313.06FU	*Hyaloscypha* sp.	Ascomycota	Helotiales
73	middle age-fire	0.0006	DQ054568	100	247	SH227682.06FU	Inocybaceae sp.	Basidiomycota	Agaricales
316	middle age-clearing	0.0148	UDB013545	77.6	321	SH236272.06FU	*Inocybe* sp.	Basidiomycota	Agaricales
82	senescent	0.021	HQ586860	100	181	SH228222.06FU	*Inocybe praetervisa*	Basidiomycota	Agaricales
250	senescent	0.0226	JQ975966	97.5	277	SH010978.06FU	*Laccaria* sp.	Basidiomycota	Agaricales
31	middle age-clearing	0.0098	FM172814	99.5	200	SH189778.06FU	*Lachnum* sp.	Ascomycota	Helotiales
336	middle age-clearing	0.03	JF937586	91.8	196	SH189784.06FU	*Lachnum virgineum*	Ascomycota	Helotiales
107	senescent	0.0074	FJ440917	98.8	168	SH197707.06FU	Lecanorales sp.	Ascomycota	Lecanorales
326	senescent	0.0266	HQ446010	96.4	165	SH006443.06FU	Lecanoromycetes sp.	Ascomycota	unidentified
299	middle age-clearing	0.0084	JN943601	100	250	SH003180.06FU	*Marasmius tricolor*	Basidiomycota	Agaricales
301	middle age-clearing	0.003	FJ553302	99.5	191	SH207199.06FU	Meliniomyces sp.	Ascomycota	Incertae_sedis
186	middle age-clearing	0.0006	AJ430223	100	191	SH229443.06FU	*Mollisia cinerea* f. *minutella*	Ascomycota	Helotiales
117	middle age-fire	0.0392	AY969671	91	188	SH214840.06FU	*Mortierella* sp.	Zygomycota	Mortierellales
86	middle age-clearing	0.0036	EU490025	100	175	SH214852.06FU	*Mortierella* sp.	Zygomycota	Mortierellales
26	middle age-clearing	0.0142	JX316376	97.9	189	SH237297.06FU	*Mortierella* sp.	Zygomycota	Mortierellales
97	middle age-clearing	0.0162	EF635743	100	174	SH211067.06FU	*Mortierella* sp.	Zygomycota	Mortierellales
152	middle age-clearing	0.0168	JX675149	97.7	176	SH218046.06FU	*Mortierella* sp.	Zygomycota	Mortierellales
2027	middle age-clearing	0.018	AY969462	96.7	181	SH214832.06FU	*Mortierella* sp.	Zygomycota	Mortierellales
130	middle age-clearing	0.0476	AB542110	98	200	SH011316.06FU	*Mortierella* sp.	Zygomycota	Mortierellales
148	senescent	0.0022	EF040832	99.5	188	SH217229.06FU	*Mortierella* sp.	Zygomycota	Mortierellales

OTUs	Origin	p-value	Accession no.	%	bp	SH	Name	Phyllum	Order
138	senescent	0.0464	JF461339	99.1	211	SH015211.06FU	*Mortierella* sp.	Zygomycota	Mortierellales
8	senescent	0.0376	FJ861399	100	195	SH020182.06FU	*Mortierella alpina*	Zygomycota	Mortierellales
2026	middle age-clearing	0.018	HQ630331	95.7	185	SH214845.06FU	*Mortierella camargensis*	Zygomycota	Mortierellales
116	middle age-clearing	0.0468	HQ630331	97.8	185	SH214845.06FU	*Mortierella camargensis*	Zygomycota	Mortierellales
855	middle age-clearing	0.0476	HQ630331	95.2	188	SH214845.06FU	*Mortierella camargensis*	Zygomycota	Mortierellales
919	middle age-clearing	0.0456	JX976111	95.7	185	SH020121.06FU	*Mortierella elongata*	Zygomycota	Mortierellales
59	middle age-fire	0.0376	JN943800	99.5	196	SH235686.06FU	*Mortierella globulifera*	Zygomycota	Mortierellales
456	senescent	0.0294	JX975908	98.4	191	SH217224.06FU	*Mortierella macrocystopsis*	Zygomycota	Mortierellales
124	middle age-clearing	0.0166	JX316327	99.1	216	SH206387.06FU	Mortierellales sp.	Zygomycota	Mortierellales
434	middle age-clearing	0.0062	JN205898	99.6	245	SH097635.06FU	*Mucor racemosus f. racemosus*	Zygomycota	Mucorales
248	middle age-clearing	0.0142	FJ455867	99.2	252	SH226030.06FU	*Mucor ramosissimus*	Zygomycota	Mucorales
748	middle age-fire	0.0414	AF062789	100	200	SH217745.06FU	*Oidiodendron chlamydosporicum*	Ascomycota	Incertae_sedis
146	middle age-fire	0.0478	AY997067	96.9	162	SH214989.06FU	*Olpidium brassicae*	Chytridiomycota	Olpidiales
577	middle age-clearing	0.0018	FR799508	100	228	SH225860.06FU	*Penicillium* sp.	Ascomycota	Eurotiales
443	senescent	0.002	AJ748692	99	202	SH201030.06FU	*Penicillium virgatum*	Ascomycota	Eurotiales
607	middle age-clearing	0.02	UDB009021	99	200	SH191052.06FU	*Pisolithus capsulifer*	Basidiomycota	Boletales
29	middle age-fire	0.0258	JX982370	98.3	177	SH196604.06FU	Pleosporales sp.	Ascomycota	Pleosporales
141	middle age-fire	0.0412	FJ475715	97.9	192	SH012526.06FU	*Rhizoscyphus ericae*	Ascomycota	Helotiales
79	senescent	0.0242	FJ013070	100	231	SH200171.06FU	*Russula* sp.	Basidiomycota	Russulales
277	senescent	0.025	FN610951	97.4	235	SH200296.06FU	Russulaceae sp.	Basidiomycota	Russulales
23	middle age-fire	0.014	AY288098	99.3	149	SH211497.06FU	*Sistotrema* sp.	Basidiomycota	Cantharellales
204	middle age-fire	0.0222	GU237890	99.4	177	SH255306.06FU	*Stagonosporopsis loticola*	Ascomycota	Pleosporales
155	middle age-fire	0.0012	EU021596	99.5	218	SH225870.06FU	*Talaromyces flavus*	Ascomycota	Eurotiales
450	senescent	0.0002	HQ625453	97.6	169	SH015141.06FU	*Talaromyces* sp.	Ascomycota	Eurotiales
445	middle age-fire	0.0364	HQ698106	100	226	SH212196.06FU	*Terfezia* aff. *olbiensis*	Ascomycota	Pezizales
280	middle age-clearing	0.0008	HM056211	94.3	227	SH212207.06FU	*Terfezia* sp.	Ascomycota	Pezizales
446	senescent	0.045	HM056211	99.5	219	SH212207.06FU	*Terfezia* sp.	Ascomycota	Pezizales
103	middle age-fire	0.0032	HF565112	100	235	SH202598.06FU	Thelephoraceae sp.	Basidiomycota	Thelephorales
188	middle age-fire	0.012	JQ991893	96.7	240	SH199069.06FU	Thelephoraceae sp.	Basidiomycota	Thelephorales
324	middle age-fire	0.0272	FJ897233	98.7	228	SH202664.06FU	Thelephoraceae sp.	Basidiomycota	Thelephorales

OTUs	Origin	p-value	Accession no.	%	bp	SH	Name	Phylum	Order
45	middle age-clearing	0.0002	UDB008827	97.2	248	SH016852.06FU	Thelephoraceae sp.	Basidiomycota	Thelephorales
866	middle age-clearing	0.0034	EU880218	96.1	229	SH202723.06FU	Thelephoraceae sp.	Basidiomycota	Thelephorales
168	middle age-clearing	0.0036	DQ233803	99.6	236	SH195956.06FU	Thelephoraceae sp.	Basidiomycota	Thelephorales
642	senescent	0.0294	JF519133	100	215	SH216217.06FU	Trechisporales sp.	Basidiomycota	Trechisporales
114	senescent	0.0004	JN053483	70.9	134	SH228996.06FU	*Tremella giraffa*	Basidiomycota	Tremellales
419	middle age-fire	0.0154	JN053476	88.7	141	SH006149.06FU	*Tremella leptogii*	Basidiomycota	Tremellales
140	middle age-fire	0.0034	HM036632	100	216	SH212235.06FU	Trichocomaceae sp.	Ascomycota	Eurotiales
344	middle age-fire	0.019	KC993077	99.1	232	SH222763.06FU	*Trichoderma* sp.	Ascomycota	Hypocreales
164	middle age-fire	0.002	EU668953	100	274	SH011997.06FU	*Tricholoma* sp.	Basidiomycota	Agaricales
300	middle age-clearing	0.0134	JN206380	99.1	220	SH205578.06FU	*Umbelopsis angularis*	Zygomycota	Mucorales
112	middle age-clearing	0.0002	DQ516528	99.1	218	SH011259.06FU	*Umbelopsis autotrophica*	Zygomycota	Mucorales
376	middle age-fire	0.0002	FJ553846	96.3	188	SH452553.06FU	uncultured Ascomycota	Ascomycota	unidentified
429	middle age-clearing	0.0002	FJ440886	99.5	188	SH014107.06FU	uncultured Ascomycota	Ascomycota	unidentified
458	senescent	0.0002	FJ440861	96.4	166	SH025803.06FU	uncultured Ascomycota	Ascomycota	unidentified
732	senescent	0.0068	JF691080	83.5	212	SH024940.06FU	uncultured Chaetothyriales	Ascomycota	Chaetothyriales
628	middle age-fire	0.0002	FJ440874	96.6	235	SH025802.06FU	uncultured *Cladophialophora*	Ascomycota	Chaetothyriales
317	middle age-fire	0.0002	KF007265	94.8	193	SH013636.06FU	uncultured ectomycorrhizal fungus	unidentified	unidentified
157	middle age-clearing	0.0056	KC966333	71.1	263	SH028068.06FU	uncultured fungus	unidentified	unidentified
543	middle age-clearing	0.0176	EU437433	100	181	SH022398.06FU	uncultured fungus	unidentified	unidentified
492	middle age-clearing	0.0188	HQ436083	94.8	172	SH027345.06FU	uncultured fungus	unidentified	unidentified
472	middle age-clearing	0.0442	GU984802	100	200	SH004703.06FU	uncultured fungus	unidentified	unidentified
1608	senescent	0.0224	HM069493	91.2	238	SH036517.06FU	uncultured fungus	unidentified	unidentified
791	senescent	0.028	KC965979	73.8	195	SH431021.06FU	uncultured fungus	unidentified	unidentified
524	senescent	0.0022	AM384981	93.4	136	SH036707.06FU	uncultured *Glomus*	Glomeromycota	Glomerales
181	middle age-clearing	0.039	EU726289	95.5	199	SH004410.06FU	uncultured Helotiales	Ascomycota	Helotiales
406	middle age-clearing	0.0008	JQ346926	94.2	206	SH026882.06FU	uncultured Herpotrichiellaceae	Ascomycota	Chaetothyriales
77	middle age-clearing	0.0162	HQ204690	71.4	185	SH005785.06FU	uncultured *Leccinum*	Basidiomycota	Boletales
185	middle age-clearing	0.003	GU083152	99	205	SH034506.06FU	uncultured soil fungus	unidentified	unidentified
534	middle age-clearing	0.0174	JX489824	94.6	205	SH431910.06FU	uncultured soil fungus	unidentified	unidentified

4. Discussion

4.1. OTU diversity

Cistaceae is a small family of plants of Holarctic distribution, which occupies considerable areas and plays an important role in the dynamics of the xeric vegetation in the Mediterranean area (Ellul et al., 2002). To the best of our knowledge, this is the first study about that the soil fungal diversity associated with the rhizosphere of *C. ladanifer*. Previous studies based on sporocarps have described fungal diversity in these ecosystems (Hernández-Rodríguez et al., 2015, 2013). However, these studies can only describe the Ascomycota and Basidiomycota species that fructifies in a given period of time, so the rest of the information of this work is entirely new in *C. ladanifer*.

Cistus spp. present great plasticity and associated mycorrhizal diversity, including both ectomicorrizas as vesicular arbuscular mycorrhizae (Comandini et al., 2006). This coexistence has many practical advantages, such as the rapid exchange of water and nutrients through mycorrhizal hyphal networks (Brundrett, 2004, 2002). In the case of this study, high nutritional versatility with the presence of many different groups of mycorrhizal fungi (e.g. Basidiomycota, Ascomycota and Glomeromycota) was appreciated. This association with mycorrhizae from different groups seems to be due to the need to adapt to the Mediterranean arid environments, needing a wide variety of nutritional collaborators, both epigeous and hypogeous.

The largest group of fungi in this study belonged to Ascomycetes (Fig. 1a). This result agrees with previous studies that found a dominance of Ascomycota taxa in different ecosystems (Geml et al., 2014a, 2014b) . However, in France, Buée et al. (2009) found a prevalence of Basidiomycetes in different forest ecosystems of hardwoods and conifers (*Picea, Pseudotsuga, Quercus, Fagus*, etc.). Also, in a global study carried out by Tedersoo et al. (2014), Basydiomycota taxa encompassed the largest proportion of sequences (55.1% of the taxa) followed by Ascomycota species (31.3%). In the same study, the prevalence of Basidiomycota taxa was also present in Mediterranean ecosystems. In a previous work of soil fungal communities in Mediterranean pine forests subjected to different wildfire frequencies, Buscardo et al. (2015) found that frequent fires induced a decrease in the abundance ratio between basidiomycetes and

ascomycetes. This fact could explain the prevalence of Ascomycota taxa in this study, since *C. ladanifer* is a pyrophytic species adapted to recurrent fires.

Among the Ascomycetes, Helotiales was the largest order in our studio (Fig. 1b). This order comprises the largest group of undescribed fungi associated with the roots (Wang et al., 2006). These fungi play a key role since they are associated with ectomicorrizas (Tedersoo et al., 2009). Also belonging to Ascomycetes phylum, a considerable number of Pleosporales appeared. This order comprises saprobes or parasites on vascular plants and coprophilous (Kruys et al., 2006).

4.2. Impact of fuel reduction treatments on soil fungal communities

The three different origins of the shrublands (middle age from fire, middle age from total clearing and senescent) are significantly different from each other considering the specific composition of soil fungal communities. However, the treatments did not significantly affect fungal composition within a single origin. This difference between the shrublands with different origin is consistent with the findings in a previous study of sporocarp production (Hernández-Rodríguez et al., 2015). However, when studying the mushroom production we found a significant effect between treatments performed in shrublands of the same origin. Therefore, although the new conditions created by eliminating the vegetation alter fungal fruiting, the mycelium of the different species in the rhizosphere remains unaltered. This is consistent with the findings of Jennings et al. (2012), which concluded that soil fungal communities are resilient to mechanical treatments.

Controlled burning had no significant effect on the presence of fungal mycelium. Prescribed fires are usually conducted under conditions that result in lower fire intensity and severity, and generally outside of the high risk conditions that are most conducive to the high intensity wildfires (Oliver et al., 2015). Thus, soil temperatures reached during a controlled burning are lower than during wildfire and therefore, the impact on the mycelium is lower, especially in the deeper layers.

Although according to Oliver et al. (2015) the season of the prescribed burning do not affect richness and diversity of the fungal communities, the seasonal difference in the distribution of mycelia could also determine the impact of fire on soil mycelium. For instance, in the case of *Boletus edulis*, mycelium is more abundant in spring-summer (when wildfires normally occur) and less abundant in autumn (when controlled burns are conducted) (de la Varga et al., 2013).

Considering the indicator species, the middle aged shrubland from fire presented a much smaller number of indicator species that the middle aged shrubland from clearing. This may suggest that most of the species found in the middle aged shrubland from fire are also found in at least one of the other shrublands. On the other hand, numerous fungal species are only present in the middle aged shrubland from clearing. This would indicate that several species are not able to adapt to the effect of fire. For example, in the results of this study we found Glomeromycota indicator species in middle aged shrublands from clearing (Acaulosporaceae and Glomeraceae) and senescent shrublands (Glomus) but not on middle aged shrublands from fire. Previous studies have shown the adverse effects of fire on communities of mycorrhizal fungi Glomeromycota in Asian arid shrublands (Rashid et al., 1997) and also in forests of South America with dry season (Longo et al., 2014).

5. Conclusions

Our work provides new information on fungal communities associated with the rhizosphere of *C. ladanifer* and the effect that different fuel reduction treatments have on them. The high number of taxa found associated with these Mediterranean ecosystems support the need for their conservation. Furthermore, our work highlights that fungal communities found in these shrublands are primarily shaped by history of major disturbance, whereas current fire fuel reduction treatments do not have a significant impact on them. Therefore, although the new conditions created by eliminating the vegetation alter fungal fruiting, the mycelium of the different species is able to remain in the soil. Although more detailed, taxon-specific studies will follow, this project provides useful information for the conservation of fungal communities in these ecosystems.

6. Acknowledges

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SUPPLEMENTARY MATERIAL
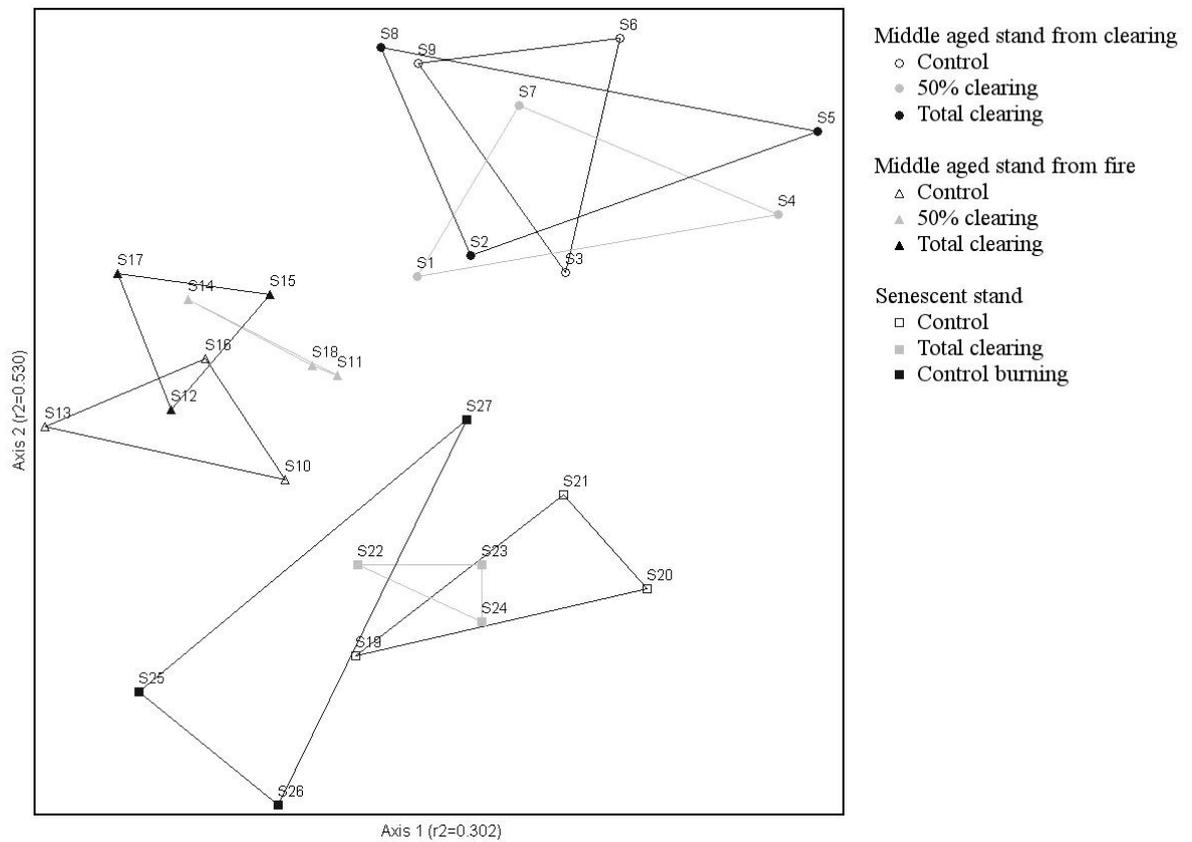
Fig. S1: Non-metric multidimensional scaling (NMDS) ordination plot for fungal communities based on the presence/absence of OTUs.
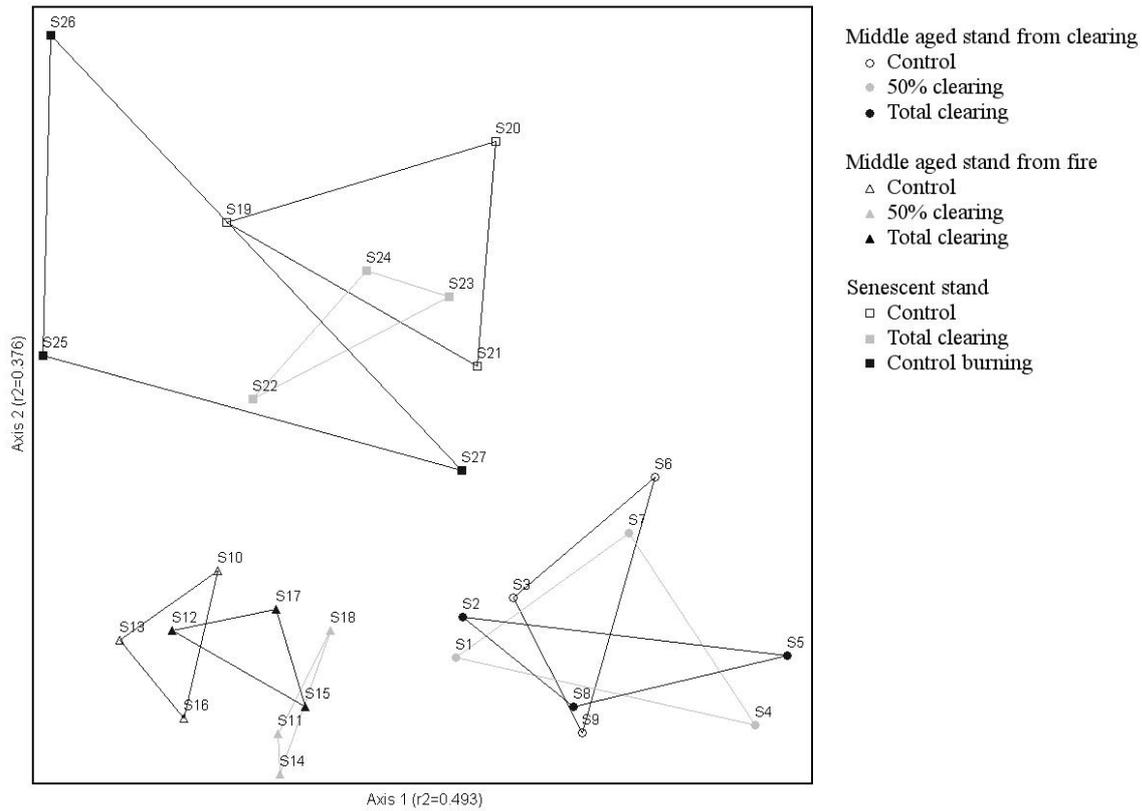
Fig. S2: Non-metric multidimensional scaling (NMDS) ordination plot for fungal communities based on the square-root transformed abundance of OTUs.
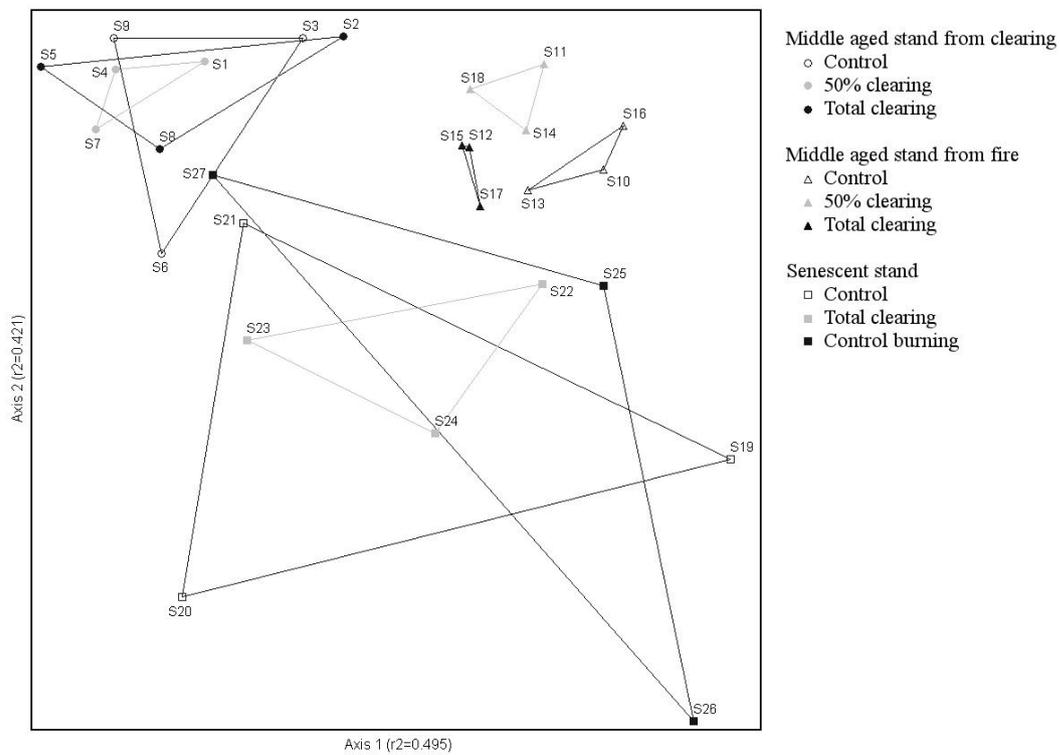
Fig. S3: Non-metric multidimensional scaling (NMDS) ordination plot for fungal communities based on the relative abundance of OTUs.