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FUNGAL COMMUNITIES AND FIRE ASSOCIATED TO *PINUS PINASTER* IN A MEDITERRANEAN REGION

DOCTORAL THESIS

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TESIS DOCTORAL

FUNGAL COMMUNITIES AND FIRE ASSOCIATED TO *PINUS PINASTER* IN A MEDITERRANEAN REGION

Presentada por Pablo Vásquez Gassibe para optar al grado de doctor por la Universidad de Valladolid

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CONTENTS

NOTE TO READERS

This thesis is based on four original works, which are referred to in the text with Roman numerals (**I**, **II**, **III**, and **IV**), published or under revision in different international journals. Each article constitutes one of the studies or chapters of the thesis. Authors, coauthors, and the stage of the publication are presented below.

Previously to the presentation of each study, the reader will find the outline of the thesis and an abstract of the thesis written in English and Spanish. Then, a summary of the whole thesis which includes an introduction with the general and specific objectives, the main methodologies and analyses employed, the main results and a general discussion. After that, the reader will find the four studies and the general conclusions of the thesis.

LIST OF ORIGINAL WORKS

I. Gassibe, P.V., Oria-de-Rueda, J.A., Martín-Pinto, P., 2014. *P. pinaster* under extreme ecological conditions provides high fungal production and diversity. Forest Ecology and Management DOI: 10.1016/jforeco.2014.11.013

II. Gassibe, P.V., Fraile-Fabero, R., Hernández-Rodríguez, M., Oria-de-Rueda, J.A., Martín-Pinto, P., 2011. Fungal community succession following wildfire in a Mediterranean vegetation type dominated by *Pinus pinaster* in Northwest Spain. Forest Ecology and Management 262: 655-662.

III. Gassibe, P.V., Fraile-Fabero, R., Hernández-Rodríguez, M., Oria-de-Rueda, J.A., Bravo-Oviedo, F., Martín-Pinto, P., 2014. Post-fire production of mushrooms in *Pinus pinaster* forests using classificatory models. Journal of Forest Research 19: 348-356.

IV. Gassibe, P.V., Oria-de-Rueda, J.A., Santos-del-Blanco, L., Martín-Pinto, P., 2014. The effects of fire severity on ectomycorrhizal inoculum and allometric features in *P. pinaster* seedlings. Annals of Forest Science (under revision).

OUTLINE OF THE THESIS

This thesis is structured in four chapters. In the first chapter richness, production, diversity and taxa composition of ectomycorrhizal and saprophytic fungal communities associated with pine trees growing under extreme Mediterranean conditions in the north-western part of the Iberian Peninsula were investigated during a long term survey of 6 years without previous ecological disturbances. The main edaphoclimatic factors responsible for the above ground appearance of the macrofungal species were identified.

In the second chapter the effects of wildfire on the fungal communities in successional terms of a Mediterranean pine forest in Northwest Spain were evaluated by comparing an undisturbed forest dominated by 50-year-old *P. pinaster* Ait. plantations with an area with the same initial characteristics of vegetation where tree and understory vegetation were totally destroyed by fire.

In the third chapter the post fire fungal production was evaluated through alternative descriptive models based on classifications according to combinations of edibility and functional groups in which fruiting body biomass production classes were the responses. These models were explained with explanatory factors that considered time after fire and climatic variables significantly related to sporocarps production.

In the fourth chapter the influence of fire severity on the ectomycorrhizal colonization and morphometric parameters of *P. pinaster* was assessed. Soil samples were collected in three sites that portrayed different fires severities to test ECM fungal colonization at two different root depths and its influence on allometric features in pine seedlings under greenhouse controlled conditions.

ABSTRACT

ABSTRACT

The aim of this investigation was to study how fungal communities in Mediterranean forests dominated by maritime pine (*Pinus pinaster* Ait.) are influenced by fire and different edaphoclimatic conditions specifically in areas where degraded and poor soils are predominant. Sporocarps were collected and identified from 100 m^2 transects during the autumn seasons of 2003-2006 period in a post-fire scenario and another from 2006 to 2012 in an undisturbed situation.

The data collected that were used to assess fungal richness, production and diversity, were grouped into categories (saprotrophic/mycorrhizal; edible/inedible) for statistical analysis and modeling procedures. On the other hand a greenhouse experiment was carried out during 2008 until 2009 where the fire severity on the ectomycorrhizal seedling colonization along its influence on plant development was also assessed.

Firstly, the results showed remarkably high fungal production and richness when associated with *P. pinaster* in extreme but undisturbed Mediterranean conditions composed by natural forests in calcareous and sandy soils and from reforestations in siliceous soils. The same trend was observed in forests associated to the effects of wildfire, an essential factor in the dynamics of Mediterranean forests.

In the undisturbed situation, total fungal production and richness were much higher in the siliceous and calcareous forests than in the inner sandy dune site. The results showed a similar trend when the production of mycorrhizal taxa and diversity index for saprotrophic fungi were also analyzed. On the other hand, the fungal community was mainly correlated with climatic variables, such as precipitation and temperature. Additionally, nitrogen and potassium soil contents significantly affected the distribution of species for both saprophytic and mycorrhizal functional groups. Some of the collected taxa were adapted to a broad range of ecological conditions such as *Lycoperdon perlatum, Russula torulosa,* and species within genus *Galerina* and *Mycena.* However, other recorded fungi were only found in very specific environmental conditions. Within this group, all the species included within *Macrolepiota* genera were collected in the calcareous soils, whereas *Laccaria laccata* and *L. bicolor* were exclusively associated with higher amounts precipitation and nitrogen in the siliceous plots.

Secondly, to assess the effect of fire we examined the succession of fungal communities following fire in a Mediterranean ecosystem in Northwest Spain, dominated by *Pinus pinaster* Ait. A large wildfire occurred on August 2002. During the autumns 2003 until 2006, fruiting bodies were collected and identified, production in both burned (early stage) and unburned (late stage) areas were measured. Data were grouped into categories (saprotrophic/mycorrhizal; edible/inedible) for statistical analysis. 115 fungal taxa were collected during the four year sampling (85 in the late stage and 60 in the early one). Mycorrhizal population not only increased the number of species from early to late stage but also shifted in composition across the chronosequence.

Fire strongly affected the production of fungal species. Thus, yields in the early stage treatment were significantly lower than those observed in the late one.

Total fungal fresh weight decreased from 209.95 kg fw ha^{-1} in late stage to 162.45 kg fw ha^{-1} in the early where richness and production of mycorrhizal species and production of edible fungi were significantly lower. Fresh weight for saprotrophic and inedible species was higher than for mycorrhizal fungi in the early stage treatment. Thirdly, from the data from burned and non burned areas from the period 2003-2006, our results provide the first classificatory simple model for fungal production in *P. pinaster* forests. Climate variables related with the sporocarp production were included in the modelling procedure (discriminant functions) to classify the productions into four yield categories. Nine discriminant classificatory models were studied to determine these four yield categories within each dependent variable according to edibility and functional group. Models for edible and edible-mycorrhizal were fitted to allow an estimate of the production class (R^2 =0.92 and 0.85 respectively).

Finally, mycorrhizal fungi in the soil play a key role in the processes of evolution of Mediterranean forests after wildfire. However, the impact of fire on these fungal communities is poorly understood. Therefore a broader understanding of an important typical pyrophitic species as *P. pinaster* and its fungal symbionts is necessary for forest restoration. We analyzed the effect of a wildfire in Central Spain that took place in 2008 comparing the ectomycorrhizal community checked in *P. pinaster* root apexes from 3 different burned severities sites. The work was conducted in a greenhouse, where *P. pinaster* seedlings were used as bioassay to examine the influence of fire severity on soil characteristics, root ectomycorrhization at two horizon depths and plant development. The ectomycorrhizal degree was higher in the unburned bioassays than in those that have been affected by the fire, for both depth layers. While that plant parameters increased their values according fire severity. This study suggests that fire affects soil properties, mycorrhizal inoculum and plant development after a year.

These findings could represent ecological and economical implications that may be important for managers to consider when seeking new options in order to optimize the management and harvesting of these more and more appreciated non wood resource, therefore adding value to these Mediterranean forests, especially into fire-impacted areas, in which ectomycorrhizal fungi play a role as a biological tool in restoring disturbed forest ecosystems. Therefore, having a positive impact on rural communities that depend on forest resources, edible mushroom production can provide supplementary economical incomes for population as complement to those obtained from wood resources in forestry areas.

RESUMEN

El objetivo de esta investigación fue estudiar cómo las comunidades de hongos de bosques mediterráneos dominados por *Pinus pinaster* Ait. están influenciados por el fuego y las diferentes condiciones edafoclimáticas que caracterizan específicamente estas diversas zonas, donde predominan los suelos degradados y pobres. La recolección de carpóforos se realizó a partir de transectos de 100 m^2 durante las estaciones de otoño, en los períodos correspondientes a 2003 - 2006 en un escenario post-incendio y otro durante 2006-2012 en uno sin perturbaciones previas.

Los datos recogidos se utilizaron para evaluar la riqueza, producción y la diversidad fúngica, los que se agruparon en categorías (saprófitos / micorrícicos; comestible / no comestible) para el análisis estadístico y la posterior modelización. Por otra parte, se llevó a cabo un experimento de invernadero durante el año 2008 hasta el 2009, donde también se evaluó la severidad del fuego sobre la infectividad de hongos ectomicorrícicos sobre plántulas de pino y su influencia en su desarrollo.

En primer lugar, los resultados mostraron una sobresalientemente alta producción y riqueza fúngica asociada a *P. pinaster* en condiciones mediterráneas extremas pero no sujetas al fenómeno del fuego en bosques naturales de suelos calcáreos y arenosos, al igual que en los provenientes de reforestaciones en suelos silíceos. La misma tendencia fue apreciada en los bosques asociados a los efectos de los incendios forestales, un factor esencial en la dinámica de los bosques mediterráneos, aunque se puso en evidencia una ostensible disminución frente a los ecosistemas no perturbados.

En la situación sin perturbaciones pero de climas mediterráneos adversos, la producción total y riqueza de hongos fueron más altos en los bosques silíceos y calcáreos que en los situados sobre dunas interiores. Los resultados mostraron una tendencia similar cuando también se analizó la producción de taxones micorrícicos y el índice de diversidad de hongos saprófitos. Por otro lado, la comunidad fúngica se correlacionó principalmente con variables climáticas, tales como la precipitación y la temperatura. Además, el contenido de nitrógeno y de potasio del suelo afectó significativamente la distribución de especies de ambos grupos funcionales. Algunos de los taxones recogidos se asociaron a una amplia gama de condiciones ecológicas, tales como *Lycoperdon perlatum*, *Russula torulosa*, y las especies pertenecientes a los géneros *Galerina* y *Mycena*. Sin embargo, otros hongos registrados sólo se encontraron en condiciones ambientales muy específicas. Dentro de este grupo, todas las especies incluidas en los géneros *Macrolepiota* se recolectaron en los suelos calcáreos, mientras que *Laccaria laccata* y *L. bicolor* se asocian exclusivamente a mayores cantidades de precipitación y de nitrógeno en suelos silíceos.

En segundo lugar, para evaluar el efecto del fuego se analizó la sucesión de las comunidades de hongos después de un incendio en un ecosistema mediterráneo en el noroeste de España, dominado por *Pinus pinaster* Ait. En este estudio presentamos los resultados de una investigación de 4 años

encaminada a describir la sucesión de las comunidades fúngicas tras un incendio en un ecosistema Mediterráneo dominado por *Pinus pinaster* en comparación con las comunidades existentes en una masa madura antes del paso del fuego. Tras un incendio forestal ocurrido en 2002, se establecieron seis transectos lineales (2x50m^2) en zonas quemadas y no quemadas que se corresponden con las etapas más tempranas y tardías de la sucesión. Se recogieron e identificaron semanalmente todos los carpóforos encontrados durante las temporadas de otoño de 2003 a 2006, anotando información de la producción (peso fresco y seco) y del modo de vida de las especies (micorrícicas o saprófitas). Se recogieron un total de 115 taxones (85 en masas maduras y 60 en zonas quemadas). El número de taxones micorrícicos se incrementó a lo largo de la sucesión, observándose también un cambio en la composición específica. La producción fúngica se vió afectada por el fuego de manera diferente según el modo de vida: para especies micorrícicas fue seis veces menor en las masas afectadas por el incendio, mientras las saprófitas incrementaban cuatro veces su biomasa.

En tercer lugar, las condiciones anteriores de las zonas quemadas y no quemadas del periodo 2003-2006, permitieron proporcionar los primeros modelos simples de clasificación para la producción micológica en bosques de *P. pinaster*. Nueve modelos alternativos para las diferentes combinaciones entre modo de vida y comestibilidad fueron ajustados definiendo previamente cuatro clases de producción como posible respuesta. El tiempo trascurrido tras el incendio y variables climáticas relacionadas significativamente con la producción fúngica se incluyeron como factores explicativos.

Los modelos obtenidos se aplicaron para analizar el efecto del fuego en la producción fúngica. La producción de especies micorrícicas y comestibles tras un incendio forestal se clasificaron dentro de la clase de producción más baja, mientras las especies saprófitas y no comestibles mostraron una tendencia contraria.

Por último, los hongos micorrícicos bajo suelo juegan un papel clave en los procesos de la evolución de los bosques mediterráneos después de un incendio forestal. Sin embargo, el impacto del fuego en estas comunidades de hongos es poco conocido. Por lo tanto, una comprensión más amplia de una importante especie pirófita como lo es *P. pinaster* y sus hongos simbiontes es necesaria para la restauración de ecosistemas forestales mediterráneos. Por lo cual, se analizó el efecto de un incendio forestal en el centro de España que tuvo lugar en 2008 comparando la colonización ectomicorrícica en ápices radiculares y el desarrollo de plántulas de *P. pinaster* según tres severidades de quemado. El experimento se realizó en condiciones de invernadero, donde la colonización ectomicorrícica fue mayor en los bioensayos no quemados que en los afectados por el fuego, para ambas capas de profundidad. Mientras que los parámetros de la planta aumentaron sus valores según la severidad del fuego. Lo que sugiere que el fuego afecta a las propiedades del suelo, al inóculo micorrícico y al desarrollo de la planta en el transcurso de un año.

Estos hallazgos podrían representar implicaciones ecológicas y económicas que pueden resultar importantes para los gestores forestales, los que podrían así considerar la búsqueda de nuevas opciones para optimizar el manejo y aprovechamiento de estos cada vez más apreciados recursos no maderables. Agregando de esta manera valor a estos bosques mediterráneos, especialmente en zonas afectadas por incendios, en las que los hongos ectomicorrícicos juegan un papel como herramienta biológica en la restauración de los ecosistemas forestales perturbados. Así mismo enfocando una gestión forestal integrada de producción maderera y micológica, la que tendría un efecto positivo sobre las comunidades rurales que dependen de este apreciado recurso forestal no maderable.

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Dedicated to the true forest dwellers of Southern Chile and Castilla y León.

“I am a forest, and a night of dark trees: but he, who is not afraid of my darkness, will find banks full of roses under my cypresses.”

Friedrich Nietzsche, Thus Spoke Zarathustra

INTRODUCTION

1. INTRODUCTION

1.1. Maritime pine and fungal communities

Pinus pinaster Ait. forests are within the most relevant representatives of Mediterranean areas, and it is one of the main forest tree species planted in Spain, Portugal and France (Majada et al., 2010), reaching a greatest economic importance (Fernandes and Rigolot, 2007), since its uses are related to wood production, pulp and paper production, resin production and soil protection (Majada et al., 2011). In Spain it occupies almost 1.700.000 ha, 0.6 million of which were established through direct planting (Alía et al., 1996), making it the second most important tree species by surface area (Rodríguez et al., 2008). The distribution of the species across the Iberian Peninsula is patchy comprising a broad spectrum of substrates (limestone, granite, schist, marly limestone, peridotite), topographies and climates, ranging from montane sub-humid to Mediterranean semi-arid with summer drought (Nicolás and Gandullo, 1967; López-Sáez et al., 2010).

In this Mediterranean area, the climate is characterized by an irregular precipitation regime and high temperatures in summer. The soils are well drained and vary from sandy to rocky. This specie is one of the best adapted in the Iberian Peninsula due to that it is native in some areas as reported by some authors that indicate that its natural origin is in the Iberian System (Blanco et al., 1997) (Fig. 1).

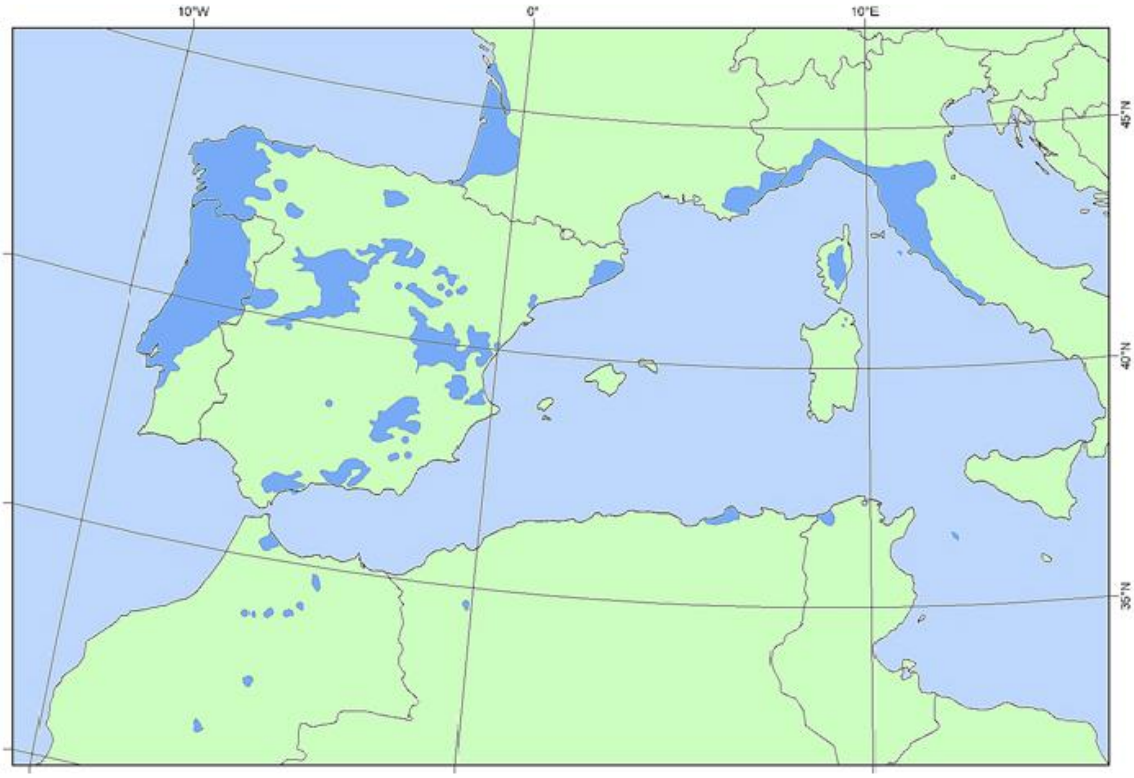

Fig. 1: Natural distribution of *Pinus pinaster* Ait. in the Mediterranean region (Alía and Martín, 2003)

P. pinaster can form closed forests that are either single-species, or a mixture of different evergreen and deciduous trees, with a wide range of understory species (Blanco et al., 1997). Therefore, it has been used widely in the reforestation of infertile, sandy, and slightly acid soils (Barčić et al., 2006; Oliveira et al., 2012). Its success in colonizing and establishing on disturbed soils and former agricultural fields may be attributed in part to its compatibility with a wide range of fungal symbionts (Carson et al., 2010; Gassibe et al., 2011; Oria-de-Rueda et al., 2010). Some ectomycorrhizal relationships are highly specific and a fungus may colonize only one or a few host species, as is true for suilloid fungi, which are restricted to mostly pines (Bruns et al., 2002; Mohatt et al., 2008). The general rule is that young seedlings typically accept a range of generalist ectomycorrhizal fungi but as the trees mature the fungal relationship shifts towards fungi more specific to the host (Buscardo et al., 2012).

Due to these relationships, artificial and natural stands of *P. pinaster* have the potential to support significant fungal production and diversity (Oria-de-Rueda et al., 2010). Previous studies have also shown these associations between fungi and this Mediterranean species (Bonet et al., 2012; Fernández-Toirán et al., 2006; Gassibe et al., 2011; Martín-Pinto et al., 2006b; Oria-de-Rueda et al., 2010). These relationships are important since fungi can play an essential ecological and economic role in these forest communities (Oria-de-Rueda et al., 2010).

Regarding fungal functional groups, mycorrhizal associations have significant effects on nutrient and water uptake, growth and plant survival (Brundrett, 2009; Courty et al., 2010), improving soil aeration and porosity (Fernández-Toirán et al., 2006), resistance to pathogens (Martín-Pinto et al., 2006a), and also provide a food source for many organisms in the forest food chain (Sato et al., 2012). On the other hand, saprobial, saprophytic or saprotrophic fungi are essential in Mediterranean forests due to the role they play in the decomposition of dead matter, and therefore in the process of nutrient cycling in forest ecosystems (Ferris et al., 2000). These previous definitions which may seem as synonyms differ, since these fungi, are characterized beyond their trophic status, according to J.A. Oria-de-Rueda (personal communication, November, 25, 2014).

Formation of sporocarps is influenced by various factors, such as host specificity (Ishida et al., 2007; Molina et al., 1992), physiological conditions, nutritional status of the mycelium (Murat et al., 2008), and environmental factors (Bonet et al., 2004). Climatic variables are especially important because they directly affect the mass of mycorrhizal mushrooms produced (Dahlberg, 1991). The development of fungal fruiting bodies is dependent on the availability of surface water and soil temperature (Bonet et al., 2010; Egli et al., 2010; Pinna et al., 2010). Rainfall and temperature are generally recognized as important factors, however not many studies have been conducted to address these variables (Straatsma et al., 2001). On the other hand, fungal species composition is strongly determined by soil chemical properties (Ruhling and Tyler, 1990), especially in the case of saprotrophic fungi due to their lack of mutualistic interaction, they are expected to be more dependent upon their respective substrates than mycorrhizal fungi (Reverchon et al., 2010). Therefore, soil temperature, nutrients and, moisture cause an important influence for both mycorrhizal and saprotrophic groups (Richard et al., 2004).

Apart from the ecological importance of fungi, much of the recent interest in mushrooms comes from their recognized economic value (Cai et al., 2011). During the last decade, there has been a sharp increase in the demand for edible fungi and in the marketing and distribution of these products (Pettenella et al., 2007), becoming an important source of rural income, especially in areas where adverse edaphoclimatic conditions are predominant, and wood production has been somewhat neglected. In this sense *P. pinaster* forests are one of the most productive in terms of mushroom production and richness, when compared to other Mediterranean pines (Oria-de-Rueda et al., 2010), therefore from an economical perspective, mycosilvicultural approaches and the development of adapted forest management practices appears to be means to improve a joint production of edible ECM mushrooms and timber (Diaz-Balteiro and Romero, 2008; Savoie and Largeteau, 2011).

1.2. Fire effect in macrofungal communities and belowground ectomycorrhizal colonization

Wildfires constitutes the main disturbance in the Mediterranean forest ecosystems (Gassibe et al., 2011), where pine forests are affected approximately at a 10-40 years interval recurrence period, furthermore an increased level of fire recurrence is projected due to the actual climate change scenario an increase of fire frequency has been projected (Buscardo, 2011). *P. pinaster* is associated to a stand-replacement fire regime and historically subjected to surface fire (Fernandes and Rigolot, 2007).

P. pinaster is considered as a main colonizer after fire (López-Sáez et al., 2010) due to its pyrophytic ecology and its high light regime requirements for regeneration and growth (Gil et al., 1990) that has adapted to fire by storing its seed in serotinous cones which are opened by the heat generated during a fire the seeds so released germinating and growing rapidly as a selection pressure from fire (Johnson, 1992; Dahlberg, 2002). Also, development and coexistence of fire related traits such as bark thickness, serotiny (Tapias et al., 2004) and precocity (Santos-del-Blanco et al., 2012) is highly variable depending on *P. pinaster* populations (Tapias et al., 2004).

Fire also strongly affects soil fungi associated to these forests (Gassibe et al., 2011; Martín-Pinto et al., 2006b; Rincón and Pueyo, 2010), both as a direct result of heating and indirectly via changes to soil characteristics such as an increase in pH, the enhancement of hydrophobicity or a change of available nutrients (Certini, 2005), in which the penetration of heat through the soil during a fire is an important factor in determining post-fire plant regeneration (Bellgard et al., 1994; Rincón and Pueyo, 2010). For soil fungi, the fire severity and regimen, and especially the soil horizon they inhabit are important elements (Bastias et al., 2006; Jiménez-Esquilín et al., 2007; Kipfer et al., 2010; Ponzetti et al., 2007). During a fire, the heat is felt closest to the surface, consequently the most affected layer of soil (Cairney and Bastias, 2007; Kipfer et al., 2010), where the majority of fungal communities are located (Buchholz and Gallagher, 1982).

However, while the direct effect of fire is mainly destructive for existing fungal communities, at the same time fire also provides large inputs of new, mainly competition-free resources and substrates for fungi to use (Penttilä and Kotiranta, 1996).

Ectomycorrhizal fungi (ECM) belowground inoculum may survive fire in infected roots, as sclerotia, or as resistant spores (Baar et al., 1999; Horton et al., 1999), likely coming from deep soil horizons (Claridge et al., 2009). In these sense, the effect of fire severity in the belowground mycoflora and edaphic conditions (Rincón et al., 2014) is crucial. Survival and growth of new seedlings depends essentially on the rapid formation of an efficient root system determined by the presence of mycorrhizal organs and by ecological conditions (Jackson et al., 2007; Palfner et al., 2008), as well as the post fire plant species composition (Mediavilla et al., 2014), seed bank availability, and the recovery of soil microbial communities after fire (Buscardo et al., 2011; Rincón et al., 2014). In this sense seedling allometric features appear to be benefited by ECM fungi as reported in previous studies (Rincón et al., 2007; Sousa et al., 2014, 2012). Furthermore, soil nutrients as nitrogen and phosphorus have been linked to ECM colonization and plant growth (Dickie et al., 2006; Gleeson and Good, 2003; Sousa et al., 2011), which are affected by different fire severities (Turrión et al., 2010).

It is known that a situation of a pine stand growing on a previously treeless site corresponds to a primary succession (Visser, 1995). This succession concept could be also applied to fungal developing in stands regenerating after natural disturbance such a wildfire as early stage fungi (Martín-Pinto et al., 2006b; Visser, 1995). On the contrary the late stage fungi would correspond to mature forest stable conditions (Gassibe et al., 2011; Hernández-Rodríguez et al., 2013). This concept of fungal succession related to temporal designations such as early stage and late stage is strongly correlated to the host species, its adaptation to fire and also the resilience capacity of the fungal communities such as post fire fungi (Visser, 1995; Claridge et al., 2009). These factors will determine what kind of fungal succession (late stage or early stage) will take place. 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).

Pre-fire fungal communities are largely eradicated by fires (Vazquez et al., 1993) and subsequently post-fire fungal succession is initiated by the first significant rain that follows disturbance. The pioneer fungal species are developed in the absence of spores typically produced during the early post fire season since their source is usually the spore bank in the soil that has been built up over time since previous fire (Claridge et al., 2009). Post fire seedlings are primarily colonized by post-fire fungi whose spores survive fire impact in deeper soil horizons (Claridge et al., 2009). However, some studies report large shifts in species abundances, decreases in diversity, large losses in ECM biomass after a severe wildfire (Gassibe et al., 2011; Grogan et al., 2000; Martín-Pinto et al., 2006b; Treseder et al., 2004), where a short term fire impact induces also a reduction of fungal species richness (Kutorga et al., 2012; Mediavilla et al., 2014; Olsson and Jonsson, 2010). After less severe burns, results vary from no changes to decreases in ECM evenness, biomass, and richness (Chen and Cairney, 2002; De Román and de Miguel, 2005; Hart et al., 2005a, 2005b; Jonsson et al., 1999; Tuininga and Dighton, 2004). The presence of mycorrhizal taxa in the early stage of succession can be explained by their survival strategy (Cairney and Bastias, 2007), as mentioned above. 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).

With respect to saprotrophic fungi, there are evidences that indicate an inverse trend than for ECM fungi (Martin-Pinto et al., 2006b; Gassibe et al., 2011), in accordance with the assessment that spore germination of certain saprotrophic post-fire fungi is stimulated by heat (Dahlberg, 2002). Furthermore, saprotrophic growth pattern could allow these fungal species to survive by persisting on the dead host systems or other organic matter (Bonello et al., 1998).

As cited before, detrimental effects of fire on fungal communities is a well known fact (Cairney and Bastias, 2007; Hart et al., 2005a; Smith et al., 2005; Tuininga and Dighton, 2004). However, the fungal fruiting phase is also strongly affected by microclimatic and macroclimatic factors including soil temperatures, evapotranspiration, relative humidity, rainfall, and subsequent water deficit or excess (Büntgen et al., 2012; Martínez-de-Aragón et al., 2007). In this sense, complex predictive equations (Bonet et al., 2010; Martínez-de-Aragón et al., 2007) and simple models (Gassibe et al., 2014) can be developed in order to know how mushroom yields are recovered following fire depending on several environmental variables. Thus, the development of tools such as simple classificatory models can help to optimize forest management and increase commercial value of forests (Bonet et al., 2004). The use of linear discriminant functions (Bravo and Montero, 2001), facilitates explicit consideration of fire impact and other relevant environmental factors on mushroom productivity when planning land-use activities (Gassibe et al., 2014).

The understanding of the different factors such as fire, environmental variables and *P. pinaster* influencing fungal communities in Mediterranean region is fundamental since some of these areas are economically depressed. Furthermore, restoration through maritime pine reforestations may be an adequate intermediate stage in the recovery of the native vegetation in forests affected by fire, degraded and abandoned farmlands (Onaindia and Mitxelena, 2009; Oria-de-Rueda et al., 2010).

1.3. Objectives

The main objective of this research was to explore the relationship of fungal communities with *Pinus pinaster* growing in Mediterranean conditions subjected to wildfires under extreme environmental conditions. This general objective was assessed through the following specific goals:

I. ***P. pinaster*** **under extreme ecological conditions provides high fungal production and diversity:** ***Study I***

Fungi have become strategic in the conservation and management of Mediterranean forest systems (Fernando Martínez-Peña et al., 2012a). A deeper knowledge of the fungal communities associated with *P. pinaster* is essential for sustainable forest management focused on both forests production and conservation (Carson et al., 2010). The goal of this study was to assess the fungal communities associated to *P. pinaster* forests in Mediterranean areas contrasting in site edaphoclimatic conditions. Our specific goals included; (i) to analyze the diversity and production of fungal fruiting bodies according to functional groups within three *P. pinaster* forests sites under different edaphoclimatic conditions and ii) to assess the influence of edaphoclimatic variables on taxa composition.

II. Fungal community succession following wildfire in Mediterranean vegetation type dominated by Pinus pinaster in Northwest Spain: Study II

Edible fungi represent an important forest economic resource, occasionally generating higher benefits than timber production (Martín-Pinto et al., 2006b). These macrofungi are strongly affected by fire in the studied areas and they also play an important succession role in the regeneration of *P. pinaster* stands after wildfire (Gassibe et al., 2011; Martín-Pinto et al., 2006b). Therefore, the aim of this study was to analyze fungal community succession following wildfire in a Mediterranean vegetation type dominated by *P. pinaster* in Northwest Spain.

III. Post-fire production of mushrooms in Pinus pinaster forests using classificatory models: Study III

Due to the importance of fungal communities behavior after a wildfire, formulate simple predictive or classificatory models (Bravo and Montero, 2001), for mushrooms yields in disturbed Mediterranean ecosystems could be useful to forest managers in order to optimize the management and harvesting of these more and more appreciated non wood resource.

The aim of this study was to develop and test classificatory models for mushroom production, applied to a particular post-fire scenario in a Mediterranean vegetation type dominated by *P. pinaster* in the northwest of Spain.

IV. The effects of fire severity on ectomycorrhizal inoculum and allometric features in P. pinaster seedlings: Study IV

The importance of post disturbance interactions between early colonizing plants and ECM fungal communities has been recognized (Rincón et al., 2014; Rincón and Pueyo, 2010) but how fire severity and edaphic constraints affect pine regeneration in vigour terms, along the influence of natural (non-inoculated) ECM seedling colonization at different depths is poorly understood in Mediterranean forest ecosystems affected by fire.

Thus, the aim of this work was to assess whether ECM fungi can enhance *P. pinaster* allometric features in a burned soil affected by fire after a year period through soil natural ectomycorrhizal colonization.

MATERIAL
AND
METHODS

2. MATERIALS AND METHODS

In order to achieve the general and specific objectives of each study, four study areas were selected in Central and Northwest Spain (Fig.2).

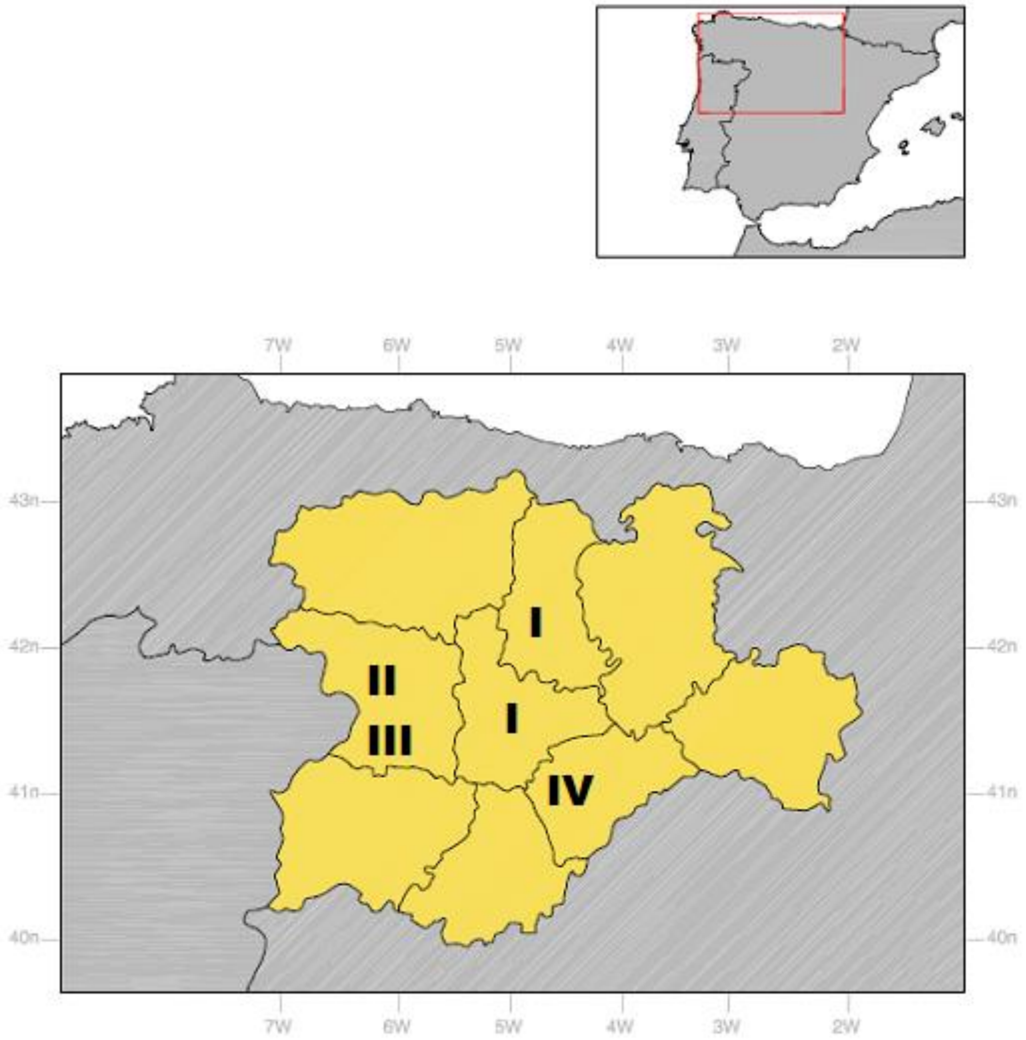

Fig. 2: Study areas of the *P. pinaster* forests. Roman numerals I, II, III and IV correspond to each Study.

2.1. Study sites (Studies I, II, III and IV)

The four studies were carried out in Mediterranean ecosystems dominated by stands of *P. pinaster* under different ecological conditions. All the studied stands in the four studies can be considered as even-aged stands of 50–60 years in the unburned sites conditions. In order to avoid different light conditions which can influence on fungal production (Clark and St. Clair, 2011), canopy cover in the studied stands was always between 70% and 80%, in the cases of non burned situations (Oria-de-Rueda et al., 2010).

In Study **I,** the analysis was carried out in three different sites, where the dominant vegetation are composed by one artificial reforestation stand and two natural stands of *P. pinaster*. These sites are located in the Palencia and Valladolid provinces (NW Spain) respectively, where Mediterranean-continental climate predominates. Nevertheless, these three sites can be distinguished according to ecological and edaphoclimatic differences (Table 1, Study **I**). The Valladolid study area is near the city of Valladolid, where two different sites can be distinguished: a) a low altitude (700 m.a.s.l.) sandy site nearby Duero Valley (Tudela del Duero) named VAS in the present study and b) a high plateau located at 890 m.a.s.l. in southeast of the province in the locality of Montemayor de Pililla (Table 1). This site is characterized by calcareous soils, hereafter called VAC. On the other hand, the Palencia (Celadilla del Río) site is located at 985 m.a.s.l. in the most elevated plateau in the north of the province (Table 1). This site is characterized by siliceous soils, hereafter named PA.

In study **II** and study **III** the analysis were carried where a large crown-fire burned 1427 hectares on August 2002. The region has a sub-Mediterranean climate with 3 months of dry season in the summer, a mean annual rainfall of 700–750 mm and mean temperatures ranging from 14.5 to 15 º C (Table 1). The soil is composed of Paleozoic metamorphic rocks, dominated by Ordovician and Silurian shales (García-Rodríguez et al., 1964), and its classification corresponds to Inceptisol suborder Xerept (Soil Survey Staff, 2006). The soils of this region are stony, acidic (pH 5.0-5.5), extremely low in calcium and phosphorus and variable in nitrogen and potassium availability. The humification degree is generally good (García-Rodríguez et al., 1964).

The area has gentle slopes that vary from 0% to 20%. Plots were selected to represent both the north and south aspects. "Late stage" or unburned site is located in an area dominated by mature *Pinus pinaster* Ait. plantations and "Early stage" or burned, is sited in an area where fire superficially affected the soil. This site is located at 750–780 m.a.s.l. in the northwest of Zamora province (NW Spain) (Table 1).

Study **IV** was performed in Central Spain, in the Autonomous Community of Castilla y León, the third European region in size and one of the most strongly damaged by wildfires according to the European Forest Fire Information System (EFFIS). A devastating crown-fire occurred in Honrubia de la Cuesta, Segovia (NW Spain) in August 2008, affecting 1200 ha of *P. pinaster* forest. This site is defined as a supra-Mediterranean climate with 3 months of dry season in the summer, where the warmest month is July and the colder January (Table 1). This area is composed of Paleozoic metamorphic rocks, dominated by Ordovician and Silurian shales (Barrenechea and Rodas, 1992). The soil is classified as Inceptisol suborder Xerept (Alvarez et al., 1993). The edaphic characteristics from the analyzed soils are shown in Table 1 of Study **IV.**

Table 1: Summarized information about the *P. pinaster* sampling sites in Central and Northwest Spain according treatments. Prec: 30 year annual precipitation; Temp: 15 year mean annual temperature.

Location	Time span	X	Y	Z (m)	Prec(mm)	Temp(ºC)
Palencia (PA)	2006-2012	354319	4719850	985	551	10,7
Valladolid (VAC)	2006-2012	381259	4593910	700	430	12,5
Valladolid (VAS)	2006-2012	357837	4605171	890	500	12
Zamora (ES)	2003-2006	706439	4632901	780	725	15
Zamora (LS)	2003-2006	706140	4632495	750	725	15
Segovia (UB)	2008-2009	444386	4592701	1135	494	11
Segovia (MSB)	2008-2009	443529	4591395	1179	494	11
Segovia (HSB)	2008-2009	442131	4591013	1190	494	11

Treatment description. (PA): siliceous soils; (VAC): calcareous soils; (VAS): sandy soils; (ES): early successional stage; (LS): late successional stage; (UB): unburned, (MSF): moderate severity fire and (HFS): high severity fire. Where, X: longitude-UTM; Y: latitude-UTM; Z: altitude.

Climatic data such as annual rainfall, mean annual temperature, mean annual soil temperature, minimum mean temperature and maximum mean temperature were provided by the closest meteorological stations in Zamora, Palencia and Valladolid for the *P. pinaster* plots located less than 15 km from the sampling plots (Table 2).

Mean monthly potential evapotranspiration (ETP) for these three studies was calculated by the empirical method of Thornthwaite and Mather (1955) for the latitude of each of the meteorological station, substituting monthly mean soil temperatures for monthly mean air temperature, since monthly mean soil temperature near the soil surface (< 10 cm) has been shown to coincide with the monthly mean air temperature. Edaphoclimatic variables for Palencia and Valladolid plots are shown in Study **I**, Table 1, whereas climatic data for the Zamora sites are shown in Study **III**, Table 1.

Table 2: Description of the meteorological stations. Data provided by Agencia Estatal de Meteorología (AEMet) and InfoRiego (IR).

Station	Location	Time span	X	Y	Z (m)	Data
Celadilla del Rio	Palencia	2006-2012	352644	4718971	986	AEMet
Cogeces del Monte	Valladolid	2006-2012	390074	4596416	887	AEMet
Finca Zamadueñas	Valladolid	2006-2012	357837	4618273	701	InfoRiego
Villardeciervos	Zamora	2003-2006	415630	617222	850	AEMet

Where, X: longitude-UTM; Y: latitude-UTM; Z: altitude.

Edaphic characteristics of Study **IV**, corresponded to nine soil samples (3 from each site), taken randomly in unburned (UB), moderate severity fire (MSF) and high severity fire (HSF) sites in the year 2009. The soil samples were taken to ITAGRA.CT (Centro Tecnológico Agrario y Agroalimentario-Universidad de Valladolid), where soil analysis were carried out (Table I, Study **IV**).

2.3. Sampling (Studies I, II, III and IV)

Randomly selected *P. pinaster* permanent plots of 2 x 50 m^2 per each site were established in accordance with previous studies conducted by Oria-de-Rueda et al. (2010) and Smith et al. (2002) in the Studies **I**, **II** and **III**. Sporocarps were collected between October and December when cold temperatures stop their emergence (Bonet et al., 2012) from the following periods: 2006–2012 (Study **I**) and 2003-2006 (Studies **II** and **III**).

In Study **I**, nine plots were selected, three by each site, which corresponded to VAS, VAC and PA respectively.

In studies **II** and **III,** twelve sampling plots were analyzed (six plots per treatment). In the early stage treatment (burned area, 1-4 years after fire), the distribution of the plots was designed to avoid direct interactions with roots from the adjacent unburned forest as much as possible. Plots in unburned areas reflected a late stage of fungal ecological succession (51-54 years old forest stand after fire). Furthermore, for modeling procedures in Study **III**, forest stand age after fire was considered as “TF” variable (Study **III**, Table 1).

In study **IV,** sampling was performed in mid-June 2009 after one year the wildfire took place. Fifteen soil intact soil blocks (22 x 22 x 20 cm) were extracted randomly at the high severity fire (HSF), moderate severity fire (MSF) and unburned (UB) sites. Fire severity was classed following Rincón and Pueyo (2010) criteria: control or unburned site was established in an adjacent *P. pinaster* forest unaffected by fire at least in the preceding 60 years, moderate fire severity with all pine crowns and upper barks burned, and high fire severity with pines, canopy and understory litter totally burned and the entire humic soil organic layer consumed.

2.4. Identification and classification. Production, diversity and richness calculations (Studies I, II and III)

During the fruiting period, all the sporocarps (both ectomycorrhizal and saprotrophic) were weekly collected and taken to the laboratory, kept at 4ºC and processed within 24 h after collection for taxonomic identification and fresh weight measurements (kg $ha^{-}1$) (Mediavilla et al., 2014). Sporocarps were dried in air-vented ovens at 35ºC/48h and then weighted in order to obtain comparable biomass data.

The sporocarps were identified at species level whenever possible through some of the following key reference books:

All of the extensive work of F.D. Calonge in the Iberian Peninsula through his publications, especially those in Sociedad Micológica de Madrid (1976-2014), Oria-de-Rueda (2007), Antonin and Noordeloos (2010), Knudsen and Vesterholt (2008), Bon (2004), Breitenbach and Krätzlin (1984; 1986;

1991; 1995; 2000; 2005) amongst others : As in previous works (Bonet et al., 2004; Martín-Pinto et al., 2006b) samples that could only be identified to the genus level were grouped into genus taxa. Data were grouped into categories (saprotrophic/mycorrhizal; edible/inedible) for further statistical analysis. Furthermore, information about mycorrhizal/saprotrophic life strategy of each taxa was obtained from various sources (e.g. Agerer, 2006; Gadd et al., 2007; Hobbie and Agerer, 2009; Taylor et al., 2003; Tedersoo et al., 2010; Trudell et al., 2004). The nomenclature source for fungal taxa names and authors used was obtained from Index Fungorum (www.indexfungorum.org) (Abrego and Salcedo, 2013).

In Study **I,** Shannon's H diversity index (Shannon and Weaver, 1949), based on dry weight of the fruiting bodies was calculated. An analysis of richness (S) (Martínez-Ruiz, 2001; Straatsma and Krisai-Greilhuber, 2003) and the similarity of the macrofungal community composition between sites was determined by calculating the Jaccard similarity index (J) (O'Hanlon and Harrington, 2012). These variables were calculated using the following formula where coefficient *pi* indicates the relative importance of each fungal species; (S) is the total number of species found and (J) is the percentage similarity of fungal assemblages between sites.

$$\mathrm{H} = \qquad -\sum pi\,(\ln pi)$$

$$\mathrm{S} = \qquad \text{number of species}$$

$$\mathrm{J} = \frac{\text{Taxa in common}}{\text{Taxa in common} + \text{Taxa only found in site } j + \text{Taxa only found in site } k} * 100$$

2.5. Mycorrhizal status and allometric measurements and statistical analysis (Study IV)

The fifteen soil blocks randomly taken from the three sites were placed into square plastic containers in a naturally lit glasshouse with controlled temperature and humidity. Twenty five *P. pinaster* seeds were used per container. All seeds were surface sterilized with hydrogen peroxide 30% for 30 min and washed tree times with sterile water. Seeds were sown into each container and tap-watered daily. No nutrients were added. To monitor potential aerial contamination of ECM fungi in the glasshouse, 5 containers of peat soil, each sown with 15 seedlings of *P. pinaster*, were used as controls. Fourteen weeks after sowing, five randomly selected seedlings were extracted from each container. Whole seedlings were wrapped with the adhering soil in moistened diary paper and stored in plastic bags for transport and storage. In the laboratory, plants were kept at 4°C before further processing up to a maximum of 2 weeks.

After completing four months in nursery conditions, each plant was rinsed with tap water in a plastic tray and gently shaken to soften and remove adhering soil. Length of tap root and shoot was measured with a ruler (Palfner et al., 2008). Dry biomass of shoots was determined after drying the plant material at 70°C during 40 h (Sousa et al., 2011). Root/shoot ratio was derived from these measurements (Palfner et al., 2008). The roots were divided into two depth segments, from 0-10 cm to 10-20 cm (Anderson et al., 2007). Subsequently, roots were separated from the shoot and cut into a sequence of sections of 50 mm length along the vertical axis. All root tips were classified morphologically and anatomically as mycorrhizal vs. non-mycorrhizal or “non vital” according to Scattolin et al. (2008), using a Leica M3 dissecting microscope according to Agerer (1987-2002, 1991) and to De Román and de Miguel (2005).

The diagnostic features of active mycorrhizal roots included the presence of a well-developed fungal mantle and intact adhering mycelial elements showing mycobiont-specific coloration, being borne on a fully turgescent root containing white cortical tissues (Montecchio et al., 2004; Palfner et al., 2005).

For each treatment, lateral roots were examined under a stereoscopic microscope at 15× magnifications to determine the ECM percentage (%M) total colonization rate for both depth levels as the number of mycorrhizal root tips per total number of root tips in an individual plant (%M) was calculated using the gridline intersect method (Brundrett et al., 1996).

2.6. Statistical analysis (Studies I, II, III and IV)

In Study **I**, fungal parameters of fungal taxa collected in the three sites were subjected to a Repeated Measures ANOVA analysis and means were compared by 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, where data on species identified to the genus level were not used for analysis because of the confusion they can cause in comparing plots (Straatsma and Krisai-Greilhuber, 2003). 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, a Canonical Correspondence Analysis (CCA) (Ter Braak, 1986) was performed to assess the effect of environmental variables on the fungal taxa assemblies of both functional groups (mycorrhizal and saprotrophic). Forward selection was used to select significant explanatory variables ($P < 0.05$). These analyses were conducted using CANOCO for Windows version 4.5 and Cano Draw 4.1. Softwares (Ter Braak and Šmilauer, 2002).

In studies **II** and **III**, fresh weights, edibility and life strategies status according (non-edible, edible, mycorrhizal and saprotrophic) and combinations such as edible mycorrhizal (EM), inedible (IE), edible saprotrophic (ES) and inedible saprotrophic (IES) were analyzed statistically and also used as dependent variables for modeling purposes. Data were subjected to a one-way ANOVAs and means were compared by LSD Fisher Tests ($P<0.05$). STATISTICA '08 Edition software (StatSoft Inc., 1984-2008) was used for the analysis.

In study **III**, each dependent variable (sporocarp production), was grouped into four yield categories in kg fw ha^{-1} according to our previous fungal yield data in Study **II** (Study **III**, Table 2). A simple classification model was obtained for each of the dependent variable using a classificatory discriminant analysis. A constant (K), time following fire (TF) and climate variables were the independent variables used in the models. Only simple and available variables (Study **III**, Table 1) from the meteorological station that contributed significantly in an ecological way to the overall model of sporocarp production were kept ($P < 0.05$) and used to classify the productions into the four yield cited categories.

When modeling fungal production, the discriminant rule was used when estimation by means of annual sporocarp production was not secure, for instance in a post-fire Mediterranean ecosystem. This discriminant rule allows classify observations into different production groups (Bravo and Montero, 2001).

Nine discriminant classificatory models were studied to determine these four yield categories within each dependent variable according to edibility and functional group. The structure of the general discriminant rules is:

$$\beta_0 + \sum \beta_1 X_i$$

Where:

- $\beta_0, \beta_1 \ldots \beta_n$ are parameters.
- X_is are "TF" and climatic variables, where mean values of the climatic variables were set as constants and only time after fire was modified.

In order to define and evaluate the classification criteria, original database was used to cross validate the model. The resulting error-count estimate has an optimistic bias and is called an apparent error rate. In order to reduce this bias, cross validation was used (Bravo and Montero, 2001). Therefore classificatory discriminant analysis was applied to know the yield category (Study **III**, Table 2) based on independent variables such as time following fire (TF) and climatic variables (Study **III**, Table 1). The original dataset was used to define and evaluate the classification criteria. The PROC DISCRIM procedure from SAS 9.1 was used.

In study **IV**, data were tested for normality and subjected to a Repeated Measures ANOVA; means were compared by LSD Fisher Tests ($P < 0.05$). STATISTICA '08 Edition software (StatSoft Inc., 1984-2008) was used for the analysis. A Principal Component Analysis (PCA) was also performed to assess the associations between allometric variables, soil parameters, severity, and mycorrhizal percentage colonization in both seedling root depths to examine for potential relationships. Data were determined using the Pearson rank-order correlation coefficient (R Development Core Team, 2011).

RESULTS

3. RESULTS

3.1. Fungal richness, diversity and production (Studies I, II and III)

P. pinaster Mediterranean forests showed to provide adequate ecological conditions for high fungal richness and production reaching 240 taxa as a total during the whole study period in the three different studied areas from Castilla y León (NW Spain) region (Tables 2,1 and 3 respectively shown in Studies **I**, **II** and **III** respectively). To get a more detailed knowledge of fungal communities associated to *P. pinaster* forests under different ecological conditions, taxa were classified according to functional groups (mycorrhizal and saprotrophic) and edibility in the studied areas. In this sense, a total of 194 taxa were found for Study **I** in the 9 sampled plots (Table 2; Study **I**,) from the three sites. Whereas fungi were classified into 115 different taxa (Studies **II** and **III,** Table 1 and Table 3 respectively). There was a taxa dominance of saprotrophic fungi over mycorrhizal in the three studies. From the total taxa list in Study **I**, seventy nine can be identified as mycorrhizal and 115 as saprotrophic fungi. On the other hand, fifty six can be identified as mycorrhizal and 59 as saprotrophic fungi (Studies **II** and **III**).

These differences in richness can also be appreciated when comparing the treatments within the studies. In the sites from Study **I**, undisturbed forests, sporocarps collected from Palencia, were classified into 103 different taxa (35 mycorrhizal; 68 saprotrophic). On the other hand, 87 taxa occurred in VAS plots (40 % mycorrhizal and 60 % saprotrophic). The highest number of total taxa was found in VAC plots where 108 taxa were collected. Forty three were classified as mycorrhizal and 65 as saprotrophic. On the other undisturbed situation (late stage successional stage treatment) as the one from Zamora of Studies **II** and **III**, 85 taxa were harvested (49 % mycorrhizal and 51 % saprotrophic).

In Study **I,** Fungal richness was significantly different when comparing mean values found in the three sites, where values in PA and VAC were significantly higher than those observed in VAS plots (Fig. 2a; Study **I**, $P < 0.05$). Similar results between PA and VAC plots were observed analysing richness and Shannon's H´ Diversity index for saprotrophic and mycorrhizal species. Within the three locations values for saprotrophic fungi were always higher than mycorrhizal regarding richness and diversity. However, no differences were found comparing mean total values for Shannon's H´ diversity index (Figs. 2a, 2b; Study **I,** $P > 0.05$). Whereas Study **II**, fungal richness varied across the chronosecuence with an increasing trend (Table 2; Study **II**).

In Study **I**, Jaccard similarity coefficients showed that VAS and VAC were the most similar sites, with 46 taxa in common. When comparing mycorrhizal and saprotrophic taxa, those sites had 16 and 31 taxa in common respectively, showing again the highest coefficient between VAS and VAC (Table 3; Study **I**). In Study **II**, 30 species were found to be common in early stage and late stage successions (Table 2; Study **II**).

Regarding taxa composition, fungal community assemblies along the three locations in Study **I** can be analyzed from the results obtained in the two canonical correspondence analyses (CCAs) (Table 4; Study **I**). The results of both CCAs are displayed in two ordination biplots (Figs. 4a, 4b; Study **I**). Where a total of 10 edaphoclimatic variables were significant (P < 0.05) in the ordination of mycorrhizal (7) and saprotrophic (10) taxa dry weight according to the forward selection process with seven variables in common (Table 5; Study **I**). For mycorrhizal taxa, Axis 1 was positively correlated with edaphic and climatic variables such as N, P, K, precipitation and temperature. Axis 2 was correlated with ETP, and a negative correlation was observed for precipitation values during fructification period, (Table 5; Fig. 4a; Study **I**). For saprotrophic taxa, similar trends were observed for Axis 1. However, Axis 2 was positively correlated with the precipitations values during fructification period (Fig. 4b; Study **I**, P < 0.05).

CCAs from Study **I** revealed that there are clear differences between fungal communities in *P. pinaster* sites (Figs. 4a, 4b; Study **I**). The two Axes explain an accumulative variance for the interaction between taxa and environment of 22.8% and 16.5% for mycorrhizal and saprotrophic groups respectively.

Regarding total sporocarp production, 447.31 kg fw ha^{-1} was collected in Study **I**, where 192.7 kg fw ha^{-1} was from PA treatment, 167.66 kg fw ha^{-1} from VAC, decreasing to 87.32 kg fw ha^{-1} in VAS treatment (Fig. 3; Study **I**). Whereas Studies **II** and **III,** reported a total production of 372.4 kg fw ha^{-1} where 209.95 kg fw ha^{-1} corresponded to the undisturbed treatment or late successional stage. While no differences were found comparing production among treatments for mycorrhizal species in Study **I**, lowest values were found for saprotrophic fungi in VAS, significantly different than those found in PA sites (Fig 3; Study **I**, P < 0.05).

Regarding edible species, in Study **I** yields were significantly different among the three studied forests where 50 of the total taxa found were edible (Table 2; Study **I**). On the other hand, undisturbed forests from Studies **II** and **III**, showed that thirty of the total taxa found were edible; nine of them are marketed in the region (Table 1; Study **II** and Table 3; Study **III**).

In Study **I**, when comparing productions amongst sites, the highest productions were observed in PA and VAC sites (67.8 and 43.3 kg fw ha^{-1} respectively). This same trend was observed regarding edible mycorrhizal species. PA and VAC sites showed the highest values (31.3 and 29.2 kg fw ha^{-1}), while production in VAS was the lowest (2.3 kg fw ha^{-1}). Finally, highest values for production of edible saprotrophic species, were recorded in PA (36.7 kg fw ha$^{-1)}$, and the lowest in VAS (4.3 kg fw ha^{-1}) (Fig. 3; P < 0.05).

In study **II,** the production for edible mycorrhizal species was significantly higher than that observed for edible saprotrophic ones (3.71 kg fw ha^{-1}), reaching its maximum of 61.57 kg fw ha^{-1} corresponding to 23 taxa (Study **II**, Table 2).

3.2. Fire effect in macrofungal communities and belowground ectomycorrhizal colonization (Studies II, III and IV)

In Studies **II** and **III**, 162.45 kg fw ha^{-1} were collected in the early stage (burned) treatment when compared to 209.95 kg fw ha^{-1} of late stage (unburned) one during the 4-year study period. Furthermore, 60 of the taxa were harvested in early stage treatment, 25 species less than in the late stage, of which 30 were found to be common in early stage and late stage successions. In early stage, 45 % mycorrhizal and 55 % saprotrophic taxa occurred. Twelve are edible and 3 of them are marketed. Mycorrhizal species were significantly affected by wildfire since they decreased from 43 in the late stage 33 in the early stage. On the other hand, saprotrophic fungi showed a similar trend decreasing from 44 in the late stage to 33 in early stage (Table 2; Study **II**).

Mycorrhizal species production was significantly affected by wildfire since decreased from 36.23 kg fw ha^{-1} in the late stage to 7.54 kg fw ha^{-1} in the early stage (Fig. 1; Study **II**). On the other hand, saprotrophic fungi showed a contrary trend increasing from 7.50 kg fw ha^{-1} in the late stage to 26.23 kg fw ha^{-1} in the early state (Fig. 1; Study **II**, $P < 0.05$).

Regarding edible species, in the burned treatment there was a decrease from 32.64 kg fw ha^{-1} in the late stage to 0.34 kg fw ha-^{1} (Fig. 2; Study **II**). For their importance as they form appreciated edible sporocarps which are marketed in the studied area (Study **II** and **III**), were especially impacted by fire. The following species had significantly lower production weights following the fire: *Chroogomphus rutilus*, *Hydnellum concrescens*, *Laccaria laccata*, *Lycoperdon molle*, *Hygrophorus gliocyclus*, *H. agasthomus*, *Lactarius deliciosus*, *Stereum hirsutum*, *Tricholoma fracticum* were carried out in both successional states. Their behaviour was common and their fresh weight productions decreased significantly following wildfire. A contrary trend was also appreciated in non edible fungi for both mycorrhizal and saprotrophic taxa.

In study **III**, a simple classification model was obtained for fungal yield using a classificatory discriminant analysis. A constant (K), time following fire (TF) and climate variables were the independent variables used in the models. K and TF were used in the nine models obtained. T MIN; T MAX; PREC and ETP were used in the total and edible mycorrhizal production models. T and ETP were used for inedible saprotrophic production models. ETP was used for inedible, saprotrophic and edible saprotrophic and inedible mycorrhizal ones. And finally, T and PREC were the variables involved in the mycorrhizal and edible species production classificatory model (Table 4; Study **III**).

For edible species production model, the correct classification is 100 % in classes I, II and IV. Also, no errors were observed for edible mycorrhizal production in classes I and II. In the model for saprotrophic species, the best classifications are observed for classes I and IV where error rates varied from 0.23 to 0.25 respectively. Similar trend was observed for mycorrhizal species where model showed adequate classifications for classes I and IV (error rates: 0.08 and 0.21). Total production model showed a total correlation R^2 of 0.65 (Study **III**, Table 5). In this case, correct classification is from 52.94% in the class III to 80% in the class II.

In Study **III**, the production classificatory models for functional groups (mycorrhizal and saprotrophic) showed a strong detrimental predicted effect of fire on mycorrhizal species. Indeed, production decreased to the minimum (production class IV) during the first 4 years after fire, whereas highest production class was reached for these species in the unburned plots according to the models (Table 7; Study **III**). A contrary trend was observed for the saprotrophic group. Thus, production was classified into class II for areas affected by fire while production was predicted to decrease to class IV in the unburned areas (Table 7; Study **III**). In addition, predicted time after fire effect on fungal production was analysed depending on edibility. Edible fungal group production decreased to class IV during the first and second years after fire and reached the highest production (class I) thereafter. Conversely, non-edible fungal species showed a contrary trend, with a post-fire production predicted to be class I and a sudden decrease to class IV after the two first years until the end of the modelled time period.

In Study **IV**, the effect of fire on belowground ECM was assessed relating ectomycorrhization and plant development according to different fire severities and soil properties (Table 1, Study **IV**) where sseedlings from high severity fire (HSF) soils reached the highest growing rates for shoot dry weight (Fig. 1; Study **IV**, $P < 0.05$). Whereas for the root system, significantly differences in length were observed among the three bioassays in which HSF treatment reached a greater value in root length (33 cm) than moderate severity fire (MSF) and unburned (UB) bioassays (Fig.1; Study **IV**, $P < 0.05$). Consequently, the seedlings mean root/shoot ratio from the both post-fire soils were significant higher (Fig.1; $P < 0.05$) than for the plants growing in UB substrate.

The percentages total mycorrhization in the seedlings from the both post-fire treatments were significantly lower than the one from UB soils ($P < 0.05$) which are presented in Study **IV**, Fig. 2.

In relation to depth, the three treatments indicated significant higher levels of mycorrhization ($P < 0.05$) in the upper layer (0-10 cm) than the below one (Fig. 2; Study **IV**). Differences among treatments at the upper level (0-10 cm), indicated a higher colonization rate in the control treatment when comparing it both of the burned treatments (Fig. 2; Study **IV**, $P < 0.05$). Same trend was followed by colonization in deeper layers (10-20 cm), but in this case no differences were observed between seedlings from MSF soils comparing to the other two bioassays.

The PCA performed on the soil characteristics and seedling vegetative variables of the three treatments showed that samples from HFS and MFS sites were grouped in the positive area of Axis 1 whereas UB ones are located in the negative area (Fig. 3; Study **IV**). The two axes explained 68% of the variation present in the samples (50% axis 1 and 19% axis 2). The corresponding eigenvalues were 8.2 and 3.4 respectively. Unburned soil seedlings appear associated with higher values of Organic Mater (OM) which was not affected by fire as in HFS and MFS sites. Furthermore a positive correlation is also shown for N, Ca and CIC with seedlings at UB treatment, but negatively to pH, Mg, Na and Conductivity which are positively correlated to the both burned (HFS and MFS) bioassays.

DISCUSSION

4. DISCUSSION

4.1. Fungal richness, diversity and production (Studies I, II and III)

Despite extreme and highly variable environmental conditions and the detrimental effects of fire, fungal communities associated with stands of *P. pinaster* are highly diverse. In this study, a total of 240 fugal taxa were collected. In comparison to other studies regarding similar Mediterranean pine species, the number of fungal taxa reported here is among the highest reported. Thus, Mediavilla et al. (2014) found a total of 91 different fungal taxa were collected in a single autumn season in forests dominated by *P. nigra* in similar conditions and area as in our study. A total richness of 119 taxa was recorded for *P. sylvestris* forests in a long term inventory in the inner northeast zone of the Iberian Peninsula (Martínez-Peña et al., 2012b). For this same species, a similar figure was found by Bonet et al. (2004), with 164 taxa in central Pyrenees. However, Martínez-de-Aragón et al. (2007) recorded only 46 taxa in pine forests of the pre-Pyrenees mountains. A lower richness value was also reported by Oria-de-Rueda et al. (2010) in *P. Sylvestris* forests in northwest of Spain, where 43 taxa were collected. Also, lower richness was found in *P. halepensis*, which is typical species present in Iberian Peninsula. For this species, Oria-de-Rueda et al. (2010) and Martínez- de- Aragón et al. (2007) found 16 and 35 taxa respectively. Furthermore, our results showed a higher richness than was observed in other *P. pinaster* studies Fernández-Toirán et al. (2006) collected 60-80 taxa in a 15 ha *P. pinaster* natural stands through a 4 and 6 year sampling in the northwest of Spain. Oria-de-Rueda et al. (2010) also found lower richness values, recording 49 taxa in a reforested 50-year old *Pinus pinaster* growing on degraded and eroded soils in north-western Spain. Richness observed in our study was also higher than the one reported by Martín-Pinto et al. (2006b), who recorded 39 taxa in a Mediterranean ecosystem dominated by *P. pinaster*.

The high richness values reported in this study can be partially attributed to the different edaphoclimatic characteristics of the four undisturbed locations included in our study. The elevated level of genetic variability in *P. pinaster*, due to an important genotype by environment interaction, has enabled this species to adapt to these extreme ecological conditions (Alía et al., 1996). *P. pinaster* can be specially adapted to high temperatures and summer-time droughts, but it is also present in areas characterized by mild temperatures and high precipitation values. In this sense we appreciate that *P. pinaster* populations have the capacity to shift their habitat range from Mediterranean to pre Eurosiberian locations (Ruiz-Labourdette et al., 2012). This plasticity could explain its compatibility with a wide range of fungal symbionts, and therefore significant fungal production and diversity observed in this study (Gassibe et al., 2011; Martín-Pinto et al., 2006b; Mediavilla et al., 2014; Oria-de-Rueda et al., 2010).

Regarding to functional groups, we found an overall ratio of about 1:2 between mycorrhizal and saprotrophic fungal species as shown in the three studies. Roberts et al. (2004) observed a similar result in six habitats in Vancouver Island, British Columbia over a five years period, whereas Straatsma et al.

(2001) found that there were about twice as many ectomycorrhizal as saprotrophic fungi in a Swiss forest formed by a mixture of deciduous trees and conifers. Other studies report higher percentages of mycorrhizal species, such as Bonet et al. (2004) with 88% of mycorrhizal taxa or Väre et al. (1996) with 59%. These studies were carried out on natural *P. sylvestris* stands in areas where optimal ecological conditions for the growth of this species are observed. Thus for *P. nigra,* Mediavilla et al. (2014) found 47 % of mycorrhizal taxa in Northern Spain.

Poor and degraded soils present in the studied area could explain the low presence of mycorrhizal species related to the saprotrophic ones as those observed in a previous research where fungal richness in poor soil conditions were also studied (Oria-de-Rueda et al., 2010). The relatively high proportion of saprotrophic compared to mycorrhizal fungi observed could be due to the presence of high amounts of organic matter in the forests, since decomposition rates are particularly low in Mediterranean ecosystems where no coincidence of high precipitations and high temperatures is observed (Oria-de-Rueda et al., 2010), furthermore *Pinus* stands are frequently associated with low decomposition rates (Senn-Irlet and Bieri, 1999), while high rates are more often found in deciduous forests of more humid regions (Oria-de-Rueda et al., 2010). Organic matter is likely to influence fungal communities through its impact on soil-moisture content and water-holding capacity (Ferris et al., 2000). Thus, higher amount of accumulated organic matter is observed in the forest soil and such conditions enhance the fruiting of saprotrophic fungi (Straatsma et al., 2001).

Taxa from both functional groups had been linked to various ecological amplitudes, whilst others are site specific, further more termophilous and hygrophilous species have been observed (Oria de Rueda et al., 2007). Moreover, climatic variables do not fully explain the presence of sporocarps (Barroetaveña et al., 2008; Egli, 2011). This is in accordance with the results observed in fungal taxa composition from Study **I**, where soil nutrients such us N and K were linked to it as previously reported by Natel and Neumann (1992). The influence of nitrogen on fungal richness distribution patterns was also previously reported. In those studies, sporome distribution showed a large degree of community specialization along the soil quality gradient (Reverchon et al., 2012, 2010).

Regarding mycorrhizal taxa, CCA from Study **I** showed a significant influence of higher nitrogen content in the siliceous site as previously showed by Avis et al. (2003). This fact may be attributed that this site is an artificial reforestation in an abandoned farmland, thus an increment of nitrogen can influence formation of mycorrhizas, production and distribution of extra radical mycelium in the soil, and sporocarp formation (Trudell and Edmonds, 2004). Furthermore, other authors noted that fungal communities can be adapted to more nitrogen-rich sites (Kranabetter et al., 2009; Toljander et al., 2006). Mycorrhizal taxa also showed a significant influence of phosphorous, which was positively correlated with the plots where mycorrhizal yields were also higher. It must be noted that some previous studies reported that high P availability can increase ECM infection (Cairney, 2011; Wallander, 2000).

On other hand, our results on saprotrophic taxa distribution revealed that some species are positively correlated with the high precipitation in siliceous habitats, which could be considered as the saprotrophic hygrophilous species (Oria de Rueda et al., 2007), whereas thermophilous species are highly correlated with rising temperatures and ETP as shown for the sandy and calcareous sites. Also, soil nutrients such as N and K affected the taxa composition of this group, specifically in the siliceous site. Higher nitrogen contents were linked to a greater abundance of the most nitrophilous species. Especially in the siliceous site, where fungal mycelium has the physiological potential to act as both an expandable reservoir and a distribution system for elevated nitrogen inputs (Watkinson et al., 2006), and may account for the responsive nitrogen absorption of the forest floor.

Climate variables affecting the fungal composition of Study **I**, related to precipitations and temperatures were also correlated to fungal yields according their functional groups as seen in the models of Study **III**, in which these variables are responsible in different degrees for mushroom yield. As noted in previous studies, climatic variables can explain 60 % to 80 % of the mass of mushrooms produced (Bonet et al., 2010; Pinna et al., 2010).

Total average fresh weight production recorded was considerably high when analyzing the average from the four undisturbed situations (328 kg fw ha^{-1}). Sporocarp productions are often very irregular over the different pine stands as reported by Mediavilla et al. (2014), where 44.7 kg ha $^{-1}$ were found in an undisturbed *P. nigra* under a similar study area.

Similar values were previously referenced by Martín-Pinto et al. (2006b), with an average plot yield of 332.7 kg fw ha^{-1} under similar tree age class and stand conditions. Our yield results were also similar than those found by Ágreda et al. (2014) who reported 330 kg fw ha^{-1} for different *P. pinaster* age classes in Mediterranean ecosystems in northwestern Spain. The average high production found in our study may be attributed to the very different edaphoclimatic conditions which characterize these habitats. Some previous studies link mushroom fructification to habitat characteristics (Bonet et al., 2010) and climate conditions (Büntgen et al., 2012), mainly soil moisture and temperature (Barroetaveña et al., 2008; Martínez-de-Aragón et al., 2007; Pinna et al., 2010). Thus, in the case of our sampled forests, *P. pinaster* is able to form fungal associations with a large number of different mushroom taxa adapted to the broad ecological conditions providing higher yields.

When comparing different soil characteristics which affect fungal yield (Kennedy and Peay, 2007; Nordén et al., 2004), the sandy soils from Study **I** reported a decrease in yield. The lower fungal production found in sandy soil environments can be explained by the substrate, since these sites are considered to be under continental dune environment with specific soil properties.

Moreover, soil moisture is an important factor in sporocarp production by macro fungi, and it also plays a key role in the productivity of mycorrhizal fungal communities (O'Dell et al., 1999). Higher precipitation could explain higher yield values, while that increase in ETP suggests lower soil humidity and fungal production (Trudell and Edmonds, 2004).

4.2. Fire effect in macrofungal communities and belowground ectomycorrhizal colonization (Studies II, III and IV)

Adverse effects of fire on the number of macrofungal species was observed, although functional groups were differently affected (Studies **II** and **III**). The decrease of many species of macrofungi after fire is a well-known fact (Hernández-Rodríguez et al., 2013; Martín-Pinto et al., 2006b; Mediavilla et al., 2014). In this sense, fire can dramatically change surface soil characteristics and erosion rates causing significant effects on soil microorganisms in general (Turrión et al., 2012, 2010). Furthermore, vegetation mortality can result in large changes in the mycorrhizal community because of the elimination of the host (Dahlberg, 2001). Thus, immediately after fire a deep decrease of fungal richness is observed since some fungi are not capable of growing in the new conditions. In this sense, burning of the vegetation can affect the spore population and the infection potential of some fungal species (Vilariño and Arines, 1991), and subsequent loss of topsoil by erosion also reduces infectivity of fungal propagules (Rashid et al., 1997). However, the presence of mycorrhizal post fire taxa that appeared immediately after fire can be explained due to its resistance structures such as infected root tips, sclerotia or resistant spores (Cairney and Bastias, 2007; Grogan et al., 2000).

On the other hand, saprotrophic fungal richness was not significantly affected. These species are adapted to the environmental conditions created by short-term increase of fertility in the soils following fire (Turrión et al., 2012; Egli et al., 2010). Also, saprotrophic species showed significant higher yields in the burned treatment, since certain saprotrophic post-fire fungi are stimulated by heat (Dahlberg, 2002).

Despite the inmediate effects of fire on fungal communities, a progressive increase in the fungal richness is observed after time, showing the adaptation of the fungal communities to the new vegetation and environmental conditions following fire (Visser, 1995). Our study revealed a higher mycorrhizal richness across the forest chronosequence as previously observed by Fernández-Toirán et al. (2006). In this sense, there are some fungi "post-fire fungi" that appear to be benefited from this disturbance since they fruit as a result of fire (Hart et al., 2005b). Their source is usually the spore bank in the soil built up over time following fire and can retain viability for many years. Some fungal spores are strongly dormant and require heat to germinate (Claridge et al., 2009; Izzo et al., 2006). This fungal species normally fruit during the first significant rain event following disturbance and they are not the product from spores dispersed during that season (Claridge et al., 2009).

In study **IV**, the burned soils showed reductions in nutrients as N, Mg and Ca. Clark and St. Clair (2011) studied late successional conifer stands with a fire history in central Utah, USA, and they also reported similar results. Fire drives the conversion of organic into inorganic forms of nitrogen that can be more easily lost by volatilization, direct leaching, erosion or denitrification, inducing the reduction of total nitrogen content in soil along the time (Certini, 2005). Also, fire can reduce surface and soil organic matter content, along with influencing nitrogen availability as previously found by Turrión et al. (2012).

With respect to mycorrhizal colonization**,** our results revealed a significantly decrease in the most recently burned site. This is in agreement with previous findings by Sousa et al. (2011) who studied inoculation of *P. pinaster* seedlings in burned and unburned soils. Rincón et al. (2014) also found similar results. They studied mycorrhizal colonization in early regeneration seedlings growing in burned and unburned *P. pinaster* forest in central-east Spain three years after a fire. ECM colonization decreasing was also reported by De Román and de Miguel (2005), who found a decreased colonization percentage at the 10 cm level in burned soils when compared to an unburned plot in *Quercus ilex* forest located in northern Spain. Other authors found similar results (Kipfer et al., 2011; LeDuc et al., 2013).

Dickie et al. (2006) described that seedling colonization was related with edaphic characteristics such as soil nitrogen, soil calcium, and soil cation exchange capacity at 0- to 20-cm root depth. Similar results were obtained by Scattolin et al. (2008) who found that ECM community structure was strongly correlated with soil characteristics especially with pH and nitrogen contents. Our results showed that nitrogen could be responsible for the higher levels of mycorrhization on the unburned treatments as reported previously by Kranabetter et al. (2009). Regarding root seedling depth colonization our study showed significant higher levels in the upper horizon. This is due to the fact that the mycelium of ECM fungi is usually most abundant in the superficial organic soil layers (Neville et al., 2002). There are some evidences that mycelium of ECM basidiomycete communities are vertically stratified in soil, and that upper organic soil layers and underlying mineral soil support distinct fungal communities (Anderson et al., 2007; Koide and Dickie, 2002; Scattolin et al., 2008). The ability of a plant to decrease colonization, in high nutrient settings as seen in the post fire treatment with certain macronutrients, indicates that the host plant may have substantial control over both root colonization and ECM species interactions (Johnson et al., 1997).

Plant development appeared to have been enhanced as a consequence of the high severe fire. Similar biometric results have been found by Pausas et al. (2003) in *P. halepensis* seedlings in eastern Iberian Peninsula under three fire severity classes. In that case, allometric features were increased by high severity fires. In our study, a short-term increasing level of P was observed in the high severity fire treatments and this would affect the allometric enhancement of seedlings in the post burned treatment as previously found (Fierro et al., 2007).

CONCLUSIONS

5. CONCLUSIONS

The following conclusions can be drawn from the studies that compose this thesis:

1) Fungal richness, diversity and production (Studies I, II and III):

- Despite extreme and highly variable environmental conditions, fungal communities associated with stands of *P. pinaster* are highly diverse and productive. This is partially due to the great plasticity of this forest species to different environmental conditions and also to the ability of fungal species to fruit under harsh ecological context.
- Species richness was greater for the saprotrophic group than the mycorrhizal one in the undisturbed stands. On the contrary, fungal yield had an inverse trend, where mycorrhizal fungi reached the highest values.
- The areas of the calcareous and siliceous soils have a higher overall mycological taxa richness, diversity and yield than in the sandy inner dune, since higher precipitations and best soil conditions favored the development of both vegetation and fungal communities.
- Some of the fungal taxa can be classified according to specific edaphic and climatic conditions, in terms of fungal assemblies: hygrophilous, nitrophilous, xerophilous, and termophilous species. Whereas some other taxa showed broader ecological amplitudes.
- An integrated forest management aimed to combine timber and mushroom production would result in economic and ecological benefits, especially in these depressed areas.

2) Fire effect in macrofungal communities and belowground ectomycorrhizal colonization (Studies II, III and IV):

- Very high fungal richness was found in the *P. pinaster* studied forests, despite the detrimental effects of fire.
- After a fire, mycorrhizal and saprotrophic fungal productions were differently affected. Mycorrhizal mushroom yields decreased after fire in contrast to the trend recorded for saprotrophic taxa.
- Edible species production was affected by fire and would significantly decrease the potential of rural populations to collect mushrooms to sell in the market place. Since this is an economically depressed area, this reduction after a fire impact regional livelihoods.
- Following fire, fungal succession can be observed. Thus, post-fire, super-pioneer, pioneer and late stage species were found.

- Classificatory simple models allow estimation of edible fungal production classes in *P. pinaster* stands in Northwest Spain after fire. The utility of the defined models for operational foresters is really interesting in these marginal Mediterranean ecosystems where wildfire is one of the major disturbance factors.
- Fire severity affected soil properties and decreased seedling mycorrhizal colonization. Plant development was enhanced after fire. This was likely due to post fire nutrient deliverance in the forest soil rather than ECM colonization.

The declines in the availability of edible mycorrhizal fungi and increased demand have encouraged research into developing methods for the sustainable productivity in natural forests, and this forest management can be useful as a tool for wildfire prevention. Furthermore, in the studied areas, as well as in many Central European countries, the commercial value of forests could be enhanced through mycosilvicultural programs. Therefore, conservation of existing edible mycorrhizal fungi ecosystems is an urgent matter, particularly in developing countries and in this sense this study may provide information to local forest managers.

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6. REFERENCES

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STUDIES

Study I: *P. pinaster* under extreme ecological conditions provides high fungal production and diversity.

Study II: Fungal community succession following wildfire in a Mediterranean vegetation type dominated by *Pinus pinaster* in Northwest Spain.

Study III: Post-fire production of mushrooms in *Pinus pinaster* forests.

Study IV: The effects of fire severity on ectomycorrhizal inoculum and allometric features in *P. pinaster* seedlings.

STUDY I

P. pinaster under extreme ecological conditions provides high fungal production and diversity

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P. pinaster under extreme ecological conditions provides high fungal production and diversity

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Abstract

The goal of this project was to study how fungal communities in Mediterranean forests dominated by *Pinus pinaster* are influenced by different edaphoclimatic conditions, characterized by three different edaphoclimatic sites composed of siliceous, calcareous, and sandy soils. Sporocarps were collected and identified from nine 100 m^2 permanent plots, three in each site, during the autumn seasons from 2006 to 2012. The data collected were used to assess fungal productivity and diversity.

Fungal community composition was mainly correlated with climatic variables, such as precipitation and temperature. Additionally, soil nitrogen and potassium significantly influenced the distribution of species for both saprotrophic and mycorrhizal functional groups. Some fungi such as *Lycoperdon perlatum*, *Russula torulosa*, and species within the genera *Galerina* and *Mycena* were adapted to a broad range of ecological conditions. Others were found only in very specific environmental conditions. All species within *Macrolepiota* were collected in the calcareous soils, whereas *Laccaria laccata* and *L. bicolor* were exclusively associated with higher amounts precipitation and nitrogen in the siliceous plots. These findings have ecological implications that are important for managers seeking new options for adding economic value and for sustaining biological diversity in these Mediterranean forests.

Key words: fungal production, fungal diversity, environmental variables, Mediterranean ecosystems

1. Introduction

Pinus pinaster Ait. forests within the Mediterranean are of great economic importance (Majada et al., 2010). In Spain, this species occupies nearly 1.7 million ha, 0.6 of which were established through direct planting (Alía et al., 1996), making it the second most important tree species by surface area in Spain (Rodríguez et al., 2008). The distribution of the species across the Iberian Peninsula is patchy. It comprises a broad spectrum of substrates (limestone, granite, schist, marly limestone, peridotite), topographies and climates, ranging from montane sub-humid to Mediterranean semi-arid with summer drought (López-Sáez et al., 2009). *P. pinaster* can form closed forests that are either monospecific, or a mixture of different evergreen and deciduous trees, with a wide range of understory species (Blanco et al., 1997).

This species is considered as a main colonizer after fire (López-Sáez et al., 2009) due to its pyrophytic ecology and its high light regime requirements for regeneration and growth (Gil et al., 1990). Consequently, it has been used widely in the reforestation of infertile, sandy, and slightly acid soils (Barčić et al., 2006; Oliveira et al., 2012). Its success in colonizing and establishing on disturbed soils and former agricultural fields may be attributed in part to its compatibility with a wide range of fungal symbionts (Carson et al., 2010; Gassibe et al., 2011; Oria-de-Rueda et al., 2010). Due to these relationships, artificial and natural stands of *P. pinaster* have the potential to support significant fungal production and diversity (Bonet et al., 2012; Fernández-Toirán et al., 2006; Martín-Pinto et al., 2006b; Oria-de-Rueda et al., 2010).

Fungi play an essential ecological role in these forest communities, where mycorrhizal associations have significant effects on nutrient and water uptake, growth and plant survival (Brundrett, 2009; Courty et al., 2010), improving soil aeration and porosity (Fernández-Toirán et al., 2006), resistance to pathogens (Martín-Pinto et al., 2006a), and also provide a food source for many organisms (Sato et al., 2012). Saprotrophic fungi are essential for decomposition of dead matter, and therefore nutrient cycling in forest ecosystems (Ferris et al., 2000).

Sporocarp formation is influenced by various factors, such as host specificity (Molina et al., 1992), physiological conditions, nutritional status of the mycelium (Murat et al., 2008), and environmental factors (Bonet et al., 2004; Luoma et al., 1991; O'Dell et al., 1999; Smith et al., 2002).Climatic variables are especially important because they explain 60 % to 80 % of the variation in mycorrhizal fungi production (Dahlberg, 1991; Luoma, 1991; Luoma et al., 1991). Specifically, the development of fungal fruit bodies is dependent on the availability of surface water and soil temperature (Bonet et al., 2010; Egli et al., 2010; Pinna et al., 2010). But despite the widely recognized importance of these two factors few studies have actually been conducted to address them (Richard et al., 2004; Straatsma et al., 2001). Additionally, fungal species composition is strongly determined by soil chemical properties (Straatsma et al., 2001). In particular for saprotrophic fungi, which are more dependent upon their respective substrates than mycorrhizal fungi (Reverchon et al., 2010).

Fungi have become a strategic component in the conservation and management of Mediterranean forest systems (Martínez-Peña et al., 2012a). Apart from the ecological importance of fungi, much of the recent interest in mushrooms comes from their recognized economic value (Cai et al., 2011). During the last decade, there has been a sharp increase in the demand for edible fungi and in the marketing and distribution of these products (Pettenella et al., 2007), which are becoming an important source of rural income. A deeper knowledge of the fungal communities associated with *P. pinaster* is essential for sustainable forest management focused on both timber production and forest conservation (Carson et al., 2010). The goal of this study was to assess the fungal communities associated with *P. pinaster* forests in Mediterranean areas contrasting in site conditions. Our specific goals included; (i) to analyze the diversity and production of sporocarps according to functional groups within three *P. pinaster* forests subject to different edaphoclimatic conditions and ii) to assess the influence of edaphoclimatic variables on taxa composition.

2. Materials and methods

2.1. Study sites and plots

The study was carried out in three Mediterranean ecosystems dominated by *P. pinaster*. These sites are located in NW Spain, in Palencia and Valladolid provinces, where Mediterranean-continental climate predominates. The three sites can be distinguished according to ecological and edaphoclimatic variables:

Site 1. Celadilla del Río (Palencia), hereafter PA study site. Located at 985 m.a.s.l. in a high plateau. Characterized by siliceous soils (Umbrisol) with a mean annual rainfall of 622 mm and mean annual temperature of 10 ºC. This site is dominated by an artificial reforestation and has a mean density of 363 trees ha^{-1} and basal area of 20 m^{2} ha^{-1}. The vegetation in the understory is composed by *Agrostis castellana* Boiss. & Reut., *Avenula marginata* (Lowe) Holub subsp. *sulcata* (J.Gay ex Delastre) Franco, *Calluna vulgaris* (L.) Hull, *Erica cinerea* L., *Erica vagans* L., *Halimium viscosum* (Willk.) P. Silva, *Lavandula stoechas* L. subsp. *pedunculata* (Mill.) Samp. ex Rozeira, *Thymus mastichina* L. and *Thymus zygis* L.

Site 2. Montemayor de Pililla (Valladolid), hereafter VAC study site. Located in a high plateau at 890 m.a.s.l. Characterized by calcareous soils (Calcareous cambisol) with a mean annual rainfall of 500 mm and mean annual temperature of 12 ºC. This site is dominated by natural *P. pinaster* which has a mean density of 153 trees ha^{-1} and basal area of 8.5 m^{2} ha^{-1}. Its understory vegetation is mainly composed by calcicole and psammophilous species: *Andryala ragusina* L., *Arrhenatherum elatius* (L.) P. Beauv. ex J. Presl & C. Presl, *Centaurea castellana* Boiss. & Reut., *Cistus laurifolius* L., *Corynephorus canescens* (L.) P. Beauv., *Juniperus thurifera* L., *Leuzea conifera* (L.) DC., *Medicago sativa* L., *Micropyrum tenellum* (L.) Link, *Ononis spinosa* L., *Ornithopus compressus* L., *Vicia laxiflora* Brot., and *Tuberaria guttata* (L.) Fourr.

Site 3. Tudela del Duero (Valladolid), hereafter VAS study site: This site is located at 700 m.a.s.l., with sandy soils, a mean annual rainfall of 430 mm and a mean annual temperature of 12.5º C. *P. pinaster* here is also natural and it has a mean density of 130 trees ha^{-1} and basal area of 7.2 m^{2} ha^{-1}. Its understory vegetation is mainly composed by psammophilous species: *Helichrysum italicum* (Roth) G. Don subsp. *serotinum* (Boiss.) P. Fourn., *Lavandula stoechas* L. subsp. *pedunculata* (Mill.) Samp. ex Rozeira, *Lupinus angustifolius* L., *Ornithopus compressus* L., *Sedum amplexicaule* DC. and *Vulpia myuros* (L.) C.C. Gmel.

All the studied sites had homogeneous forest structures and were even-aged and 50–60 years old. Within each of the three sites we randomly selected three permanent plots at the beginning of the study.

These nine sampling plots consisted in transects of 2 m × 50 m, established in accordance with previous studies (Oria-de-Rueda et al., 2010; Smith et al., 2002).

2.2. Climatic and soil data

Climatic data for the period 2006-2012 were provided by the closest meteorological stations. Mean monthly potential evapotranspiration (ETP), was calculated by the empirical method of Thornthwaite and Mather (1955) for the latitude of the three meteorological stations. Soil samples were taken in each plot for all three sites in mid-May 2014 before the fruiting season. Six soil samples were extracted per plot using a cylindrical (2 cm radius, 20 cm deep, 250 cm^3) soil borer (De la Varga et al., 2012; Taylor, 2002) and after carefully removing the overlying litter and humus layer. A minimum distance of 30 cm apart from any tree trunk was kept (De la Varga et al., 2012).

Table 1: Edaphoclimatic variables for the three sampling locations.

SITE	PA	VAS	VAC
N (g/100g)	0.17a	0.036b	0.050c
P (mg/kg)	4.83a	2.66b	2.66b
K (mg/kg)	63a	22b	67a
pH	5.23a	6.34b	6.60c
Organic matter (%)	4.84a	0.67b	1.24c
Na (meq/100g)	0.023a	0.033b	0.01c
Mg (meq/100g)	0.64a	0.21b	0.46c
Ca (meq/100g)	2.73a	1b	2.96a
Conductivity (mS/cm)	0.06a	0.02b	0.04c
C/N ratio	25.33a	15.24b	20.48c
T (ºC)	10.63a	12.11b	12.45b
TMAX (ºC)	16.70a	19.01b	18.38b
TMIN (ºC)	4.53a	5.88b	6.51b
Prec (mm)	561.18a	374.62b	373.24b
ETP (mm)	640.40a	695.60b	629.80a
PAN (mm)	206.30a	151.80b	147.24b
PAS (mm)	50.34a	36.40a	39.84a
TAN (ºC)	12.85a	14.79b	14.54b

T= monthly mean soil temperature; TMIN= monthly mean minimum temperature; TMAX= monthly mean maximum temperature; TAN= (August + September + October + November) mean temperature; Prec= annual mean precipitation; PAN= (August + September + October + November) accumulated precipitation; PAS= (August + September) accumulated precipitation; ETP= annual mean ETP according to Thornthwaite (1955).

2.3. Sampling and identification

Field sampling was carried out weekly between October and December 2006-2012, when cold temperatures deter the emergence of sporocarps (Bonet et al., 2012). During the fruiting period, all the mushrooms (both ectomycorrhizal and saprotrophic) with a cap diameter wider than 1 mm were collected in the study plots. Mushrooms were right after taken to the laboratory, kept at 4ºC and processed within 24 h after collection for identification, fresh weight measurements and drying (Bonet et al., 2012). Sporocarps were dried in an air-vented oven at 35ºC and were then weighed in order to obtain comparable biomass data. For statistical purposes, data were grouped into the following categories: saprotrophic/mycorrhizal, and edible/inedible.

Sporocarps were identified at the species level. This was not possible for all samples, which were grouped into genus taxa as described by Bonet et al. (2004) and Martín-Pinto et al. (2006b). Information about mycorrhizal/saprotrophic life strategy of each taxa was obtained from various sources (e.g. Agerer, 2006; Gadd et al., 2007; Hobbie and Agerer, 2009; Taylor et al., 2003; Tedersoo et al., 2010; Trudell et al., 2004).

The nomenclature source for fungal taxa names and authors used was obtained from Index Fungorum (www.indexfungorum.org).

The species edibility classification was based on Martínez-de-Aragón et al. (2007) criteria. Inedible (IE) are all species listed as non-edible in the literature consulted and are not traditionally consumed in the study region. Edible (E) are all species that are listed as edible in the majority of literature consulted or are traditionally consumed in the study region.

2.4. Production, diversity and richness calculations and statistical analysis

Fresh weights, edibility and life strategies of fungal taxa collected in the three sites were subjected to a Repeated Measures ANOVA analysis and means were compared by LSD Fisher Tests ($P < 0.05$). STATISTICA '08 Edition software (StatSoft Inc., 1984-2008) was used for the analysis. Wild mushroom production in Mediterranean areas is strongly heterogeneous, mainly due to the great variations in yearly precipitation (Ágreda et al., 2014; Fernández-Toirán et al., 2006). This makes it difficult for variables to achieve the parametric criteria of normality and homocedasticity that ANOVA requires (Ágreda et al., 2014); therefore data were log-transformed when needed.

Shannon's H diversity index (Shannon and Weaver, 1949) was calculated based on fruit body dry weight (Dahlberg, 1991) according to:

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

where pi indicated the relative importance of each fungal species. Species richness (S) was defined as the total number of species found (Martínez-Ruiz et al., 2001; Straatsma and Krisai-Greilhuber, 2003)

Similarity of the macrofungal community composition between sites was determined by calculating the Jaccard index of similarity (Chao et al., 2005; O'Hanlon and Harrington, 2012a): $J = (A / A+B+C)$, where A is the number of species in both sites, B the number of species present in site *j* and C is the number of the species in the site *k*.

Species composition was analyzed using ordination techniques. Firstly, mycorrhizal and saprotrophic taxa dry weight data were subjected to a detrended correspondence analysis (DCA) (Ter Braak and Prentice, 1988). Since the length of the extracted gradient was larger than 3 SD units in both analyses (4.185 and 5.569 respectively), canonical correspondence analysis (CCA) (Ter Braak, 1986) was used to assess the effect of edaphoclimatic variables. 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 (Hernández-Rodríguez et al., 2013). These analyses were conducted using CANOCO for Windows v.4.5 software and CCA results were displayed by ordination diagrams drawn with Cano Draw 4.1 software (Ter Braak and Šmilauer, 2002).

3. Results

3.1. Richness, diversity and sporocarps production

A total of 193 taxa were found across all 9 sampled plots after seven sampling seasons (Table 2). From the total taxa list, 75 were mycorrhizal and 118 saprotrophic. Forty six of the total taxa found were edible. In Palencia (PA) site, we found 103 different taxa, 34 of which were mycorrhizal and 69 saprotrophic. Additionally, eighteen of the total collected taxa were edible (Fig. 1). In Valladolid-sandy (VAS) plots 86 taxa occurred (37 % mycorrhizal and 63 % saprotrophic), of which 18 were edible. The greatest number of taxa was found in Valladolid-calcareous (VAC) plots, with a total of 107 taxa. Thirty nine were classified as mycorrhizal and 68 as saprotrophic. Edible fungi represented 24% of the total (26 taxa), (Fig. 1).

Table 2: Total taxa collected from *P. pinaster* forests.

Taxa	Code	PA	VAS	VAC	G	E
Agaricus cupreobrunneus(Jul. Schäff. & Steer) Pilát	Agcu		+		S	E
Agaricus impudicus(Rea) Pilát	Agim		+		S	E
Agaricus porphyrizon P.D. Orton	Agpo		+		S	E
Agaricus sp.	Agsp		+		S	
Agaricus sylvaticus Schaeff.	Agsy		+		S	E
Agrocybe arenaria Fayod	Agrsp			+	S	
Amanita muscaria (L.) Lam.	Ammu	+			MY	
Amanita ovoidea (Bull.) Link 1833	Amov			+	MY	E
Arrhenia lobata (Pers.) Kühner & Lamoure ex Redhead	Arrlo		+		S	
Arrhenia obatra (J. Favre) Redhead, Lutzoni, Moncalvo & Vilgalys	Arrob			+	S	
Arrhenia obscurata (D.A. Reid) Redhead, Lutzoni, Moncalvo & V.	Arrobs			+	S	
Auriscalpium vulgare Gray	Auvu	+			S	
Baeospora myosura (Fr.) Singer	Bamy	+	+	+	S	
Bovista aestivalis (Bonord.) Demoulin	Boae			+	S	E
Bovista plumbea Pers.	Bopl			+	S	E
Cantharellula umbonata (J.F. Gmel.) Singer	Caum		+	+	MY	E
Chroogomphus rutilus (Schaeff.) O.K. Mill.	Chru			+	MY	E
Clavulina rugosa (Bull.) J. Schröt.	Clru	+			MY	E
Clitocybe sp	Clsp	+	+		S	
Clitocybe costata (Kühner & Romagn.)	Clco	+			S	E
Clitocybe dealbata (Sowerby) P. Kumm	Clde	+	+		S	
Clitocybe diatreta (Fr.) P. Kumm.	Cldi	+			S	
Clitocybe ditopa (Fr.) Gillet	Cldit	+			S	
Clitocybe fragrans (With.) P. Kumm.	Clfr	+	+	+	S	
Clitocybe metachroa (Fr.) P. Kumm.	Clme	+	+	+	S	
Clitocybe obsoleta (Batsch) Quél.	Clob	+	+	+	S	
Clitocybe phaeophthalma (Pers.) Kuyper	Clph	+			S	
Clitocybe subalutacea (Batsch) P. Kumm.	Clsu		+		S	
Clitocybe vibecina (Fr.) Quél.	Clvi	+	+		S	
Collybia cirrhata (Pers.: Fr.) Quélet	Coci		+	+	S	
Collybia cookei (Bres.) J.D. Arnold	Coco	+		+	S	
Collybia erythropus (Pers.) P. Kumm.	Coer	+			S	
Collybia sp.	Cosp	+			S	
Conocybe sp.	Consp	+			S	
Cortinarius brunneus (Pers.) Fr.	Corbr	+			MY	
Cortinarius cinnabarinus Fr	Corci	+			MY	
Cortinarius cinnamomeus (L.) Fr	Corcin		+	+	MY	
Cortinarius croceus (Schaeff.) Gray	Corcr	+			MY	
Cortinarius subgen Dermocybe (L.) Gray	Corsecc.	+			MY	
Cortinarius sp.	Corsp	+	+	+	MY	
Cortinarius sp. Subgen.*Telamonia*	CorsubTe			+	MY	
Crinipellis scabella (Alb. & Schwein.) Murrill	Crsc		+	+	S	

(Continued on the next page)

Table 2: continued

Taxa	Code	PA	VAS	VAC	G	E
Crucibulum laeve (Huds.) Kambly	Crla			+	S	
Cystoderma amianthinum (Scop.) Fayod	Cyam	+		+	S	
Cystoderma carcharias (Pers.) Fayod	Cycar	+			S	
Cystoderma sp.	Cysp	+			S	
Cystoderma superbum Huijsman	Cysu		+		S	
Cystoderma terreyi (Berk. & Broome) Harmaja	Cyte	+	+		S	
Cystodermella cinnabarina (Alb. & Schwein.) Harmaja	Cycin		+	+	S	
Cystodermella granulosa (Batsch) Harmaja	Cygr		+	+	S	
Entoloma araneosum (Quél.) M.M. Moser	Enar		+		MY	
Entoloma cetratum (Fr.) M.M. Moser	Ence	+			MY	
Entoloma dysthales (Peck) Sacc.	Endy			+	MY	
Entoloma formosum (Fr.) Noordel	Enfo	+			MY	
Entoloma hirtipes (Schumach.) M.M. Moser	Enhi	+	+		MY	
Entoloma infula (Fr.) Noordel.	Enin			+	MY	
Entoloma minutum (P. Karst.) Noordel.	Enmi		+		MY	
Entoloma mougeotii (Fr.) Hesler	Enmo	+		+	MY	
Entoloma sepium (Noulet & Dass.) Richon & Roze	Ense		+	+	MY	
Entoloma sericeum Quél.	Enser	+	+	+	MY	
Entoloma sp	Ensp	+	+	+	MY	
Entoloma vernum S. Lundell	Enve		+		MY	
Entoloma versatile (Gillet) M.M. Moser	Enver		+		MY	
Flammulaster sp.	Flsp			+	S	
Galerina badipes (Pers.) Kühner	Gaba	+	+	+	S	
Galerina embolus (Fr.) P.D. Orton	Gaem	+	+	+	S	
Galerina fallax A.H. Sm. & Singer	Gafa			+	S	
Galerina marginata (Batsch) Kühner	Gama	+	+	+	S	
Galerina paludosa (Fr.) Kühner	Gapa			+	S	
Galerina sp.	Gasp	+	+	+	S	
Galerina uncialis (Britzelm.) Kühner	Gaun	+	+	+	S	
Galerina vittiformis (Fr.) Singer	Gavi	+	+		S	
Gymnopilus penetrans (Fr.) Murrill	Gype	+	+		S	
Gymnopus androsaceus (L.) J.L. Mata & R.H. Petersen	Gyan			+	S	
Gymnopus aquosus (Bull.) Antonín & Noordel.	Gyaq	+			S	E
Gymnopus brassicolens (Romagn.) Antonín & Noordel	Gybr	+			S	
Gymnopus dryophilus (Bull.) Murrill	Gydr	+	+	+	S	E
Gymnopus erythropus (Pers.) Antonín, Halling & Noordel.	Gyer	+		+	S	E
Gymnopus ocior (Pers.) Antonín & Noordel.	Gyoc	+			S	E
Hebeloma cistophilum Maire	Heci			+	MY	
Hebeloma cylindrosporum Romagn.	Hecy		+		MY	
Hebeloma mesophaeum (Pers.) Quél.	Heme		+	+	MY	
Hebeloma psammophilum Bon	Heps		+	+	MY	
Hebeloma sp	Hesp		+	+	MY	
Hemimycena lactea (Pers.) Singer	Hela	+			S	

(Continued on the next page)

Table 2: continued

Taxa	Code	PA	VAS	VAC	G	E
Hohenbuehelia petaloides (Bull.) Schulzer	Hope			+	S	E
Hygrocybe sp.	Hysp	+			MY	
Hygrophoropsis aurantiaca (Wulfen) Maire	Hyau	+			S	
Hygrophorus hypothejus (Fr.) Fr.	Hyhy	+			MY	E
Hypholoma fasciculare (Huds.) P. Kumm.	Hyfa	+			S	
Inocybe lacera (Fr.) P. Kumm.	Inla			+	MY	
Inocybe maculata Boud.	Inma		+		MY	
Inocybe nitidiuscula (Britzelm.) Lapl	Inni		+		MY	
Inocybe pelargonium Kühner	Inpe		+		MY	
Inocybe pyriodora (Pers.) P. Kumm	Inpy			+	MY	
Inocybe rimosa (Bull.) P. Kumm.	Inri			+	MY	
Inocybe dunensis P.D. Orton	Insp		+	+	MY	
Laccaria laccata (Scop.) Cooke	Lala	+			MY	E
Lactarius aurantiacus (Pers.) Gray	Laau	+			MY	E
Lactarius deliciosus (L.) Gray	Lade		+	+	MY	E
Lactarius hepaticus Plowr.	Lahe	+		+	MY	
Lentinellus micheneri (Berk. & M.A. Curtis) Pegler	Lemi		+	+	S	
Lepiota castanea Quél.	Leca			+	S	
Lepiota cristata (Bolton) P. Kumm	Lecr			+	S	
Lepiota sp.	Lesp		+		S	
Lepista nuda (Bull.) Cooke	Lenu		+	+	S	E
Leucoagaricus pilatianus (Demoulin) Bon & Boiffard	Lepi			+	S	
Limacella illinita (Fr.)	Liil		+		MY	E
Lycoperdon lividum Pers.	Lyli			+	S	E
Lycoperdon molle Pers.	Lymo		+	+	S	E
Lycoperdon perlatum Pers.	Lype	+	+	+	S	E
Macrolepiota excoriata (Schaeff.) M.M. Moser	Maex			+	S	E
Macrolepiota konradii (Huijsman ex P.D. Orton) M.M. Moser	Mako			+	S	E
Macrolepiota mastoidea (Fr.) Singer	Mama			+	S	E
Marasmius anomalus (L.) Fr.	Maan	+	+	+	S	
Marasmius curreyi Berk. & Broome	Macu	+		+	S	
Marasmius ventalloi Sing.	Masp		+		S	
Marasmius undatus (Berk.) Fr.	Maun		+	+	S	
Mycena acicula (Schaeff.) P. Kumm.	Myac	+			S	
Mycena aetites (Fr.) Quél.	Myae	+	+	+	S	
Mycena alcalina (Fr.) P. Kumm.	Myal	+	+	+	S	
Mycena ammoniaca (Fr.) Quél.	Myamm		+		S	
Mycena arcangeliana Bres.	Myar	+			S	
Mycena aurantiomarginata (Fr.) Quél.	Myaur	+			S	
Mycena capillaripes Peck	Myca			+	S	
Mycena cinerella (P. Karst.) P. Karst.	Myci	+			S	
Mycena clavicularis (Fr.) Gillet	Mycl	+	+	+	S	
Mycena epipterygia (Scop.) Gray	Myep	+	+	+	S	

(Continued on the next page)

Table 2: continued

Taxa	**Code**	**PA**	**VAS**	**VAC**	**G**	**E**
Mycena epipterygia var. pelliculosa (Fr.) Maas Geest.	Myepvar		+	+	S	
Mycena filopes (Bull.) P. Kumm.	Myfi	+	+	+	S	
Mycena flavoalba (Fr.) Quél.	Myfl	+			S	
Mycena galericulata (Scop.) Gray	Myga	+		+	S	
Mycena inclinata (Fr.) Quél.	Myin	+			S	
Mycena leptocephala (Pers.) Gillet	Myle	+	+	+	S	
Mycena polygramma (Bull.) Gray	Mypo	+	+	+	S	
Mycena pseudopicta (J.E. Lange) Kühner	Myps		+	+	S	
Mycena pura (Pers.) P. Kumm.	Mypu	+	+	+	S	
Mycena pura f. lutea (Gillet) Arnolds	Mypurl			+	S	
Mycena rosella (Fr.) P. Kumm.	Myro	+		+	S	
Mycena seynesii Quél.	Myse	+	+	+	S	
Mycena sp.	Mysp	+	+	+	S	
Mycena ustalis Aronsen & Maas Geest.	Myus			+	S	
Mycena viridimarginata P. Karst.	Myvi	+			S	
Mycena vulgaris (Pers.) P. Kumm.	Myvu	+			S	
Mycetinis scorodonius (Fr.) A.W. Wilson & Desjardin	Mysc	+		+	S	
Omphalina pyxidata (Bull.) Quél.	Ompy		+	+	S	
Omphalina sp.	Omsp		+		S	
Phanerochaete sanguinea (Fr.) Pouzar	Phsa	+			S	
Psathyrella hydrophila (Bull Ex Merat) Mre.	Pshy	+			S	
Psathyrella ammophila (Fr.) Quél.	Pssp			+	S	
Psilocybe luteonitens (Fr.) Park.-Rhodes	Pslu			+	S	
Rhizopogon luteolus Fr. & Nordholm	Rilu		+	+	MY	E
Rhizopogon sp.	Rhsp		+		MY	E
Rhodocollybia butyracea (Bull.) Lennox	Rhbu	+	+	+	S	E
Rhodocollybia butyracea f. asema (Fr.) Antonín, Halling & Noordel.	Rhbuf	+			S	E
Rickenella mellea (Singer & Clem.) Lamoure	Rime	+			MY	
Russula adusta (Pers.) Fr.	Ruad	+		+	MY	
Russula cessans A. Pearson	Ruce	+		+	MY	
Russula delica Fr.	Rude			+	MY	
Russula heterophylla (Fr.) Fr.	Ruhe			+	MY	E
Russula integra (L.) Fr.	Ruin	+		+	MY	E
Russula olivacea Pers.	Ruol	+		+	MY	E
Russula romellii Maire	Ruro			+	MY	E
Russula sanguinea Fr.	Rusa	+			MY	
Russula sardonia Fr.	Rusar	+			MY	
Russula sp.	Rusp	+	+	+	MY	
Russula torulosa Bres.	Ruto	+	+	+	MY	
Russula xerampelina (Schaeff.) Fr.	Ruxe	+			MY	
Stropharia coronilla (Bull. ex DC.) Quél.	Stco			+	S	E
Suillus bellinii (Inzenga) Watling	Sube			+	MY	E
Suillus granulatus (L.) Roussel	Sugr	+			MY	E

(Continued on the next page)

Table 2: continued

Taxa	Code	PA	VAS	VAC	G	E
Suillus luteus (L.) Roussel	Sulu	+			MY	E
Tapinella atrotomentosa (Batsch) Šutara	Taat	+			S	
Tapinella panuoides (Batsch) E.-J. Gilbert	Tapa	+			S	
Tephrocybe rancida (Fr.) Donk	Tera		+		MY	
Thelephora caryophyllea (Schaeff.) Pers.	Thca			+	MY	
Thelephora terrestris Ehrh.	Thte			+	MY	
Tremella mesenterica Schaeff.	Trme	+			S	
Tricholoma equestre (L.) P. Kumm.	Treq	+	+		MY	
Tricholoma focale (Fr.) Ricken	Trfo			+	MY	
Tricholoma gausapatum (Fr.) Quél.	Trga		+		MY	E
Tricholoma myomyces (Pers.) J.E. Lange	Trmy		+	+	MY	E
Tricholoma portentosum (Fr.) Quél.	Trpo	+			MY	E
Tricholoma sp.	Trsp			+	MY	
Tricholoma terreum (Schaeff.) P. Kumm.	Trte		+	+	MY	E
Tricholomopsis rutilans (Schaeff.) Singer	Trru	+			S	
Tubaria sp.	Tusp	+	+	+	S	
Xylaria hypoxylon (L.) Grev.	Xyhy			+	S	

MY: mycorrhizal; S: saprotrophic; E: edible

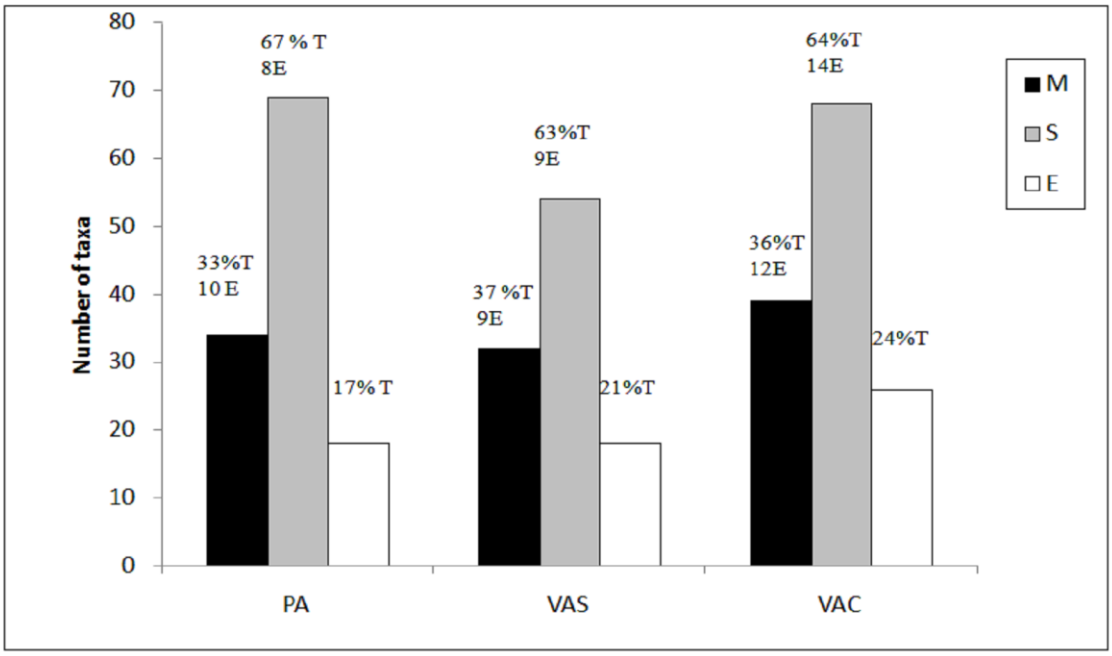

Fig. 1. Number and percentage of taxa found in the studied forest sites depending on vital strategy (mycorrhizal or saprotrophic). (T %): Percentage of total taxa. (E): number of edible taxa.

Fungal richness from all three sites was different. Values in PA and VAC were significantly higher than those observed in VAS plots (Fig. 3; $P_{\text{PA-VAS}} = 0.029$; $P_{\text{VAC-VAS}} = 0.003$).

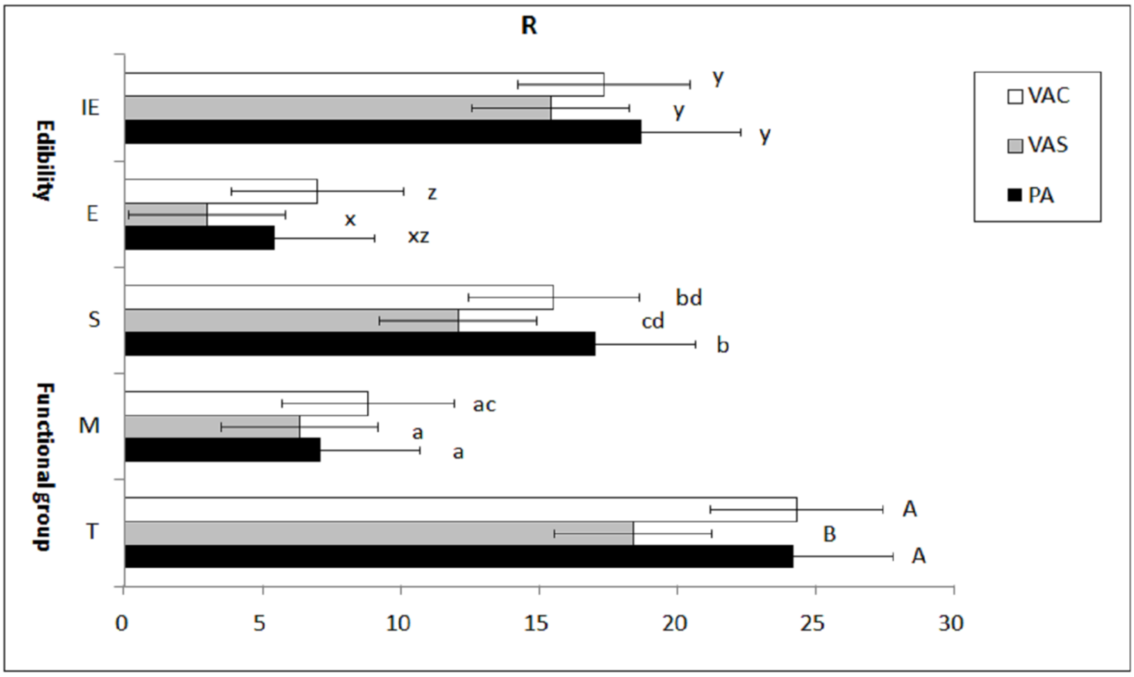

Fig. 2a. Richness variable analysed depending on functional groups and edibility. (S): Richness. (S/M): Saprotrophic/Mycorrhizal (E/IE): Edible/Inedible.

Similar results between PA and VAC plots were observed when analysing richness and Shannon's *H´* Diversity index for saprotrophic and mycorrhizal species. Within the three locations, both richness and diversity values were always higher for saprotrophic fungi compared to mycorrhizal fungi. However, no differences were found when comparing mean total values for Shannon's *H´* diversity index (Figs. 2a and 2b; $P > 0.05$).

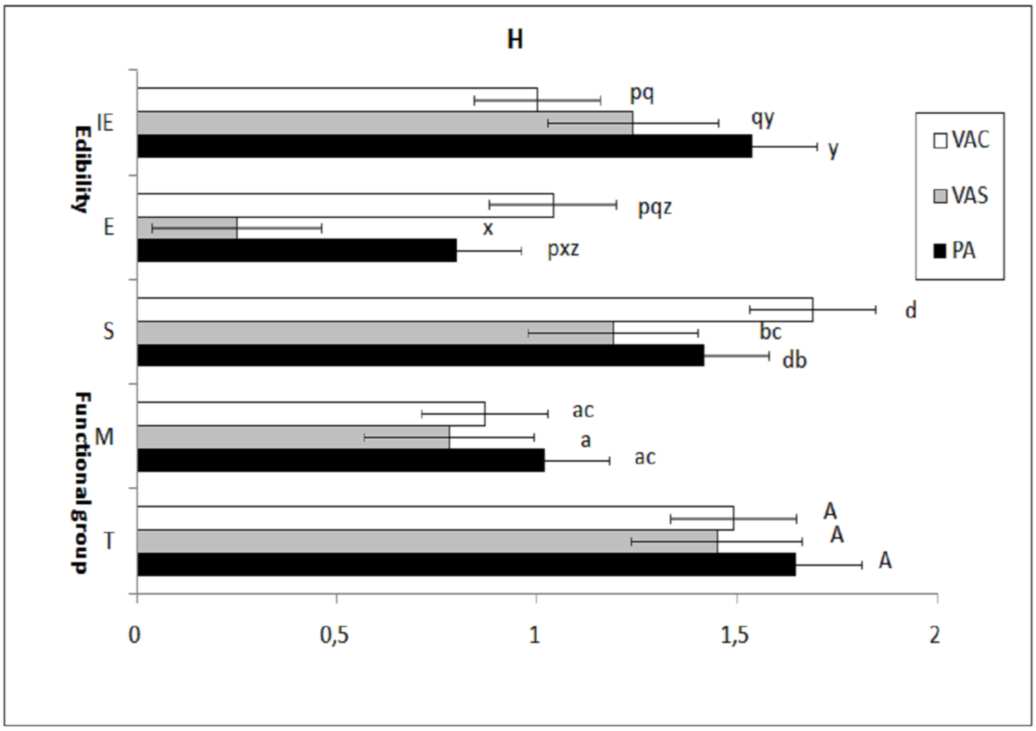

Fig. 2b. Diversity variable analysed depending on functional groups and edibility. (H): Shannon index. (S/M): Saprotrophic/Mycorrhizal; (E/IE): Edible/Inedible

A total production of 447.3 kg fresh weight (fw) ha^{-1} (39.4 kg ha^{-1} dry weight) was collected over the 7-year study period, where 192.7 kg fw ha^{-1} were from PA site, 167.7 kg fw ha^{-1} from VAC, and 87.3 kg fw ha^{-1} from VAS site (Fig. 3). While production of mycorrhizal species showed no differences among sites, production of saprotrophic fungi was lowest in VAS and significantly different from that in PA (Fig. 3; P = 0.003).

Regarding edible species, yields were significantly different among the three studied sites. The highest production was observed in PA and VAC sites (67.8 and 43.3 kg fw ha^{-1} respectively). While lower values (6.4 kg fw ha^{-1}) were collected in VAS (Fig. 3; $P_{\text{PAC-VAS}}$ = 0.000; $P_{\text{VAC-VAS}}$ < 0.001). This same trend was observed regarding edible mycorrhizal species. PA and VAC sites showed the highest values (31.3 and 29.2 kg fw ha^{-1}), while production in VAS was the lowest (2.3 kg fw ha^{-1}) (Fig. 3; $P_{\text{PA-VAS}}$ = 0.007; $P_{\text{VAC-VAS}}$ = 0.011). Finally, highest values for production of edible saprotrophic species, were again recorded in PA (36.7 kg fw ha$^{-1)}$, and the lowest in VAS (4.3 kg fw ha^{-1}) (Fig. 3; P = 0.043).

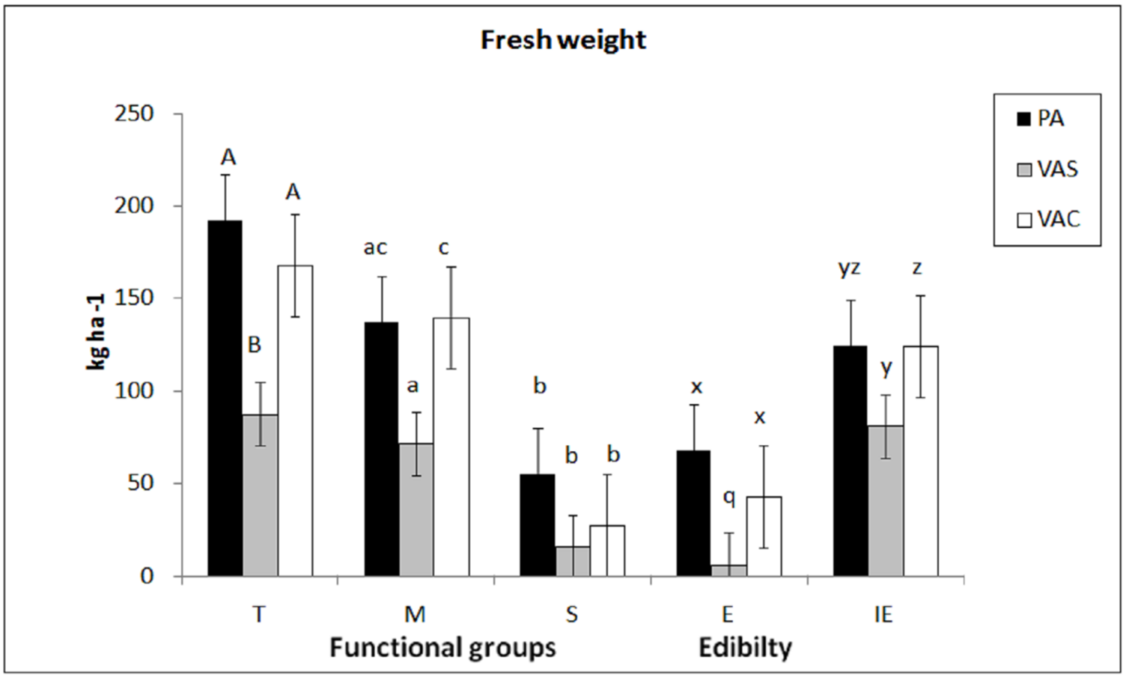

Fig. 3. Production of carpophores according to functional groups and edibility. (kg fw ha^{-1}). M: Mycorrhizal, S: Saprotrophic, E: Edible, IE: Inedible. The data are mean results ± standard error of the mean. Independent comparisons were carried out within functional groups and edibility. Values with the same letter are not significantly different.

3.2. Taxa composition

Paired Jaccard similarity coefficients showed that VAS and VAC were the most similar sites, with 46 taxa in common. When comparing mycorrhizal and saprotrophic taxa, those sites had 16 and 31 taxa in common respectively, showing again the highest coefficient between VAS and VAC (Table 3).

Table 3: Jaccard similarity coefficients (lightface) and the numbers of taxa in common (boldfaces) among sites.

Total	PA	VAS	VAC
PA		0.246	0.239
VAS	**32**		0.370
VAC	**35**	**45**	
Mycorrhizal	PA	VAS	VAC
PA		0.111	0.114
VAS	**6**		0.290
VAC	**7**	**16**	
Saprotrophic	PA	VAS	VAC
PA		0.333	0.313
VAS	**25**		0.442
VAC	**27**	**31**	
Edible	PA	VAS	VAC
PA		0.214	0.162
VAS	**6**		0.233
VAC	**6**	**7**	

Fungal community assemblies along the three sites 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 (Figs. 4a and 4b). Axis 1 separated PA plots from VAC and VAS sites, and Axis 2 showed further differences between the three sites, especially between VAS and VAC sites where there was a relative species overlap. VAS mycorrhizal taxa tended to concentrate in the middle (Fig. 4a); on the contrary VAS saprotrophic taxa were dispersed towards the extreme of Axis 2 (Fig. 4b). Axis 1 explained 7.1 and 6.9 % of the variation in the data (mycorrhizal and saprotrophic taxa respectively), and the two axes together explained an accumulative variance for the interaction between taxa and environment of 22.8% and 16.5% for mycorrhizal and saprotrophic groups respectively.

Table 4: Summary of canonical correspondence analysis of fungal taxa presence and environmental factors according functional groups for the three sites.

Functional group	**Mycorrhizal**		**Saprotrophic**	
Axes	1	2	1	2
Eigenvalues :	0.605	0.436	0.560	0.547
Species-environment correlations :	0.965	0.942	0.949	0.960
Cumulative percentage variance				
of species data :	7.1	12.3	6.9	13.6
of species-environment relation:	22.8	39.3	16.5	32.5

A total of 10 edaphoclimatic variables were significant ($P < 0.05$) in the ordination of mycorrhizal (7) and saprotrophic (10) taxa dry weight according to the forward selection process with seven variables in common (Table 5). A Monte Carlo permutation test was significant for the first axis ($P = 0.002$) and for all canonical axes ($P = 0.002$) regarding the both mycorrhizal and saprotrophic taxa compositions. For mycorrhizal taxa, Axis 1 was positively correlated with edaphic variables such as N, P and K. Also mean precipitation was correlated with this axis. Furthermore, this axis was positively correlated with temperature conditions, including mean temperatures, and temperatures during the autumn fungal production period. Axis 2 was correlated with ETP, and a negative correlation was observed for precipitation values during fructification period such as the accumulated precipitation from August to November (PAN), (Table 5, Fig. 4a). For saprotrophic taxa, similar trends were observed for Axis 1. However, Axis 2 was positively correlated with PAS and PAN (Fig. 4b; $P = 0.002$ in both cases).

Regarding mycorrhizal species, in VAS and VAC, species were positively affected by temperatures from August to November (TAN), while PA was affected by the precipitation (Prec and PAN) and edaphic variables such as N, P and K. Additionally, Axis 2 indicated an almost inverse correlation between ETP and PAN (Fig. 4a). In the case of saprotrophic species, the PA plots were positively correlated to precipitation, N and K, while the VAC and VAS sites were influenced by temperature and ETP (Fig. 4b).

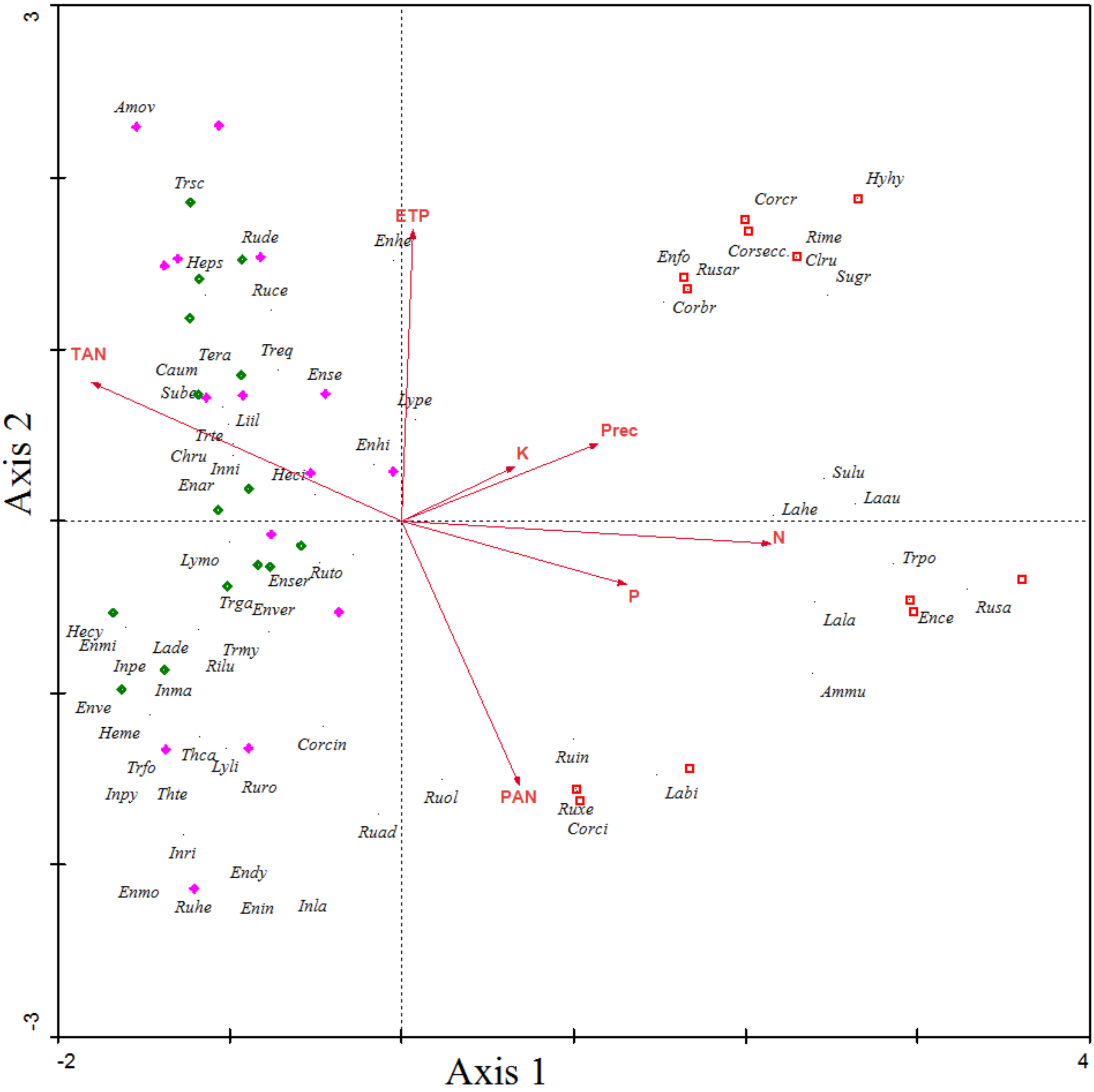

Fig. 4a. Canonical Correspondence Analysis ordination plots showing: mycorrhizal taxa (dots), PA (squares), VAS (rhomboids) and VAC (stars) and edapho-climatic factors (arrows). TAN= (August + September + October + November) mean temperature; Prec= annual mean precipitation; PAN= (August + September + October + November) accumulated precipitation; ETP= annual mean ETP according to Thornthwaite (1955). N= soil nitrogen; K= soil potassium; P= soil phosphorus. Fungal taxa are identified by the code shown in Table 2.

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

Functional group	Variables	F-ratio	P-value
Mycorrhizal	N	2.86	0.002
	PAN	2.14	0.002
	ETP	1.98	0.002
	K	1.73	0.004
	Prec	1.57	0.008
	TAN	1.40	0.020
	P	1.34	0.048
Saprotrophic	PAN	2.91	0.002
	N	2.74	0.002
	K	2.30	0.002
	Prec	2.08	0.002
	TMIN	1.65	0.012
	T	1.68	0.048
	TMAX	2.43	0.002
	TAN	1.76	0.004
	PAS	1.69	0.002
	ETP	1.61	0.026

Only significant variables are included. T= monthly mean soil temperature; TMIN= monthly mean minimum temperature; TMAX= monthly mean maximum temperature; TAN= (August + September + October + November) mean temperature; Prec= annual mean precipitation; PAN= (August + September + October + November) accumulated precipitation; PAS= (August + September) accumulated precipitation; ETP= annual mean ETP according to Thornthwaite (1955). N= soil nitrogen; K= soil potassium; P= soil phosphorus.

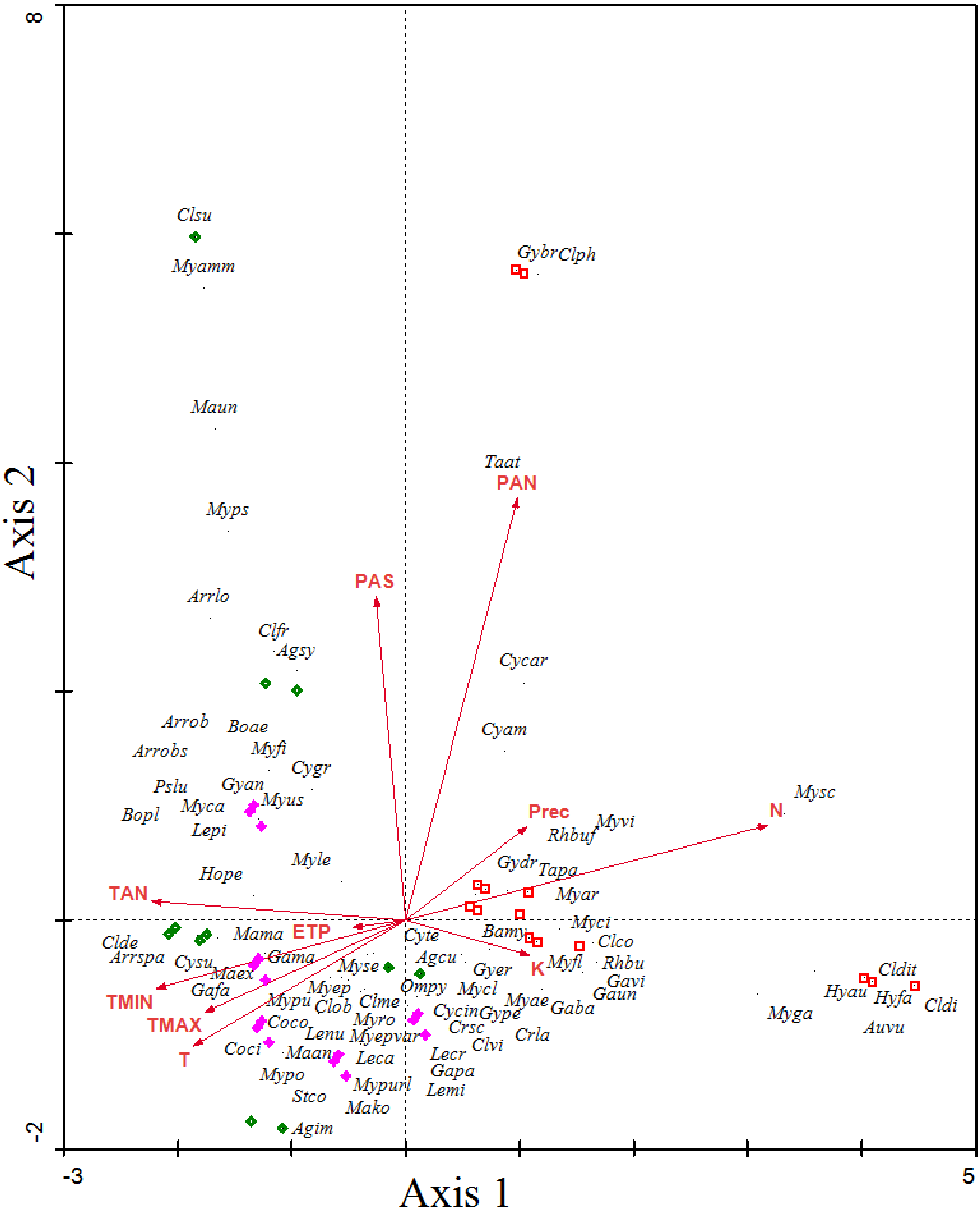

Fig. 4b. Canonical Correspondence Analysis ordination plots showing: saprotrophic taxa (dots), PA (squares), VAS (rhomboids) and VAC (stars) and edapho-climatic factors (arrows). T= monthly mean soil temperature; TMIN= monthly mean minimum temperature; TMAX= monthly mean maximum temperature; TAN= (August + September + October + November) mean temperature; Prec= annual mean precipitation; PAN= (August + September +October + November) accumulated precipitation;

PAS= (August + September) accumulated precipitation; ETP= annual mean ETP according to Thornthwaite (1955). N= soil nitrogen. K= soil potassium. Fungal taxa are identified by the code shown in Table 2.

4. Discussion

4.1. Richness, diversity and sporocarps production

Despite extreme and highly variable environmental conditions, fungal communities associated with *P. pinaster* are highly diverse and productive. In this study, a total of 193 fugal taxa were collected. The number of fungal taxa reported here is among the highest reported in the literature, since other studies on single-species pine stands of approximately 0.1 ha, showed lower richness of fungal species (15-35) (Bruns et al., 2002). A total of 119 taxa was recorded from 18 permanent plots in *P. sylvestris* forests during a 15 years long term inventory in the inner northeast zone of the Iberian Peninsula (Martínez-Peña et al., 2012b). For this same species, a similar figure was reported by Bonet et al. (2004), who collected 164 taxa from a three years inventory carried out weekly during autumn season in the central Pyrenees. Martínez-de-Aragón et al. (2007) recorded only 46 taxa in pine forests of the pre-Pyrenees mountains based on a 5 years sampling from 34 plots. A low species richness value was also reported by Oria-de-Rueda et al. (2010) in *P. sylvestris* forests in northwest Spain, where 43 taxa were collected. Finally even lower richness have been reported from *P. halepensis* Mediterranean forests in the Iberian Peninsula, with 16 (Oria-de-Rueda et al., 2010) and 35 taxa (Martínez-de-Aragón et al., 2007). The different results when comparing previous studies suggest that ecological factors such us soil, climate and host species are decisive factors affecting fungal richness as previously described by Oria-de-Rueda et al. (2010).

Furthermore, our results showed a higher richness than previously reported in other *P. pinaster* studies. Fernández-Toirán et al. (2006) collected 60-80 taxa in a 15 ha *P. pinaster* natural stands through a 4 and 6 year sampling in the northwest of Spain. Oria-de-Rueda et al. (2010) also found lower richness values, recording 49 taxa in a reforested 50-year old *P. pinaster* growing on degraded and eroded soils in north-western Spain. Richness observed in our study was also higher than the one reported by Martín-Pinto et al. (2006b), who recorded 39 taxa in a Mediterranean ecosystem dominated by *P. pinaster*. Our study showed also higher richness than that reported by Gassibe et al. (2011), who recorded 115 fungal taxa in a *P. pinaster* forest located in poor, degraded, and stony soils with few disperse individuals of *Cistus ladanifer.*

The high richness values reported in this study can be partially attributed to the different edaphoclimatic characteristics of the three sites included in our study. The studied sites cover a wide range of ecological variables, from extreme Mediterranean xerophilous conditions present in VAS and VAC sites, to a pre Eurosiberian ecosystem in PA. The elevated levels of genetic variability in *P. pinaster*, together with an important genotype by environment interaction, has enabled the species to adapt to these extreme ecological conditions (Alía et al., 1996). This adaptation causes increased ecological plasticity, enabling this species to grow in a large range of soil and climatic conditions (Rodríguez-García et al., 2010). For example, *P. pinaster* can adapt to high temperatures and summer droughts, but it is also present in areas characterized by mild temperatures and high precipitation values. In this sense *P. pinaster* populations have the capacity to thrive well in both Mediterranean and pre Eurosiberian locations (Ruiz-Labourdette et al., 2012). This plasticity could explain its compatibility with a wide range of fungal symbionts, and therefore significant fungal production and diversity observed in this study (Gassibe et al., 2011; Martín-Pinto et al., 2006b; Oria-de-Rueda et al., 2010).

Total average fresh weight production recorded was considerably high (447.3 kg fw ha^{-1}). Similar values were previously referenced by Oria-de-Rueda et al. (2010), with an average plot yield of 476.3 kg fw ha-1. However, lower values of 332.7 kg fw ha^{-1} had been found by Martín-Pinto et al. (2006b) and 209.9 kg fw ha^{-1} by Gassibe et al. (2011) under similar age class and stand conditions. Our yield results were also higher than those found by Ágreda et al. (2014) who reported 330 kg fw ha^{-1} for different *P. pinaster* age classes in Mediterranean ecosystems in northwestern Spain. Despite the fact that these researches also reference *P. pinaster* forests, the high production found in our study may be attributed to the very different edaphoclimatic conditions which characterize the three sampled sites (Table 1). Some previous studies linked mushroom fructification to habitat characteristics (Bonet et al., 2010) and climate conditions, mainly soil moisture and temperature (Barroetaveña et al., 2008; Martínez-de-Aragón et al., 2007; Pinna et al., 2010). Thus, in the case of our sampled forests, *P. pinaster* is able to form fungal associations with a large number of different mushroom taxa adapted to the broad ecological conditions providing higher yields.

Regarding functional groups, we found an average species ratio of 1:2 for mycorrhizal and saprotrophic taxa. This could be due to the presence of high amounts of organic matter in the forests, since decomposition rates are particularly low in these ecosystems of limited precipitation and mild temperatures. *Pinus* stands are frequently associated with low decomposition rates (Senn-Irlet and Bieri, 1999), while high rates are more often found in deciduous forests of more humid regions (Oria-de-Rueda et al., 2010). Our results showed significantly higher values for organic matter content and lower values for ETP in both PA and VAC sites (Table 1). Organic matter can also influence fungal communities through its impact on soil-moisture content and water-holding capacity (Ferris et al., 2000).

Moreover, despite there was no significant difference in mean precipitation between VAS and VAC sites, saprotrophic and mycorrhizal richness and diversity were higher in VAC, although not significant ($P > 0.05$) for the mycorrhizal taxa. These non significant differences comparing mycorrhizal richness among sites may be attributed to host species and stand age, since this group of taxa is mainly affected by these variables (Ágreda et al., 2014; Bonet et al., 2012), and in our work the ages of the studied forests were similar. However, significant differences ($P < 0.05$) for the Shannon diversity index were observed between these two locations regarding saprotrophic taxa. ETP values in VAS were significantly higher than in VAC, affecting soil humidity, which could explain the differences regarding saprotrophic diversity between these two sites.

The results for sporocarp production showed a similar trend regarding total, mycorrhizal and saprotrophic functional groups. Significantly higher values were found in both PA and VAC sites compared to those found in VAS. Nonetheless, the lower values for VAS can also be related to the substrate, a continental sand dune with particular soil properties. Here, the soil is very sandy, with low water holding capacity and fertility, and characterized by significantly lower C/N ratio, nitrogen, and organic matter percentages. These conditions create a harsh environment for the fructification of the fungal communities (Høiland, 2012).

Moreover, soil moisture is an important factor in sporocarp production by macrofungi, and it also plays a key role in the composition and productivity of mycorrhizal fungal communities (O'Dell et al., 1999). Higher precipitation in PA could explain higher yield values, whereas evapotranspiration differences between VAC and VAS suggest lower soil humidity and fungal production in VAS soils. Indeed, higher evapotranspiration reduces soil moisture (Trudell and Edmonds, 2004) and could negatively influence fungal production.

Yield results according to functional groups showed the same trend in the three studied locations where a higher mycorrhizal production was always observed. This is in accordance with Richard et al. (2004) who found high mycorrhizal production when C/N ratios were similar to those observed in PA and VAC plots. Higher production for mycorrhizal taxa was previously reported in *P. pinaster* 50-60 year old forests in Northwest Spain (Gassibe et al., 2011; Oria-de-Rueda et al., 2010). This finding can also be related to the fact that *P. pinaster* is considered as a fast-growing species that quickly produces high amounts of biomass and mycorrhizal production depends directly on the amount of forest biomass (Oria-de-Rueda et al., 2010). Mycorrhizal production strongly depends on the number of plant individuals in the forest community (Simard et al., 1997). As stand densities were different in our study sites this could have an effect on the mycorrhizal production. as reported on previous mycosilvicultural studies regarding different thinning intensities effects on the yield of edible mycorrhizal fungi such as *Lactarius sanguifluus*, *Lactarius semisanguifluus*, *Lactarius salmonicolor* and

Lactarius vinosus associated to 50-year-old *P. pinaster* stands in Northeastern Spain (Bonet et al., 2012). Furthermore, Pilz et al. (2006) has also reported the impact of these silvicultural practices on the production of *Cantharellus formosus* and *Cantharellus subalbidus* in 50-year-old Douglas-fir stands, Oregon, USA.

For rural communities dependent on forest resources, edible mushroom production can provide supplementary economical incomes as a complement to those obtained from timber. In our study we accounted for a total of 117 kg fw ha^{-1} of edible mushrooms. This result was similar to another obtained in *P. sylvestris* forests in north-eastern Spain (Martínez-Peña et al., 2012b), where the host species is considered to be highly productive. Therefore, it could be important to manage these forests including both timber and mushroom potential incomes (Diaz-Balteiro and Romero, 2008).

4.2. Taxa composition

Large similarities were found between VAS and VAC sites for total species, functional groups, and edibility. However, they both showed dissimilarities regarding macromycetes in the PA site. The main environmental variables affecting this result were linked to climatic data. Temperature (T, TMAX, TMIN and TAN) and precipitation (Prec, PAN and PAS) were similar within the VAS and VAC sites, but were significantly different to those in PA. As noted in previous studies, soil temperature and moisture conditions appear to be specifically linked to the phenology of each fungal species (Pinna et al., 2010). Similarly, Ferris et al. (2000) confirmed the site and plot-specific nature of fungal communities in 12 stands of planted *P. sylvestris* and *Picea abies* across lowland England. In their study, few species were found in common between the sites.

Our CCA also showed a significant effect of the climatic variables on fungal communities. Thus, the results found support those of Humphrey et al. (2000) reported that climatic variables influenced fungal community composition. The influence of precipitation and temperature has been previously studied. O'Dell et al. (1999) observed an increase in species richness with higher precipitations. Other studies related fungal yield to climatic variables (Bonet et al., 2012; Gassibe et al., 2014; Martínez-de-Aragón et al., 2007). In our study, climate variables such us T, TMIN, TMAX, TAN, ETP, were correlated to the fungal composition at functional level along climate gradients.

Moreover, climatic variables do not fully explain the presence of sporocarps (Barroetaveña et al., 2008; Egli, 2011). This is in accordance with the results observed where differences in fungal taxa composition were also correlated to superficial soil nutrients such us N and K. An increasing trend of these variables is found in the PA plots. This may be a consequence of the management practices, since the PA stand was located in previous agricultural area. Therefore, the inputs of organic matter are reflected in soil nutrient availability, particularly in N. In contrast, both VAC and VAS plots are situated in natural forests, which are generally characterized by lower fertility properties (Barrico et al., 2010).

Our result showing N and K influence on fungal composition is in accordance with those obtained by Natel and Neumann (1992), who found a close correlation between basidiomycete community composition and soil variables. The influence of Nitrogen on fungal richness distribution patterns was also previously reported. In those studies, sporome distribution showed a large degree of community specialization along the soil quality gradient (Reverchon et al., 2012, 2010). Increased nitrogen can influence formation of mycorrhizas, production and distribution of extra radical mycelium in the soil, and sporocarp formation

(Trudell and Edmonds, 2004). Furthermore, other authors noted that fungal communities can be adapted to more nitrogen-rich sites (Kranabetter et al., 2009; Toljander et al., 2006)

Regarding mycorrhizal taxa, the CCA showed a significant influence of phosphorous, which was positively correlated with those sites where mycorrhizal yields were also higher. Interestingly, some previous studies reported that high P availability can increase ECM infection (Cairney, 2011; Wallander, 2000).

Some mycorrhizal genera recorded in the study, such as *Cortinarius*, *Inocybe, Entoloma, Russula* and *Tricholoma* included a large number of species, as also reported by other studies focused on pine species (Ágreda et al., 2014; Gassibe et al., 2014; Oria-de-Rueda et al., 2010). *Cortinarius*, *Entoloma* and *Russula* showed a great ecological range since they were notably present in the three studied forests under significantly different edaphoclimatic conditions. Likewise, *Cortinarius* and *Russula* genera were frequently found in four different Irish forests characterized by particular soil factors (O'Hanlon and Harrington, 2012b). Ubiquity of Russulaceae species was previously observed by Azul et al. (2011) in southern Portugal. This result was also reported in other Mediterranean areas (Bergemann and Garbelotto, 2006; Richard et al., 2011, 2004). This is however unsurprising as this family comprises a large range of species which are able to fruit in very diverse ecological environments. The taxonomical diversity and cosmopolitan distribution within Russulaceae further give a better understanding of the putative role of these mutualist fungi in soil processes in Mediterranean ecosystems, such as stabilization following disturbance and drought stress (Azul et al., 2011). Even more, at the species level, *Russula torulosa* was found to form symbiotic associations with *P. pinaster* in the three sites. *R. torulosa* was also found by Bonet et al. (2004) and Gassibe et al. (2011) in diverse edaphoclimatic conditions.

Regarding *Entoloma* species, although highly frequent in our study, they fruit most commonly in summer, and therefore their fruiting is less constant (Heilmann-Clausen and Vesterholt, 2008).

With respect to Boletaceae, *Suillus bellini* occurred in VA, where xerophitic conditions are present, while *S. granulatus* and *S. luteus* were exclusively collected in PA, linked to higher precipitation and lower temperatures. *S. granulatus* occurrence was positively correlated to ETP and negatively to TAN, as also reported by Martínez-de-Aragón et al. (2007) who found a negative correlation between production for these species based on temperatures and a positive relationship with the potential evapotranspiration in September. In the case of *S. luteus,* our results showed a positive correlation between this taxa and the annual precipitation. On the contrary, its distribution was negatively correlated to TAN. A similar result was reported by Martínez-de-Aragón et al. (2007). These authors linked this species to water deficits and precipitation from September to November in Mediterranean conditions.

Laccaria laccata and *L. bicolor* were positively correlated to N, P as well to annual precipitation. Similar results were obtained by Azul et al. (2011) where *L. laccata* was closely related to undisturbed and fertile soils. Reverchon et al. (2012), showed positive correlations between P and N nutrients for both species in a Mexican pine site. These results contradict those obtained by Buée et al. (2011), who stated the negative impact of fertilisation on *Laccaria bicolor*, in coniferous stands in France.

All the species within the *Inocybe* genus were exclusively collected in the Valladolid sites. When CCA Axis 2 was analysed, a positive correlation between these species and PAN variable was observed. A similar trend was observed for *Hebeloma* genus, where *H. mesophaeum* and *H. cylindrosporum* were linked to PAN, while *H. psammophilum* was correlated to TAN. Martínez-de-Aragón et al. (2007) found similar trends when correlating species within these genera and climatic variables.

Some species from *Tricholoma* genus showed opposite trends. For example, *Tricholoma portentosum* can be considered as a hygrophilous species that fructifies in more humid years (Hernández-Rodríguez et al., 2013), on the contrary, *Tricholoma terreum* is a xerophilous species typical of Mediterranean pine forests, these findings corroborate the ecological amplitude of this genus in diverse edaphoclimatic conditions. Our results on saprotrophic taxa assemblies revealed a correlation with the higher precipitation values at PA. Taxa from VAC and VAS sites were highly correlated to rising temperatures, however differences between these two locations could be caused by potential evapotranspiration, creating more extreme xerophitic conditions in the VAS location. Also, soil nutrients such as N and K affect taxa composition. Higher nitrogen contents observed in PA plots result in a greater abundance of the most nitrophyllous species. Fungal mycelium has the physiological potential to act as both an expandable reservoir and a distribution system for elevated nitrogen inputs (Watkinson et al., 2006), and may account for the responsive nitrogen absorption of the forest floor (Currie, 1999).

When analyzing saprotrophic taxa by genera, we found several genera such as *Mycena*, *Galerina,* and *Cystoderma* which appeared in the three studied sites. The most represented genus was *Mycena,* which included 28 species. This genus is able to fruit under very different ecological conditions (Moore et al., 2008). Widespread occurrence of *Galerina* corresponds to the findings of Heilmann-Clausen and Vesterholt (2008), who defined it as a genus with broad ecological amplitude. The presence of *Cystoderma* species could be explained by a higher availability of substrate in mature stands like ours, where conditions of temperature and moisture are more adequate for fungal fruiting (Fernández-Toirán et al., 2006; Hernández-Rodríguez et al., 2013). At species level, *Lycoperdon perlatum* was found in the three locations. This same species was also present in other recent studies that were carried out under different ecological environments (Ágreda et al., 2014; Azul et al., 2011).

Species within *Macrolepiota* genus exclusively appeared in VAC plots. The Mediterranean ecology of the three recorded species can explain these findings (Ortega and Esteve-Raventós, 2005). *M. excoriata, M. konradii* and *M. mastoidea* are characterized by fruiting during the autumn season in forests areas of high light exposure, and prefer calcareous soils (Rotheroe, 2001).

It should also be noted that in these Mediterranean ecosystems, we found some species, of both mycorrhizal (*Gyroporus ammophilus* and *G. cyanescens* var. *lacteus*) and saprotrophic (*Conocybe dunensis*) taxa, that until now had only been reported in coastal sandy dunes.

Some of previously cited as hygrophilous species (Oria de Rueda et al., 2007) were found in Palencia due to its more humid characteristics such as *Auriscalpium vulgare*, *Cortinarius brunneus*, *Cortinarius croceus*, *Cystoderma amianthinum*, *Entoloma cetratum*, *Entoloma formosum*, *Hygrophorus hypothejus*, *Hygrophoropsis aurantiaca*, *Hypholoma fasciculare*, *Lactarius aurantiacus*, *Mycena flavoalba*, *Mycena sylvae-nigrae, Phanerochaete sanguinea*, *Psathyrella hydrophylla*, *Rickenella mellea*, *Suillus granulatus, Suillus luteus*, *Tapinella atrotomentosa*, *Tapinella panuoides*, *Tricholoma portentosum*, *Tricholomopsis rutilans*, *Russula sanguinea*, *Russula sardonia*, and *Russula xerampelina*. The results reported here show the fungal communities associated to *P. pinaster* Mediterranean forests under different environmental conditions. It is noteworthy the high fungal production and diversity that can be found associated to these forests. This is partially due to the great plasticity of this forest species under extreme Mediterranean conditions and also to the ability of fungal species, to fruit under harsh environmental conditions. These stands can be considered highly productive in terms of fungal yield and diversity, especially for Mediterranean ecosystems. Thus, an integrated management of these *Pinus* stands including a mycosilvicultural approach, aimed for combined timber and mushroom production, would also result in economic and ecological benefits, especially in these depressed areas. Therefore forest management practices should consider the influence of different ecological conditions on fungal production and diversity related to *P. pinaster*.

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STUDY II

Fungal community succession following wildfire in a Mediterranean vegetation type dominated by *Pinus pinaster* in Northwest Spain

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Fungal community succession following wildfire in Mediterranean vegetation type dominated by *Pinus pinaster* in Northwest Spain

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Abstract

This study examined the succession of fungal communities following fire in a Mediterranean ecosystem dominated by *Pinus pinaster* Ait. in northwestern Spain. A large wildfire occurred in August 2002. During the autumn seasons from 2003 to 2006, fruiting bodies were collected and identified, production in burned (early stage) and unburned (late stage) areas was measured. For statistical analysis, data were grouped into the following four categories: saprotrophic, mycorrhizal; edible and inedible. A total of 115 fungal taxa were collected during the four sampling periods (85 in the late and 60 in the early stage). The number of mycorrhizal species increased from early to late succession and there were shifts in community composition. After fire, pyrophytic species such as *Pholiota carbonaria, Peziza violacea, Rhizopogon luteolus* and *Rhizopogon sp.* appeared. Fire strongly affected the production of fungal species in the studied area. Thus, yields in the early stage treatment were significantly lower than those observed in the late stage. Total fungal fresh weight decreased from 209.95 kg fw ha^{-1} in the late stage to 162.45 kg fw ha^{-1}in the early stage when richness and production of mycorrhizal species and production of edible fungi were significantly lower. Fresh weight for saprotrophic and inedible species was higher than for mycorrhizal fungi in the early stage treatment. The results obtained can be useful to forest managers for optimization of management and harvesting of these increasingly appreciated non-timber resources.

Key words:

Fungal community succession, fungal production, fire, Mediterranean ecosystem

1. Introduction

Pinus pinaster Ait. is widely distributed in the Mediterranean region and is able to form fungal associations with a large number of different mushroom species. It is a typical pyrophytic species that has adapted to frequent wildfires, the main source of ecosystem disturbance in this region. *P. pinaster* stores its seeds in serotinous cones and their release is triggered by the heat generated during a fire. The released seeds germinate and grow rapidly as a selection pressure from fire (Dahlberg, 2002; Johnson, 1992). However, pines require mycorrhizal associates and the presence of active EM fungal propagules in a soil after fire is crucial for survival and later regeneration of seedlings (Dahlberg et al., 2001). Consequently, fungi are an integral part of forest function, most notably for their essential role in water and nutrient acquisition by trees (Smith and Read, 1997). Despite their important role in forest ecosystems, much is yet to be discovered about the response of EM fungal communities to fire (Bastias et al., 2006).

High-intensity fires, common in Mediterranean forests, strongly affect vegetation and fungal communities (Torres and Honrubia, 1997). These communities are also conditioned by the modifications of physical, chemical and biochemical soil properties caused by fire, mainly due to the transfer of heat into the soil. In this sense, high soil temperatures destroy soil organic matter, and alter soil nutrient and water status. Also pH increase is observed following fire favouring microbial community as found by Turrión et al. (in press) in a *P.pinaster* forest in Mediterranean area. In that study, total organic C concentrations increased mainly from dry leaves and partially burned plant materials. In this sense, saprotrophic fungal species can be favoured after fire.

Also following fire, an increase of available nutrients in soil is observed, mainly as water-soluble components of ash become available after fire. This effect is in part due to the increase in soil pH frequently observed after a fire. However, in general, bacteria are more tolerant to heat than fungi; therefore, it is usually observed that burning favours bacteria over fungi (González-Pérez et al., 2004)

Pre-fire fungal communities are largely eradicated by fires (Vázquez et al., 1993.) Subsequently, post-fire fungal succession is initiated by the first significant rain event that follows a disturbance. The pioneer fungal species are developed in the absence of spores typically produced during the early post-fire season. Their source is usually the spore bank in the soil that has built up over time since previous fire (Claridge et al*.,* 2009).

Fungal communities in early stage and late stages of succession are often similar, not only due to the similarity in host species, but also to the resilience of the fungal communities (Claridge et al., 2009; Visser, 1995).

Pine stands growing on a previously treeless site correspond to a primary succession and late stage fungi correspond to mature forest stable conditions (Visser, 1995). This succession concept can be applied to fungal development in stands that have regenerated after natural disturbances such as wildfires (Danielson, 1984; Visser, 1995).

Secondary succession beginning after fire depends on multiple factors such as initial plant species composition, fire intensity, seed bank availability, and ability of soil microbial communities to recover (Hart et al., 2005; Rodrigo et al., 2004). Thus, fungi play a fundamental role in the recovery of plant communities following fires through soil stabilization and restoration of soil microflora (Claridge et al*.,* 2009).

Moreover, edible fungi represent an important forest economic resource, occasionally generating higher benefits than timber production (Martín-Pinto et al., 2006). These fungi also play an important succession role in the regeneration of *Pinus pinaster* stands after wildfire (Martín-Pinto et al., 2006; Milne, 2002).

Therefore, the aim of this study is to analyse fungal community succession following wildfire in a Mediterranean vegetation type dominated by *Pinus pinaster* in Northwest Spain.

2. Materials and methods

2.1. Study site

The analysis was carried in a Mediterranean ecosystem dominated by Maritime pine (*Pinus pinaster*) where a large wildfire burned 1427 ha in August 2002. The spread of fire was mainly as an active crown fire, causing total tree mortality. However, under the canopy cover, fire impacts were quite different since the burning of *P.pinaster* needles resulted in the rapid spread of a low intensity fire.

This site is located at 750-780 m.a.s.l. in the northwest of Zamora province (NW Spain). The region has a sub-Mediterranean climate with 3 months of dry season in the summer, a mean annual rainfall of 700-750 mm and mean temperatures ranging from 14.5-15ºC.

The area has gentle slopes that vary from 0 to 20%. At the two sites, plots were selected to represent both the north and south aspects.

This area is composed of Paleozoic metamorphic rocks, dominated by Ordovician and Silurian shales (García Rodríguez et al., 1964). The soil is classified as Inceptisol suborder Xerept (Soil Survey Staff, 2006). The soils of this region are stony, acidic (pH 5.0 - 5.5), extremely low in calcium and phosphorus and variable in nitrogen and potassium availability. The humification degree is generally good (García Rodríguez et al., 1964).

The location is at 0706140-0706439 longitude-UTM, 4632495-4632901 latitude-UTM. “Late stage” treatment was conducted in an area dominated by 50-year-old *P. pinaster* Ait. plantations established by the Spanish Forest Services in previously deforested areas. These plantations had a sparse understory composed mainly of *Cistus ladanifer* L., *Quercus ilex* L. subsp. *ballota* (Desf.) Samp. and *Quercus pyrenaica* Willd. “Early stage” treatment was located in an area with the same initial characteristics of vegetation where tree and understory vegetation were totally destroyed by fire. However, partially burned plant materials were observed in the burned studied area.

Also, the soil in the burned plots was superficially affected and natural post fire regeneration of Maritime pine seedlings with 15% canopy cover was found together with *Cistus ladanifer L.* representing 60% canopy cover.

2.2. Sampling

Data for the study were collected during four autumn sampling campaigns between October and December 2003 to 2006 of twelve randomly selected *P. pinaster* stands. The study consisted of two treatments; early and late stage fungal communities after the fire. Six plots of 2 x 50 m per each treatment were established in accordance with previous studies conducted by Dahlberg and Stenlid (1994) and Ohenoja (1989). In the early stage treatment (burned area), plot distribution was designed to avoid direct interactions with roots from the adjacent unburned forested zones. Fungi were collected once a week as described by Dalhberg (1991) and Ohenoja (1984). Following Bonet et al. (2004) methods, sampling was carried out on Fridays to reduce error due to mushroom removals by recreational weekend collectors. At each sampling date, fungi were completely removed with stem facilitating the identification of fungal species and diminishing disturbance in the production calculation. Fruiting bodies were stored in the laboratory at 4ºC and processed within 24 hours after collection for identification and fresh weight (kg fw ha^{-1}) measurements.

2.3. Identification and classification

The sporocarps were identified at the species level according to the following keys: Andrés-Rodríguez (1990); Andrés-Rodríguez et al. (1999); Antonin and Noordeloos (2010); Arrillaga et al. (2000); Breitenbach and Kratzlin (1984; 1986; 1991; 1995; 2000; 2005); Bon (1987), Knudsen and Vesterholt (2008), Lage et al. (1981); Mendaza and Díaz (1994), Moser (1980), and Muñoz (1998). Some samples were only identified to the genus level and were grouped into genus taxa as described by Bonet et al. (2004) and Martín-Pinto et al. (2006). For statistical purposes, data were grouped into the following categories: saprotrophic/mycorrhizal, and edible/inedible.

2.4. Statistical analysis

Statistical analyses were conducted by data groups: an early stage period (1-4 year-old forest stands) postfire occurrence, and a late stage period (51-54 year-old stands).

Fresh weights, edibility and life strategies of the two treatments were analysed statistically. Data were subjected to a Repeated Measures ANOVA analysis and means were compared by LSD Fisher Tests ($P<0.05$). STATISTICA '08 Edition software (StatSoft Inc., 1984-2008) was used for the analysis.

3. Results

3.1. Early/late stage comparison

Fungi were classified into 115 different taxa (Table 1). According to Martín-Pinto et al. (2006) complete identification was not possible for some generic level taxa, most of which included more than a single species. In our study, the following taxa were not classified to the species level: *Clitocybe sp., Collybia sp.,* *Coprinus sp., Cortinarius sp., Entoloma sp., Hebeloma sp., Inocybe sp., Mycena sp., Omphalina sp., Rhizopogon sp., Russula sp.* and *Tricholoma sp.*

Table 1: Total taxa collected from *Pinus pinaster* plots

Taxa	Early stage				Late stage				Group	Edible	Market
	1	2	3	4	51	52	53	54			
Amanita muscaria (L.) Hook.				x					MY		
Baeospora myosura (Fr.) Singer	x	x		x	x	x	x	x	S		
Bjerkdera adusta (Willd. ex Fr.) Karst.							x		S		
Cantharellus cibarius Fr.	x								MY	E	M
Chroogomphus rutilus (Schaeff.) O.K. Mill.				x	x			x	MY	E	
Clavulina cristata (Holmsk.) J. Schröt.					x				MY	E	
Clavulina rugosa (Bull.) J. Schröt.								x	MY	E	
Clitocibe sp. (Tragthat.)							x		S		
Clitocybe angustissima (Lasch) Kumm.			x				x		S		
Clitocybe diatreta (Fr.) P. Kumm.				x		x		x	S		
Clitocybe obsoleta (Batsch) Quél.								x	S		
Clitocybe rivulosa (Pers.) P. Kumm.				x				x	S		
Clitocybe sect. fragantes								x	S		
Clitocybe sp. (Tragthat.)	x				x	x		x	S		
Clitocybe vibecina (Fr.) Quél.					x	x			S		
Collybia butyracea (Bull.: Fr.) Kumm.					x	x		x	S	E	
Collybia confluens (Pers.) P. Kumm.							x		S		
Collybia maculata (Alb & Schw.: Fr.) Kumm					x	x		x	S		
Collybia sp. (Fr.: Fr.) Staude							x	x	S		
Conocybe sp. Fayod							x		S		
Coprinus micaceus sensu Lange, auct.	x								S		
Coprinus sp Fr.			x				x		S		
Cortinarius sp. Fr.	x		x		x		x		MY		
Cortinarius trivialis (J.E. Lange) A.H. Sm.		x							MY		
Craterellus cornucupioides (L.:Fr.) Pers					x				MY	E	M
Cystoderma amianthinum (Scop.:Fr.) Fay					x			x	S		
Cystoderma cinnabarinum (Scop.:Fr.) Fay								x	S		
Dacrymyces stillatus Nees				x					S		
Dacrymyces variisporus Mc Nabb					x				S		
Dermocybe cinnamomeoluteus P.D.Orton						x			MY		
Entoloma sp. (Fr.:Fr.) Kumm.						x	x		S		
Entoloma subgen nolanea				x				x	S		
Exidia saccharina (Alb & Schw.) Fr.	x								S		
Galerina marginata (Batsch) Kühner								x	S		
Gomphidius roseus (Fr.) Fr.						x			MY		
Gymnopilus spectabilis (Fr.) Sing.		x	x	x					S		
Gymnopilus picreus (Pers.) P. Karst.				x					S		
Hebeloma mesophaeum (Pers.) Quél.			x					x	MY		
Hebeloma sp. Kummer			x			x	x		MY		
Hydnellum concrescens (Pers.) Banker					x	x		x	MY		

(Continued on the next page)

Table 1: continued

Taxa	Early Stage				Late Stage				Group	Edible	Market
	1	2	3	4	51	52	53	54			
Hydnum rufescens Fr.					x	x			MY	E	M
Hygrophorus agathosmus (Fr.) Fr.			x		x	x	x	x	MY	E	
Hygrophorus chrysodon (Fr.)				x					MY	E	
Hygrophorus gliocyclus (Fr.)				x	x	x		x	MY	E	
Hygrophorus hypothejus (Fr.: Fr.) Fr.					x			x	MY	E	
Hypholoma fasciculare (Cooke) E. Horak			x	x					S		
Inocybe geophilla Kummer								x	MY		
Inocybe maculata Boud.		x				x			MY		
Inocybe sp. Fr.		x	x	x	x	x	x	x	MY		
Inocybe sp2 Fr.				x					MY		
Inocybe sp3 Fr.								x	MY		
Inocybe sp 4 Fr.				x				x	MY		
Laccaria amethystina Cooke		x							MY	E	
Laccaria laccata (Scop. ex Fr.) Bk. & Br.	x		x						MY	E	
Lactarius deliciosus (L.: Fr.) S.F. Gray					x	x		x	MY	E	M
Lactarius tesquorum Malençon			x	x					MY		
Leccinum corsicum (Rolland) Bresinsky & Manfr. Binder				x					MY	E	
Lycogala epidendrum (J.C. Buxb. ex L.) Fr.			x						S		
Lycoperdon foetidum Bonord.								x	S		
Lycoperdon molle Pers.								x	S	E	
Lycoperdon perlatum Pers,: Pers.					x	x	x	x	S	E	M
Lyophyllum decastes (Fr.) Singer				x				x	MY	E	M
Lyophyllum fumosum (Pers.) P.D. Orton								x	MY		
Marasmius androsaceus (L.)Fr					x	x	x		S	E	
Marasmius collinus (Scop.) Singer						x			S		
Marasmius scorodonius (Fr.) A.W. Wilson						x		x	S	E	
Merulius tremellosus Schrad.				x					S		
Micromphale foetidum (Sowerby) Singer				x					S		
Mycena alcalina (Fr.) Kummer			x			x	x		S		
Mycena epipterygia (Scop.) Gray						x		x	S		
Mycena flavoalba (Fr.) Quél.				x				x	S		
Mycena galopus (Pers.) P. Kumm**.**		x				x			S		
Mycena haematopus (Pers.) P. Kumm.								x	S		
Mycena pura (Pers, Ex Fr.) Kummer			x			x	x	x	S		
Mycena seynii Quél	x	x	x	x	x	x	x	x	S		
Mycena sp (Fr.) Kummer				x				x	S		
Mycena sp1 (Fr.) Kummer			x				x		S		
Mycena sp2 (Fr.) Kummer							x		S		
Mycena viridimarginata P. Karst.				x					S		
Mycena vulgaris (Pers.) P. Kumm**.**				x				x	S		
Myxomphalia maura (Fr.) Hora		x	x						S		
Omphalina sp Lamoure								x	S		
Paxillus panuoides (Fr.) Fr.				x					S		

(Continued on the next page)

Table 1: continued

Taxa	Early Stage				Late Stage				Group	Edible	Market
	1	2	3	4	51	52	53	54			
Peziza violacea sensu Relhan (Fl. cantab.)	x								MY		
Pholiota carbonaria (Fr.) Sing.	x	x	x	x					S		
Psathyrella chondroderma (Berk. & Broom.) A.H. Sm.							x		S		
Pseudoclitocybe cyathyformis (Bull.: Fr.) Sing				x					S		
Ramaria gracilis (Pers.) Quél.				x					MY		
Rhizopogon luteolus Fr. & Nordh.	x				x				MY	E	
Rhizopogon roseolus (Corda) Th. Fr.						x			MY	E	
Rhizopogon sp Fr.				x					MY	E	
Russula torulosa Bres.						x			MY		
Russula heterophylla (Fr.) Fr.								x	MY	E	
Russula sp 1 Pers,: S.F. Gray		x							MY		
Russula sp grupo foetens		x							MY		
Russula sp. Pers,: S.F. Gray			x	x	x	x		x	MY		
Russula sp 2 Pers,: S.F. Gray						x			MY		
Russula sp3 Pers,: S.F. Gray						x			MY		
Russula sp.4 Pers,: S.F. Gray						x			MY		
*Russula sp.*5 Pers,: S.F. Gray						x			MY		
Russula sp 6 Pers,: S.F. Gray						x			MY		
Schizophyllum commune Fr.	x	x				x			S		
Sistotrema confluens Pers.					x				MY		
Stereum hirsutum (Willd. Ex Fr.)S.F. Gray		x	x	x		x	x	x	S		
Suillus bellinii (Inzenga) Watling					x	x	x	x	MY	E	M
Suillus collinitus (Fr.) Kuntze								x	MY	E	
Tremella mesenterica Schaeff.	x	x		x	x	x	x	x	S	E	
Tricholoma bufonium (Pers.) Gillet			x						MY		
Tricholoma equestre (L.) P. Kumm.					x	x		x	MY	E	
Tricholoma batschii Gulden					x	x		x	MY		
Tricholoma portentosum (Fr.) Quél.					x				MY	E	M
Tricholoma sp. (Sc.:Fr.) Kumm					x				MY		
Tricholoma terreum (Sch.:Fr.) Kumm					x	x	x	x	MY	E	M
Tricholomopsis rutilans (Sch.:Fr.) Kumm				x				x	S		

MY: mycorrhizal; S: saprotrophic; E: edible; M: marketed.

Sixty of the taxa were harvested in early stage treatment and 85 in late stage (Table 2), of which 30 were found to be common in early stage and late stage successions. Taxa were classified according to functional groups (mycorrhizal and saprotrophic), edibility and commercial status in the studied areas. From the total taxon list, 56 were identified as mycorrhizal and 59 as saprotrophic fungi. Thirty of the total taxa found were edible of which nine are marketed in the region (Table 2).

Table 2: Total taxa collected from *Pinus pinaster* plots, classified into species groups

Species groups	Early stage (years)				Late stage (years)				Total early stage	Total late stage
	1	2	3	4	51	52	53	54		
Mycorrhizal	5	6	9	14	21	22	6	23	27	40
Saprotrophic	8	9	12	20	11	19	19	27	33	45
Edible	4	2	2	7	17	13	6	17	12	23
TOTAL	13	15	21	34	32	41	25	50	60	85

In the early stage, 60 taxa were identified (45 % mycorrhizal and 55 % saprotrophic) of which 12 are edible and two sold in the markets. Conversely, 85 taxa were collected in the late stage of which 49 % were mycorrhizal and 51 % saprotrophic. Edible fungi represented 27 % (23 taxa) and eight were sold in the markets (Table 2).

Mycorrhizal species were significantly affected by wildfire; their number decreased from 40 in the late stage to 27 in the early stage. Saprotrophic fungi showed a similar trend decreasing from 45 in the late stage to 33 in early stage.

3.2. Production according to functional groups and edibility

A total production of 372.4 kg fw ha^{-1} was collected over the 4-year study period, where 162.45 kg fw ha^{-1} was from the early stage treatment and 209.95 kg fw ha^{-1} from the late stage treatment. Mycorrhizal species production was significantly affected by wildfire with a decrease from 36.23 kg fw ha^{-1} in the late stage to 7.54 kg fw ha^{-1} in the early stage (P-value = 10^{-6}, Fig. 1). Conversely, saprotrophic fungi showed an increase in production from 7.50 kg fw ha^{-1} in the late stage to 26.23 kg fw ha^{-1} in the early state (P-value = 0,00024; Fig. 1).

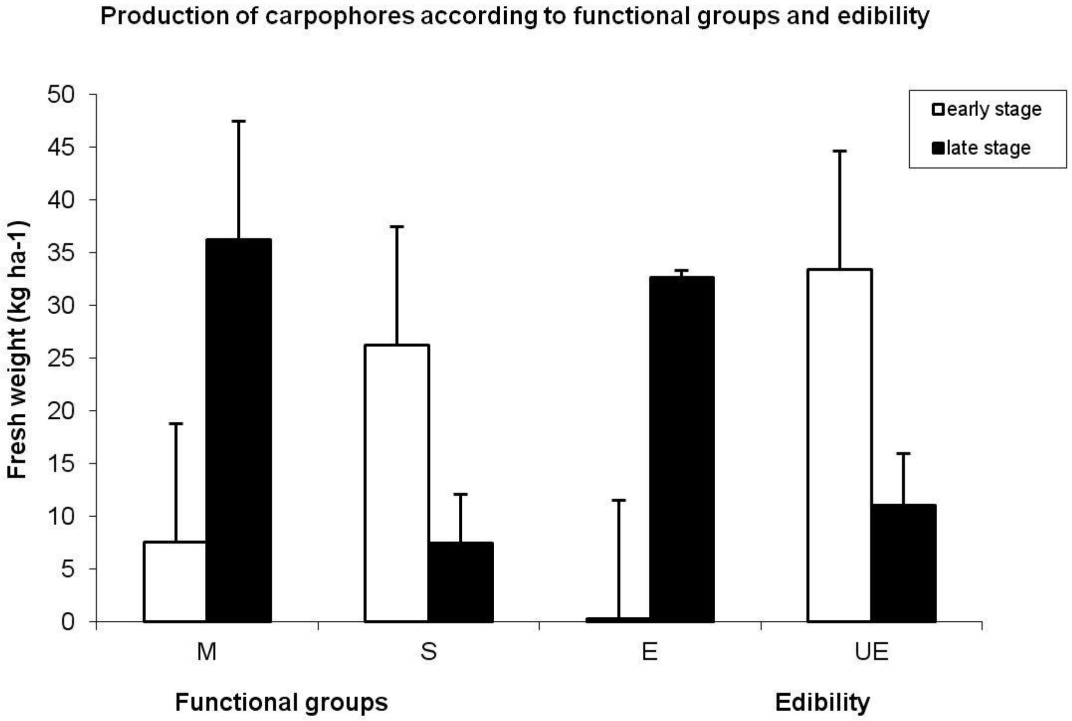

Fig. 1. Production of carpophores according to functional groups and edibility. (kg fw ha^{-1}). M: Mycorrhizal, S: Saprotrophic, E: Edible, IE: Inedible. The data are mean results ± standard error of the mean. Independent comparisons were carried out within functional groups and edibility. Values with the same letter are not significantly different.

Edible species yields were also strongly affected by wildfire, decreasing from 32.64 kg fw ha^{-1} in the late stage to 0.34 kg fw ha^{-1} of early stage production (P-value < 10^{-6} ; Fig. 1). For edible mycorrhizal species, the 4-year mean production of 20.54 kg fw ha^{-1} was significantly higher in the late stage (61.57 kg fw ha^{-1}) than in the early treatment (0,46 kg fw ha^{-1}) (Fig. 2). During the early stage period, production for edible mycorrhizal species was stable and no differences were found among the four analysed years. However, significant differences were observed during the late stage where fresh weight production varied from 1.45 to 113.42 kg fw ha^{-1}(P-value <10^{-6}).

Production for edible mycorrhizal species was significantly higher than observed edible saprotrophic species. Also, no differences were found for edible saprotrophic species before and following fire. 3.71 kg fw ha^{-1} were collected in the late stage and 0.22 kg fw ha^{-1} in the early stage (Fig. 2)

Several edible mushrooms sold in the local markets were especially impacted by fire. The following species had significantly lower production weights following the fire: *Chroogomphus rutilus, Hydnellum concrescens*, *Laccaria laccata, Lycoperdon molle Hygrophorus gliocyclus, H. agathosmus,, Lactarius deliciosus*, *Stereum hirsutum, Tricholoma fracticum.* The fresh weight production of these species decreased significantly following wildfire.

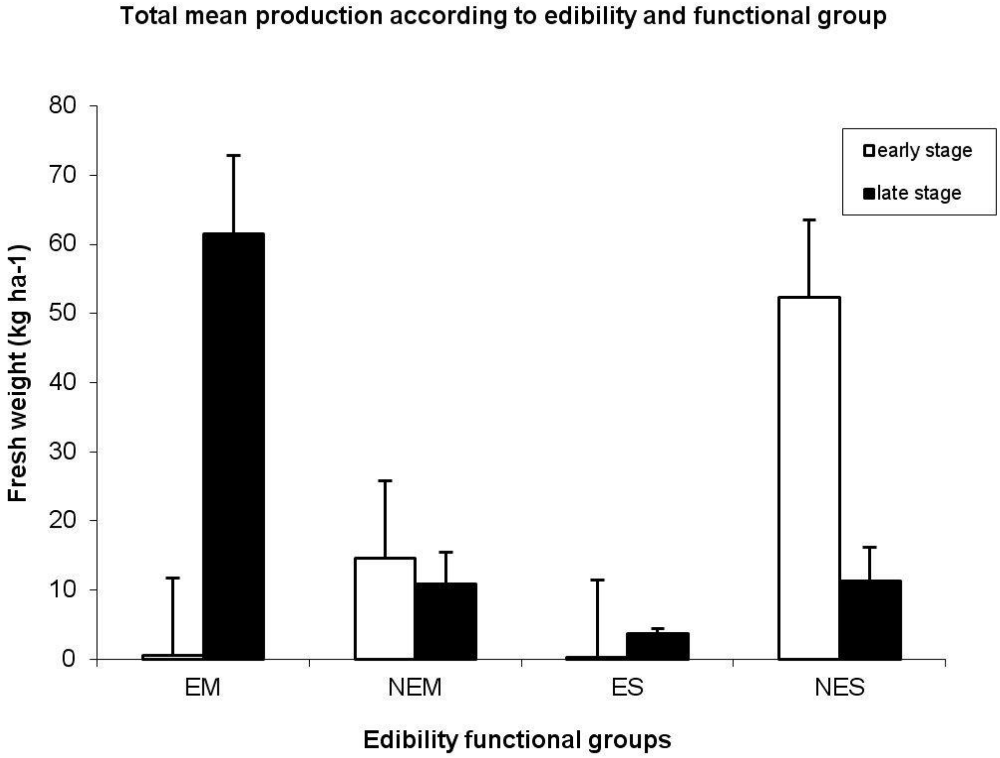

Fig. 2. Mushroom production regarding to edibility combined with functional groups. (kg fw ha^{-1}). EM: Edible-Mycorrhizal, IEM: Inedible-Mycorrhizal, ES: Edible-Saprotrophic, IES: Inedible-Saprotrophic. The data are mean results ± standard error of the mean. Values with the same letter are not significantly different.

4. Discussion

4.1. Early/late stage comparison

Fungal diversity and production have utility to describe the general biodiversity in forest ecosystems (Arnolds, 1992, Hawksworth, 2001). Although the study plots are located on extremely poor, degraded and stony soils, 115 fungal taxa were recorded during the four-year sampling period. A high proportion (85 out of 115) was late stage fungi. This result reveals a very high fungal richness, compared to the 49 taxa found by Oria-de-Rueda et al. (2010) in a 50-year old reforested *P. pinaster* areas growing on degraded and eroded soils in northwestern Spain were also studied. Moreover, Bruns et al. (2002) found lower species richness (15-35 fungal species) in a 0.1 ha pine stand.

Our results also showed a higher richness than that observed by Fernández-Toirán et al. (2006), who obtained 60-80 taxa in a 15 ha *P. pinaster* natural stands through 4 and 6-year sampling periods in the northwest of Spain. Our results are in agreement with Allen et al. (1995), who noted that natural conifer forests have high fungal richness. Even though the analysed *P. pinaster* reforested areas are located on very poor and stony soils, the large number of collected species could be due to the siliceous conditions where a high number of fungal species is frequently collected.

In addition, *Cistus ladanifer* plants found growing in the study area contributed towards the maintenance of high fungal richness. These shrubs act as a bridge for ectomycorrhizal fungi as many of the fungal species can establish symbiotic relationships with a wide range of hosts, forming mycorrhizal relationships with both shrubs and trees (Milne, 2002; Oria-de-Rueda et al., 2008). *Arctostaphylos* species have been reported to facilitate the establishment of mycorrhizal communities in the coniferous forest stands in the chaparral areas in California (Horton et al., 1999).

Adverse effects of fire on the number of fungal species in the early stage were observed (data not shown), in accordance with our previous study of the same *Pinus pinaster* stand when first-year data were analysed (Martín-Pinto et al*.,* 2006). Studies conducted by Allen et al. (1984); Martín-Pinto et al. (2006); Parke et al. (1984); Salerni and Perini (2004), confirmed the decrease in abundance of many macrofungal species after fires.

Fire can dramatically change surface soil characteristics and erosion rates (Amaranthus, 1989) and also strongly affect fungal communities causing significant effects on soil microorganisms in general (Perry et al*.,* 1987). Furthermore, vegetation mortality can result in large changes in the mycorrhizal communities as a result of the elimination of the host (Dahlberg et al*.,* 2001). The reduction of fungal species suggests that burning of vegetation affects spore production and infection potential of some fungal species (Vilarino and Arines, 1991). Subsequent loss of topsoil by erosion also reduces infectivity of fungal propagules (Rashid et al*.,* 1997). Other studies have also found a decline of fungal species richness after disturbances (Durall et al*.,* 1999; Mah et al*.,* 2001) as it usually occurs after high-intensity fire.

Overall, we found that functional groups had an approximate to 1:1 ratio between mycorrhizal and saprotrophic fungal species. Roberts et al. (2004) observed a similar result in six habitats in Vancouver Island, British Columbia over a five-year period, whereas Straatsma et al. (2001) found that there were about twice as many ectomycorrhizal as saprotrophic fungi in a Swiss forest formed by a mixture of deciduous trees and conifers. Other studies report higher percentages of mycorrhizal species, for example Bonet et al. (2004) reported 88% and Väre et al. (1996) 59% mycorrhizal taxa. Those studies were carried out on natural *P. sylvestris* stands in areas with optimal ecological conditions for the growth of this species. The relatively high proportion of saprotrophic compared to mycorrhizal fungi observed in the late stage could be due to the presence of high amounts of organic matter in the forests. Decomposition rates are particularly low in the Mediterranean ecosystems characterized by no coincidence of high precipitation levels and high temperatures (Oria-de-Rueda et al*.,* 2010).

In our study, the number of fungal species recorded was affected by the fire and decreased from the late to the early stage. Higher mycorrhizal richness across the forest chronosequence was previously observed by Fernández-Toirán et al. (2006). These results are not in agreement with other previous works

(Dighton and Mason, 1985). Decreasing mycorrhizal richness in old growth forests was observed due to litter accumulation and eutrophication that makes these stands susceptible to higher intensity fires.

Immediately after fire, a sharp decrease in fungal richness is observed since some fungi are not capable of growing under the new conditions. Eventually, however, a progressive increase in fungal richness is observed. The first year of study, 13 species were collected, whereas 34 were found in the fourth year in the early stage treatment and 50 species were found in the late stage. These data support the ability of fungi to adapt to the new vegetation and environmental conditions following fire (Danielson, 1984; Visser, 1995). In this sense, there are some post-fire fungi that appear to benefit from this disturbance since they fruit as a result of fire (Hart et al*.,* 2005). Their source is usually the spore bank in the soil built up over time from previous fires and capable of retaining viability for many years. Some fungal spores are dormant and require heat treatment to germinate (Claridge et al., 2009; Izzo et al*.,* 2006). Such fungal species normally fruit during the first significant rain event following disturbance and are not the product of spores dispersed during that season as the same species fruiting elsewhere have yet to produce spores (Claridge et al., 2009).

Post-fire fungi employ different life strategies, including carbonicolous fruiting on charcoal or partially burned organic debris; terrestrial fruiting on ash, or heated soil and some fungi appear to be mycorrhizal symbionts with pathogens on tree roots (Claridge et al*.,* 2009; Egger and Paden, 1986). Carbonicolous ascomycetes act as early colonizers of new plants after fire as a result of elimination of other competing fungi and the new conditions created by the perturbance (Torres and Honrubia, 1997).

These post-fire fungi can grow as early-stage fungi following fire. Post-fire species such as *Pholiota carbonaria, Peziza violacea, Rhizopogon luteolus* and *Rhizopogon sp.* were harvested in *Pinus* early stage plots. This result is in accordance with those of previous research conducted by Claridge et al. (2009); Fujimura et al. (2004); Martín-Pinto et al. (2006); Rochadi et al. (1999), who found these species or genera. The role of post-fire fungi is essential to a healthy post-fire recovery of forests as they actively develop soon after wildfire (Hart et al., 2005 and Jiménez Esquilín et al., 2007). Other well known pioneer fungal species were collected in the early stage treatment, such as *Laccaria amethystina, L. laccata, Mycena* spp.*, Gymnopilus spectabilis and G. picreus*. These species, which could be considered as super-pioneer species, appear in the first years of succession (Fernández-Toirán et al., 2006; Torres and Honrubia, 1997). Although *Lactarius deliciosus* or *Suillus bellini* are considered pioneer species, they were not present in the early stage treatment (1-4 years following fire). This result is in agreement with Fernández-Toirán et al. (2006) who found *L. deliciosus* to be a dominant species in 11-20-years old stand.

Some saprotrophic species such as *Lycoperdon* spp. and *Marasmius* spp. were dominant in the late stage treatment. These species are adapted to the environmental conditions created by the great amount of available organic matter in the soils of mature pine stands (Senn-Irlet and Bieri, 1999).

Mycorrhizal populations not only increased the number of species from early to late stage but also shifted in composition across the chronosequence, as previously reported by Kranabetter et al. (2005); Fernández-Toirán et al. (2006). Nevertheless, some of the mycorrhizal species such as *Hebeloma* spp. and *Inocybe* spp. were frequently collected in both stages. This observation is in agreement with Fleming (1983) and Mason et al. (1983).

4.2. Production according to functional groups and edibility

High total fresh weight production (372.4 kg fw ha^{-1}) was harvested during this research. Lower production values were referenced in our first year analysis (Martín-Pinto et al., 2006) with an average plot yield of 332.7 kg ha^{-1}. Similarly, lower results were reported by Bonet et al. (2004) in *P. sylvestris* natural stands with a total fresh weight production of 60.6 kg fw ha^{-1}.

The high average fresh weight observed in the late stage plots (209.95 kg fw ha^{-1}fresh weight) could have resulted from the quick growth rates obtained for this host species and the active metabolism especially in poor soils with low pH values frequently observed in burned areas.

Fire strongly affected the production of fungal species in the studied area. Yields in the early stage treatment were significantly lower than those observed later. The negative effect of fire on fungal production has been investigated in previous studies by Cairney and Bastias (2007); Claridge and Trappe (2004); Dahlberg et al. (2001); Hart et al. (2005); Jiménez Esquilín et al. (2007); Martín-Pinto et al. (2006); Torres and Honrubia (1997) and Vernes et al. (2004). Lower yields found in the early stage plots (162.45 kg fw ha^{-1}) can be also explained by the presence of a lower vegetative biomass present in the ecosystem.

The reduction of sporocarp production for mycorrhizal species following fire was also detected, similar to a finding reported by Martín-Pinto et al. (2006). A pontential explanation for this finding is that mycorrhizal fungi require more energy from the host plant and have slower growth rates and therefore, are less competitive after fire events (Torres and Honrubia, 1997). A decrease in fungal populations following disturbance is indicated in various studies (Arnolds, 1988; Salerni and Perini, 2004). As indicated by Fellner and Peskova, (1995) and Parke et al. (1984), this phenomenon is more commonly reported among mycorrhizal species. However, saprotrophic species showed significantly higher yields in the early stage treatment, in accordance with the assessment that spore germination of certain saprotrophic post-fire fungi is stimulated by heat (Dahlberg, 2002). Furthermore, saprotrophic growth pattern allows some of these fungal species to survive short periods of live-host absence by persisting on the dead host root systems or other organic matter (Bonello et al., 1998).

Regarding edible taxa, harvest of mushrooms from forests can be an important source of rural income, in some cases generating higher revenues than timber production (Oria-de-Rueda et al., 2010). During the last decade, there has been a sharp increase in the demand for edible fungi. The commercial value of forests can be increased through well-planned timber removals that may improve the habitat for commercially valuable edible mushrooms and provide wood and employment at the same time (Bonet et al., 2004; Pilz et al., 1999; Pilz et al., 2003; Wang and Hall, 2004). In this study, fresh weight production of edible taxa represented 26% of the total taxa but comprised 42.55% (158.36 kg fw ha^{-1}) of total fresh weight yield. Oria-de-Rueda et al. (2010) reported 294.8 kg fw ha^{-1} of edible fungal species production was recovered in *P. pinaster* stands located in the North Plateau of Spain.

Fresh weight production for edible species was also strongly affected by fire decreasing significantly from 32.64 kg fw ha^{-1} in the late stage to 0.34 kg fw ha^{-1} in the early stage. In the study region, *Pinus* ecosystems provide a habitat for high production of total fungi including species in demand, such as *Hygrophorus gliocyclus*, *H. agasthomus, Suillus bellini, Tricholoma portentosum* and *Lactarius deliciosus.*

In conclusion, very high fungal richness was found in the *P. pinaster* studied forests, characterized by extremely poor and degraded soils. Following fire, fungal succession can be observed. Thus, post-fire, super-pioneer, pioneer and late stage species were found. After a fire, mycorrhizal and saprotrophic fungal productions were differently affected. In this study, mycorrhizal mushroom yields increased from early to late stage in contrast to the trend recorded for saprotrophic taxa. Finally, edible species production was affected by fires and would significantly decrease the potential of rural populations to collect mushrooms to sell in the market place. Since this is an economically depressed area, this reduction after a fire will impact regional livelihoods.

To counter the impacts of fire on the production of fungi in these forests, several strategies may be worth pursuing. Stands can be used to improve tree health and vigour, which is likely to increase the production of mycorrhizal fungi. Management may also prevent or alleviate stand-replacing wildfire in these Mediterranean forests.

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STUDY III

Post-fire production of mushrooms in *Pinus pinaster* forests using classificatory models.

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Post-fire production of mushrooms in *Pinus pinaster* forests using classificatory models

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Abstract

This study was aimed at describing post-fire mushroom production in a Mediterranean ecosystem dominated by *Pinus pinaster* Ait. in the northwest of Spain and assessing the results by classificatory models. During the autumn periods of 2003 to 2006, fruit bodies from 115 fungal taxa were collected in burned and unburned areas and were further grouped into the following categories: saprotrophic/mycorrhizal; edible/non-edible. After wildfires, a significant reduction in the number of fungal species and fruit body biomass production was observed.

Based on this relevant information the first simple classificatory model for this aim is provided. Nine alternative models based on classifications according to combinations of edibility and functional groups were fitted and four fruiting body biomass production classes were defined as possible responses. As explanatory factors, time after fire and climatic variables significantly related to fruit body production were included. The best predictive results were obtained for edible and edible-mycorrhizal models, for which the correct classification rate of production classes was between 92 and 85%

Moreover, obtained models were applied to analyse the effect of time after fire on fungal production. Mycorrhizal and edible fungal production after fire was classified into the lowest class, whereas saprotrophic and non-edible species followed a contrary trend. The classificatory models can be useful to optimize management and harvest of these increasingly appreciated non-timber forest resources.

Key words Classificatory models, Fire, Mushroom production, *Pinus pinaster*

1. Introduction

Pinus pinaster Ait. is a conifer widely distributed in the Mediterranean region able to associate symbiotically with a large number of different fungal species (Gassibe et al., 2011). It is a typical pyrophytic species adapted to frequent wildfires, the main source of disturbance in Mediterranean ecosystems. Among other fire related traits, *P. pinaster* is partially serotinous, as it stores some of its seeds in cones which only open by heat generated during a fire allowing for a rapid post-fire regeneration of the stands (Tapias et al., 2004).

High-intensity fires, common in Mediterranean ecosystems, strongly affect vegetation and fungal communities (Torres and Honrubia, 1997). After fire, pyrophytic fungi are key in soil stabilization, habitat restoration, recovery of damaged plants, or nutrient recycling from dead vegetation (Claridge et al., 2009). Fungi also play an important role in the regeneration of *Pinus pinaster* stands after wildfire (Martín-Pinto et al. 2006) although overall, the number of fungal species, their fruit body biomass production and their economic value are negatively affected by fire (Hart et al., 2005; Gassibe et al., 2011).

Besides detrimental effects of fires on fruit body production the fruiting phase is also affected by microclimatic and macroclimatic factors. Many efforts have been made to assess the relative importance of environmental factors (Vogt et al., 1992) and complex predictive equations for mushroom yields, including soil temperatures, evapotranspiration, relative humidity, rainfall, and subsequent water fluctuations (Martínez de Aragón et al., 2007). However, rainfall and temperature are generally recognized as the most important factors in several studies. Martínez de Aragón et al. (2007) found total production positively correlated to mean annual precipitation, although this correlation improved from precipitation months just before and during the fruiting season. In addition, Straatsma et al. (2001) reported a correlation between productivity and precipitation from June to October in a mixed forest in Switzerland, and Bonet et al. (2010) found that mushroom production is correlated with precipitation from August until November in pine forests in north-eastern Spain. Concerning temperature, Martínez-Peña (2003) found a negative influence between *Boletus* production and the maximum temperature two weeks before sampling week. Traditional wisdom from commercial harvesters from the study zone, has found that summer rainfall is a salient factor influencing location and quantity of fall-fruiting mushrooms. However, these broad generalizations can be improved by taking into account some features of the life history of fungal species. For example, Straatsma et al. (2001) found that mycorrhizal and saprotrophic species have different cues for fruiting time, as mycorrhizal species fruit earlier than saprotrophic ones when summer temperatures are higher.

Different approaches have been used to test the effect of environmental variables upon fungal fruiting such as correlations (Vogt et al., 1992), stepwise multiple regressions (Martínez de Aragón et al., 2007) or mixed models (Bonet et al., 2010). Despite the usefulness of some of these models, fungal fruit body production is still regarded as a highly stochastic phenomenon and thus, predicting an exact fungal production value remains elusive. The use of linear discriminant functions facilitates explicit consideration of fire impact and other relevant environmental factors on mushroom productivity when planning land-use activities. This approach has been previously used, for instance, to classify timber productivity (Bravo et al., 2011), soil types (Wall and Westman, 2006) or habitat selection (Rodríguez et al., 2007). By using discriminant functions we can interpret the ecological variables that characterize groups, to predict group assignment, validate the model and score new observations into groups with known probability. Both interpretation and classification uses can be achieved with discriminant functions.

As a result, from the point of view of mushroom production, the development of tools such as simple classificatory models can help to optimise forest management and increase commercial value of forests (Pilz et al., 1999; Bonet et al., 2004; Yun and Hall, 2004). Apart from their ecological importance, much of the recent interest on fungi resides on their direct economic value (Cai et al., 2011) as during the last decade, there has been a sharp increase in the demand for edible fungi. Indeed, mushroom harvesting, as a non-timber forest product can be an important source of rural income, generating higher revenues than timber production in some cases (Oria-de-Rueda et al., 2010).

Therefore, the aim of this study was to develop and test classificatory models for mushroom production, applied to a particular post-fire scenario in a Mediterranean vegetation type dominated by *Pinus pinaster* in the northwest of Spain.

2. Materials and methods

2.1. Study site

The study was carried out in a Mediterranean ecosystem dominated by 50-year old *P. pinaster* Ait. plantations established by the Spanish Forest Services. The study site is located at, 750-780 m above sea level in northwest Zamora province (6.518056-6.503611W longitude, 41.820278-41.831111N latitude). This region has a sub-Mediterranean climate with a 3-month dry season in the summer, a mean annual rainfall of 700-750 mm and mean temperatures ranging from 14.5-15ºC. The soil is classified as Inceptisol.

A large wildfire burned 1.427 hectares on August 2002 leaving some areas unaffected. The understory in unburned sites was composed mainly of *Cistus ladanifer* L., *Quercus ilex* L. subsp. *ballota* (Desf.) Samp. and *Quercus pyrenaica* Willd. (Martín-Pinto et al., 2006). Fire also affected the soil surface unevenly, and our study plots were located in areas where fire affected the soil only superficially. There, natural post-fire regeneration of Maritime pine seedlings with 15% canopy cover was found together with *Cistus ladanifer L.* representing 60% canopy cover in the last year of study.

2.2. Sampling

In autumn 2003, six (2x50) m^2 plots were randomly located within burned and unburned areas in accordance with previous studies (Ohenoja, 1989; Dahlberg and Stenlid, 1994). Overall, plots were evenly distributed in the stand so average distance of plots within burned or unburned areas was not significantly different from distance of plots from burned and unburned areas. In the burned area, plot distribution was designed to avoid direct interactions with roots from the adjacent unburned forested zones. Plots in unburned areas reflected a late stage of fungal ecological succession while those in areas affected by fire showed the earliest stages of succession.

Fruit bodies found in the plots were collected weekly between October and December from 2003 to 2006 as described by Dahlberg (1991). Following Bonet et al. (2004), Friday was established as the sampling day in order to reduce error due to mushroom removals by recreational weekend collectors. Each individual fruit body was completely removed from the ground including the base of the stem, facilitating the identification of fungal species and diminishing bias in biomass production estimation. Fruit bodies were stored in the laboratory at 4ºC and subsequently identified and weighed (fresh weight per species, plot and week) within 24 hours after collection.

2.3. Climatic data

Detailed climatic data including temperatures and rainfall from the period 2003-2006 were provided by the closest meteorological station (Villardeciervos, (6.278465W longitude, 41.943442N latitude, 850 m above sea level, Spanish Meteorological Agency) located 13 km from the farthest plots (Table 1).

Table 1: Climate variables tested for relationship with sporocarp production

TF		*T(ºC)*	*T min(ºC)*	*T max (ºC)*	*P(mm)*	*ETP(mm)*
B	*UB*					
1	51	11.95	6.65	16.17	961.73	701.64
2	52	11.18	5.76	15.56	489.94	673.12
3	53	11.23	5.37	16.08	448.90	684.33
4	54	12.01	6.63	16.35	1032.23	704.21

TF: Time after fire; *B:* Burned plots; *UB:* Unburned plots; *T:* Monthly mean soil temperature; *Tmin*: Monthly mean minimum temperature; *Tmax:* Monthly mean maximum temperature; *P:* Annual mean precipitation; *ETP*: Annual mean *ETP* according to Thornthwaite (1955)

2.4. Statistical analysis

Fresh weight production data were subjected to a Repeated Measures ANOVA comparing means by LSD Fisher Tests ($P < 0.05$) with STATISTICA '08 Edition software (StatSoft Inc., 1984–2008).

Fruit body fresh biomass weight per area (kg fw ha^{-1}) was summed across weeks for each year and biomass data based on edibility (non-edible and edible), life strategy (mycorrhizal and saprotrophic) and combinations such as edible mycorrhizal (*EM*), inedible mycorrhizal (*IEM),* edible saprotrophic (*ES*) and inedible saprotrophic (*IES*) were obtained. Data from nine different classifications (Table 2) were used as dependent variables in the modelling process. For each dependent variable, fruit body biomass production was divided into four yield categories and quantified into kg fw ha^{-1} according to our previous fungal yield data in the studied area (Table 2).

Table 2: Sporocarp production classes (kg fw ha^{-1}).

CLASSES	*T*	*E*	*IE*	*M*	*S*	*EM*	*IEM*	*ES*	*IES*
I	> 100	>43	>57	>56	> 44	>40	>16	>3	>41
II	50-100	21.5-43	28.5-57	28-56	22-44	20-40	8-16	1.5-3	20.5-41
III	10 -50	4.3-21.5	5.7-28.5	5.6-28	4.4-22	4-20	1.6-8	0.3-1.5	4.1-20.5
IV	< 10	<4.3	<5.7	<5.6	<4.4	<4	<1.6	<0.3	<4.1

T=Total; *E*=Edible; *IE*=Inedible; *M*=Mycorrhizal; *S*=Saprotrophic; *EM*= Edible Mycorrhizal; *IEM*= Inedible Mycorrhizal; *ES*= Edible Saprotrophic; *IES* = Inedible Saprotrophic

A simple classificatory model was obtained for each dependent variable using discriminant analysis. Time after fire (*TF*) and climate variables were the independent variables used in the models. Forest stand age (in years) after fire (*TF)* took values between 1 and 4 for the burned plots and a between 51 and 54 for the unburned plots.

Mean potential evapotranspiration (*ETP*), was calculated for the latitude of Villardeciervos according to Thornthwaite and Mather (1955), substituting monthly mean soil temperatures for mean air temperature (Martínez de Aragón et al. 2007) since mean soil temperature near the soil surface (<10 cm) has been shown to coincide with the monthly mean air temperature.

As a result, nine discriminant classificatory models were fitted to determine the most likely of the four yield categories within each dependent variable. The structure of the general discriminant rules was:

$$\beta_0 + \sum \beta_i X_i$$

Where:

$\beta_0, \beta_1, \ldots \beta_n$ are parameters

X_is are time after fire (*TF)* and climatic variables

In order to define and evaluate the classification criteria, the original database was used to cross validate the model. The resulting error-count estimate has an optimistic bias and is described as an apparent error rate (Bravo and Montero 2001).

The PROC DISCRIM procedure from SAS 9.1 was used.

Finally, the different models were applied in order to evaluate the estimated influence of time after fire on fungal production. With this purpose, mean values of the climatic variables were set as constants and only time after fire was modified.

3. Results

Total mean annual fresh weight production was 67.6 kg ha^{-1} in the burned plots and 87.5 kg ha^{-1} in the unburned treatment. These values did not differ statistically (P=0.13). Considering species classification according to the life strategy and the edibility, total fresh weight production for burned and unburned plots were: edible-mycorrhizal (unburned: 61.6 kg ha^{-1}, burned: 0.5 kg ha^{-1}, P<0.001); inedible-mycorrhizal (unburned: 10.9 kg ha^{-1}, burned: 14.6 kg ha^{-1}, P=0.47); edible-saprotrophic (unburned: 3.7 kg ha^{-1}, burned: 0.2 kg ha^{-1}, P=0.49); inedible-saprotrophic (unburned: 11.3 kg ha^{-1}, burned: 52.3 kg ha^{-1}, P<0.001). Table 3 shows the most ecologically and economically important taxa found during the study and the fraction of total biomass represented by them, classified according to edibility and life strategy.

Table 3 Fungal taxa collected from *Pinus pinaster* plots and fraction (percentage) of total biomass represented by each species.

Group	Taxa	B	U	Group	Taxa	B	U
EM	*Cantharellus cibarius* Fr.	3,1		ES	*Lycoperdon molle* Pers.		1,2
	Chroogomphus rutilus (Schaeff.) O.K. Mill.	4,6	7,9		*Lycoperdon perlatum Pers.*		2,6
	Clavulina cristata (Holmsk.) J. Schröt.		0,7		*Tremella mesenterica* Retz.	0,6	1,7
	Clavulina rugosa (Bull.) J. Schröt.		0,3	IES	*Baeospora myosura* (Fr.) Singer	1,4	1,3
	Craterellus cornucopioides (L.) Pers		1,5		*Bjerkdera adusta* (Willd. ex Fr.) Karst.		1
	Hydnum rufescens Pers.		1,9		*Clitocybe vibecina* (Fr.) Quél.		0,9
	*Hygrophorus gliocyclus*Fr.	2,5	3,6		*Collybia confluens* (Pers.) P. Kumm.		0,8
	Hygrophorus hypothejus (Fr.) Fr.		1,4		*Collybia maculata* (Alb & Schwein.) P. Kumm.		0,5
	Laccaria laccata (Scop.) Cooke	3,2			*Coprinellus truncorum* (Scop.) Redhead	0,2	
	Lactarius deliciosus (L.) Gray		4,5		*Cystoderma amianthinum* (Scop.) Fayod		1,2
	Leccinum corsicum (Rolland) Singer	18			*Cystoderma cinnabarinum* (Alb. & Schwein.) Fayod		1
	Lyophyllum decastes (Fr.) Singer	5,9	2,7		*Exidia saccharina* Fr.	0,4	
	Rhizopogon luteolus Fr.	6,8	4,2		*Galerina marginata* (Batsch) Kühner		0,5
	Rhizopogon roseolus (Corda) Th. Fr.		2,7		*Gymnopilus picreus* (Pers.) P. Karst.	1,7	
	Russula heterophylla (Fr.) Fr.		1,9		*Hypholoma fasciculare* (Huds.) P. Kumm.	4,1	
	Suillus bellinii (Inzenga) Watling		12		*Lycogala epidendrum* (J.C. Buxb. ex L.) Fr.	0,1	
	Suillus collinitus (Fr.) Kuntze		4,8		*Lycoperdon foetidum* Bonord.		0,3
	Tricholoma equestre (L.) P. Kumm.		1,9		*Marasmius androsaceus* (L.)Fr		0,1
	Tricholoma portentosum (Fr.) Quél.		3		*Mycetinis scorodonius* (Fr.) A.W. Wilson & Desjardin		0,2
	Tricholoma terreum (Schaeff.) P. Kumm.		4,3		*Merulius tremellosus* Schrad.	0,6	
IEM	*Amanita muscaria* (L.) Lam.	4,9			*Mycena alcalina* (Fr.) P. Kumm.	0,7	1,3
	Collybia butyracea (Bull.)P. Kumm.		3,5		*Mycena epipterygia* (Scop.) Gray		0,5
	Cortinarius trivialis J.E. Lange	2,1			*Mycena galopus* (Pers.) P. Kumm.	0,8	1,1
	Dermocybe cinnamomeoluteus P.D.Orton		1,7		*Mycena pura* (Pers.) P. Kumm.	1,1	1
	Gomphidius roseus (Fr.) Fr.		0,6		*Mycena seynii* Quél	1,8	2,3
	Hebeloma mesophaeum (Pers.) Quél.	1,7	2		*Mycena vulgaris* (Pers.) P. Kumm.	0,6	0,9

Group	Taxa	B	U	Group	Taxa	B	U
IEM	*Hydnellum concrescens* (Pers.) Banker		0,7	IES	*Myxomphalia maura* (Fr.) Hora	1,3	
	Inocybe geophylla (Fr.) P. Kumm.		0,4		*Paxillus panuoides* (Fr.) Fr.	1,8	
	Lactarius tesquorum Malençon	5,2			*Phaeolepiota aurea* (Matt.) Maire	3,9	
	Lyophyllum fumosum (Pers.) P.D. Orton		0,8		*Pholiota carbonaria* (Fr.) Sing.	15	
	Ramaria gracilis (Pers.) Quél.	2,9			*Pseudoclitocybe cyathyformis* (Bull.) Singer	0,6	
	Russula torulosa Bres.		1,9		*Schizophyllum commune* Fr.	1,7	0,5
	Tricholoma batschii Gulden		7,3		*Stereum hirsutum* (Willd.) Pers.	1,2	0,9

EM: Mycorrhizal and edible; ES: Saprotrophic and edible; IEM: Mycorrhizal and inedible; IES: Saprotrophic and inedible; B: Burned plots; U: Unburned plots

3.1. Simple classificatory models for fungal production

Nine simple classification models (table 4) were obtained, one for each of the dependent variables (production by mushroom type and total production) using a classificatory discriminant analysis. $T_{\min}$; $T_{\max}$; *PREC* and *ETP* were used in the total and edible mycorrhizal production models. *T* and *ETP* were used for inedible saprotrophic production models. *ETP* was used for inedible, saprotrophic, edible saprotrophic and inedible mycorrhizal models. *T* and *PREC* were used as independent explanatory variables in the mycorrhizal and edible species production classificatory model (Table 4).

Table 4: Sporocarp production (kg fw ha $^{-1}$) classificatory models according to discriminant rules.

D1

	K	*TF*	*T*	*Tmin*	*Tmax*	*PREC*	*ETP*
T	-1.72E+09	-13.23	-	6.33E+08	7.46E+08	1.15E+06	-1.67E+07
E	-46,770	100.60	8926	-	-	-13.09	-
IE	-1,800	-17.79	-	-	-	-	5.31
S	-2,405	-21.63	-	-	-	-	7.15
M	-44,682	71.09	8526	-	-	-12.52	-
EM	-1.77E+09	-4.21	-	6.49E+08	7.65E+08	-1.18E+06	-1.71E+07
ES	-1,547	-0.54	-	-	-	-	4.49
IEM	-1,631	- 0.03	-	-	-	-	4.68
IES	-5,954	1.24	-816.47	-	-	-	31.06

D2

	K	*TF*	*T*	*Tmin*	*Tmax*	*PREC*	*ETP*
T	1.72E+14	-11.63	-	6.33E+08	7.46E+08	1.15E+06	-1.67E+07
E	-47,313	101.00	8978	-	-	-13.16	-
IE	-1,898	-19.3162	-	-	-	-	5.46
S	-2,548	-22.45	-	-	-	-	7.35
M	-45,194	72.37	8575	-	-	-12.60	-
EM	-1.77E+09	-4.22	-	6.49E+08	7.65E+08	-1.18E+06	-1.71E+07
ES	-1,487	-0.51	-	-	-	-	4.40
IEM	-1,568	- 0.02	-	-	-	-	4.59
IES	-6,078	1.27455	-818.84	-	-	-	31.27

D3

	K	*TF*	*T*	*Tmin*	*Tmax*	*PREC*	*ETP*
T	-1.72E+09	-13.51	-	6.33E+08	7.46E+08	1.15E+06	-1.67E+07
E	-47,390	102.00	8985	-	-	-13.18	-
IE	-1,858	-19.35	-	-	-	-	5.40
S	-2,512	-22.79	-	-	-	-	7.31
M	-45,247	72.71	8580	-	-	-12.61	-
EM	-1.77E+09	-4.28	-	6.49E+08	7.65E+08	-1.18E+06	-1.71E+07
ES	-1,499	-0.52	-	-	-	-	4.42
IEM	-1,605	- 0.03	-	-	-	-	4.64
IES	-6,111	1.34	-819.57	-	-	-	31.34

D4

	K	*TF*	*T*	*Tmin*	*Tmax*	*PREC*	*ETP*
T	1.72E+09	-13.51	-	6.33E+08	7.46E+08	1.15E+06	-1.67E+07
E	-47,410	102.83	8987	-	-	-13.19	-
IE	-1,893	-19.58	-	-	-	-	5.45
S	-2,578	-23.44	-	-	-	-	7.40
M	-45,185	71.84	8574	-	-	-12.60	-
EM	-1.77E+09	-4.52	-	6.49E+08	7.65E+08	-1.18E+06	-1.71E+07
ES	-1,562	-0.68	-	-	-	-	4.52
IEM	-1,621	- 0.02	-	-	-	-	4.67
IES	-6,137	1.3241	-820.74	-	-	-	31.39

E: Edible; *IE*: Inedible; *M*: Mycorrhizal; *S*: Saprotrophic; *EM*: Mycorrhizal and edible; *ES:* Saprotrophic and edible; *IEM:* Mycorrhizal and inedible; *IES*: Saprotrophic and inedible; *T:* Total.

Independent variables: *K*: Constant; *TF* = Time after fire; *T*= monthly mean soil temperature; *Tmin*= monthly mean minimum temperature; *Tmax*= monthly mean maximum temperature; *P*= annual mean precipitation; *ETP*= annual mean ETP according to Thornthwaite (1955).

D1, D2, D3, D4: Discriminant rules to classify observations into production classes I, II, III, IV.

The error rates of the discriminant rules ranged for the nine models from 8% for the edible *(E*) to 70 % for inedible mycorrhizal (*IEM*) classes (Table 5). The leave-one-out cross-validation of the production classes for the selected classificatory models is shown in Table 6.

Table 5: Error rates by production classes for the nine models.

	I	II	III	IV	Total	CCR
E	0.00	0.00	1.00	0.00	0.08	0.92
IE	0.17	1.00	1.00	0.00	0.39	0.61
S	0.23	0.63	0.82	0.25	0.43	0.57
M	0.08	1.00	1.00	0.21	0.41	0.59
EM	0.00	0.00	1.00	0.17	0.15	0.85
ES	0.36	1.00	0.66	0.17	0.33	0.67
IEM	0.64	0.45	1.00	0.81	0.70	0.30
IES	0.23	1.00	1.00	0.63	0.34	0.66
Total	0.25	0.20	0.47	0.35	0.35	0.65

E: Edible; *IE*: Inedible; *M*: Mycorrhizal; *S*: Saprotrophic; *EM*: Mycorrhizal and edible; *ES*: Saprotrophic and edible; *IEM*: Mycorrhizal and inedible; *IES*: Saprotrophic and inedible; T: total; CCR: Correct Classification Rate. I, II, III, IV: Production classes

The leave-one-out cross-validation of the production classes for the selected classificatory models is shown in Table 6.

Table 6: Cross validation of the production classes for the nine models using fresh weight and climate data of the time series of Zamora pine stands.

E	I	II	III	IV
I	100%	0.00%	0.00%	0.00%
II	0.00%	100%	0.00%	0.00%
III	0.00%	50.00%	0.00%	50.00%
IV	0.00%	0.00%	0.00%	100%

IE	I	II	III	IV
I	83.33%	0.00%	11.11%	5.56%
II	25.00%	0.00%	12.50%	62.50%
III	50.00%	0.00%	0.00%	50.00%
IV	0.00%	0.00%	0.00%	100%

M	I	II	III	IV
I	92.31%	7.69%	0.00%	0.00%
II	0.00%	0.00%	50.00%	50.00%
III	23.08%	15.38%	0.00%	23.08%
IV	21.43%	0.00%	17.24%	78.57%

S	I	II	III	IV
I	76.92%	7.69%	15.38%	0.00%
II	25.00%	37.50%	0.00%	37.50%
III	36.36%	18.18%	18.18%	27.27%
IV	0.00%	0.00%	25.00%	75.00%

EM	I	II	III	IV
I	100%	0.00%	0.00%	0.00%
II	0.00%	100%	0.00%	0.00%
III	0.00%	50.00%	0.00%	50.00%
IV	0.00%	0.00%	17.24%	82.76%

ES	I	II	III	IV
I	64.00%	0.00%	27.27%	9.09%
II	66.67%	0.00%	33.33%	0.00%
III	66.67%	0.00%	33.33%	0.00%
IV	17.86%	0.00%	0.00%	82.14%

IEM	I	II	III	IV
I	35.71%	14.29%	0.00%	50.00%
II	0.00%	54.55%	0.00%	45.45%
III	14.29%	42.86%	0.00%	42.86%
IV	37.50%	43.75%	0.00%	18.75%

IES	I	II	III	IV
I	76.92%	0.00%	15.38%	7.79%
II	40.00%	0.00%	0.00%	60.00%
III	28.57%	0.00%	0.00%	71.43%
IV	0.00%	0.00%	25.00%	37.50%

Total	I	II	III	IV
I	75.00%	0.00%	11.67%	13.33%
II	20.00%	80.00%	0.00%	0.00%
III	17.65%	11.76%	52.94%	17.65%
IV	14.29%	0.00%	21.34%	64.29%

E: Edible; *IE:* Inedible; *M*: Mycorrhizal; *S:* Saprotrophic; *EM*: Mycorrhizal and edible; *ES*: Saprotrophic and edible; *IEM:* Mycorrhizal and inedible; *IES*: Saprotrophic and inedible; I, II, III, IV: Production classes

For the edible species production model, the correct classification was 100 % in classes I, II and IV; misclassification in class III was concentrated in the contiguous production classes II and IV. No errors were observed for edible mycorrhizal production in classes I and II and the misclassifications in class III were distributed between adjacent classes II and IV (50% - 50% respectively). In the model for saprotrophic species, the best classifications were observed for classes I and IV where error rates varied from 0.23 to 0.25 respectively (Table 5). A similar trend was observed for mycorrhizal species where production in classes II and III was accurately predicted and in classes I and IV the error rate was low (error rates: 0.08 and 0.21). Total production model showed a total correct classification rate of 65% (Table 5). In this case, correct classification ranged from 52.94% in class III to 80% in class II.

Model application for mushrooms according to functional groups and edibility

The models for functional groups (mycorrhizal and saprotrophic) showed a strong detrimental predicted effect of fire on mycorrhizal species. Indeed, production decreased to the minimum (production class IV) during the first four years after fire, whereas highest production class was reached for these species in the unburned plots according to the models (Table 7). A contrary trend was observed for the saprotrophic group. Thus, production was classified into class II for areas affected by fire while production was predicted to decrease to class IV in the unburned areas (Table 7).

Table 7: Prediction the succession of production classes according to time after fire

TF	*E*	*IE*	*M*	*S*
1	IV	I	IV	II
2	IV	I	IV	II
3	I	IV	IV	II
4	I	IV	IV	II
51	I	IV	I	IV
52	I	IV	I	IV
53	I	IV	I	IV
54	I	IV	I	IV

TF: Time after fire, *E*: Edible; *IE*: Inedible; *M:* Mycorrhizal; *S*: Saprotrophic. I, II, III, IV: Production classes

In addition, predicted time after fire effect on fungal production was analysed depending on edibility. Edible fungal group production decreased to class IV during the first and second years after fire and reached the highest production (class I) thereafter. Conversely, non-edible fungal species showed a contrary trend, with a post-fire production predicted to be class I and a sudden decrease to class IV after the two first years until the end of the modelled time period.

4. Discussion

4.1. Simple classificatory models for fungal production

To our knowledge, our results provide the first classificatory simple model for fungal production in *P. pinaster* forests.

Occasionally, forest stand variables which can be managed are used to define predictive fungal production models (Bonet et al. 2010). Although climate variables cannot be modified by the managers, they can explain between 60% and 80% of the variation in mycorrhizal fungal sporocarp production (Dahlberg 1991). The influence of precipitation and temperature has been previously studied in different countries (Dahlberg 1991; Straatsma et al. 2001; Martínez-Peña 2003; Martínez de Aragón et al. 2007; Bonet et al. 2010). In our study, climate variables, T, $T_{\min}$, $T_{\max}$, P and ETP, were used to explain the production rates of the different fungal groups. In addition, inclusion of time following fire in all the obtained models was useful to determine the time necessary to allow edible fungal production to recover after fire.

Overall 92% accuracy of classification has been found using the model developed for edible fungi. Several other researchers have also published predictive models for fruit body biomass production, following different approaches. Using mixed modelling applied to edible species data, Bonet et al. (2010) reported a R^2 coefficient of 0.214 including “plot” as a factor and an improvement up to 0.710 when “year” was accounted for. In that case, dominant tree species, slope and regeneration method appeared to be non-significant in the mixed model. R^2=0.52 was obtained in the model proposed by Martínez de Aragón et al. (2007) regarding edible species in different *Pinus* species stands, where P (September + October), *ETP (*September + October) and $T_{\min}$ (August) were the climatic variables included in the model.

Time after fire, $T_{\min}$, $T_{\max}$, *PREC*, and *ETP* were the variables involved in the classificatory simple model for total fungal production for which a correct classification rate of 65% was achieved. For total production, Bonet et al. (2010) found R^2=0.239 and R^2= 0.774 per plot and year factor models respectively, using the same variables as those in edible models. Martínez de Aragón et al. (2007) observed a R^2=0.66 in their predictive models for total mushroom production.

As noted by Martínez de Aragón et al. (2007), precipitation solely does not adequately reflect the amount of water available to plants and fungi. Thus, temperature plays an essential role by influencing the *ETP* process. Martínez-Peña (2003) found the maximum true evapotranspiration (*ETRMP)* in October to be the most significant climate variable positively correlated with production of some *Boletus* spp. in *P. sylvestris* forest stands.

Water availability has a positive correlation with host biomass productivity and higher photosynthetic activity is associated with higher production of mycorrhizal fungal species (Ortega-Martínez et al. 2011). Thus, factors providing adequate conditions for tree growth should be included in the yield predictive or classificatory models since they also influence fungal communities associated with their hosts.

Model application for mushrooms according to functional groups and edibility

A reduction of mycorrhizal species fruit body production following fire was reported in previous studies in the same area (Martín-Pinto et al., 2006). In this sense, mycorrhizal fungi are poorly competitive after fire events since they require more energy from the host plant and have slower growth rates (Torres and Honrubia, 1997). A decrease in fungal populations following forest disturbance has been shown in various studies (Arnolds, 1988; Salerni and Perini, 2004). As proposed by Fellner and Pešková (1995), this phenomenon is intensified among mycorrhizal species. Fire strongly affects fungal communities with significant consequences on soil microorganisms in general (Perry et al., 1987). Vegetation mortality can result in large changes in the mycorrhizal community because of host elimination (Dahlberg et al., 2001). Decrease of fungal species production suggests that vegetation burning affects spore concentration and infection potential of some mycorrhizal fungal species (Vilarino and Arines, 1991). Subsequent loss of topsoil by erosion also reduces infectivity of fungal propagules (Rashid et al., 1997).

Saprotrophic species showed higher yields in the burned treatment, in accordance with the observations that spore germination of certain saprotrophic post-fire fungi is stimulated by heat (Dahlberg, 2002). Furthermore, the saprotrophic growth pattern allows some of these fungi to survive without a live host by persisting on the dead host root systems or other organic matter (Bonello et al., 1998).

Higher production of saprotrophic compared to mycorrhizal fungi observed in the burned treatment can be due to the presence of high amounts of organic matter left after fire from *Cistus ladanifer* and *Pinus pinaster* woody debris, which are very difficult to breakdown as decomposition rates are particularly low in Mediterranean ecosystems. Consequently, many saprotrophic fungi are considered post-fire species that appear to benefit from this disturbance since they fruit as a result of fire (Hart et al., 2005). Their inoculum is usually the spore bank in the soil built up over the time since a previous fire which can retain viability for many years. Some fungal spores are dormant and require heat treatment to germinate (Claridge et al., 2009). Such fungal species normally fruit during the first significant rain event following (Claridge et al., 2009).

Harvests of many edible mushrooms have declined over the past century, because of changes in their natural environment caused by various natural and social factors (Yun and Hall, 2004). Our results demonstrate that fresh weight production for edible mushrooms was strongly affected by fire during the two years following fire. In the studied region, *Pinus* ecosystems provide habitat for a great production of total fungi including these species.

A better understanding of the ecology of fungi can help to establish an adequate management in these forest stands which is likely to improve the production of mycorrhizal fungi (Savoie and Largeteau, 2011). Management may also prevent or alleviate stand-replacing wildfire in these Mediterranean forests.

Although these models could provide a useful tool for forest managers, many ecological factors are involved in the fungal production process and further analysis is needed for ecological and management implications.

However, the utility of the defined models is really interesting in these marginal Mediterranean ecosystems where wildfire is one of the major disturbance factors. Since the studied area is one of the most disturbed by wildfires in Spain these models are useful for operational foresters.

Moreover, non-timber forest resources are in higher demand, and they occasionally provide as much income as those obtained from timber harvests. In this sense, classificatory models to estimate edible mushroom production can be used to optimize the management of these forests.

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STUDY IV

The effects of fire severity on ectomycorrhizal inoculum and allometric features in *P. pinaster* seedlings

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The effects of fire severity on ectomycorrhizal inoculum and allometric features in *P. pinaster* seedlings

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Abstract

- **Context** Mycorrhizal fungi in Mediterranean forests play a key role in the complex process of recovery after wildfires. A broader understanding of an important typical pyrophitic species as *Pinus pinaster* and its fungal symbionts is thus necessary for forest restoration purposes.
- **Aims** This study aims to assess the effects of ECM on pine seedlings and how fire severity and soil depth affect ECM propagules.
- **Methods** We studied *P. pinaster* root apexes from seedlings grown in soils collected one year after fire in undisturbed sites, sites moderately affected by fire and, sites highly affected by fire. Natural ectomycorrhization was observed at two root depths (0-10 cm and 10-20 cm). We also measured several *P. pinaster* seedlings morphometric parameters, which were used to examine the influence of fire severity and soil chemistry upon them.
- **Results** The ectomycorrhizal infection in the unburned soils for both root depths was higher than in soils that had been affected by fire. Inversely, seedling vegetative size increased according to fire severity.
- **Conclusion** Fire severity affected soil properties and mycorrhizal colonisation one year after occurrence, thus affecting plant development. These considerations can result as an approach for the successful establishment *of P. pinaster* in Mediterranean after wildfire.

Key words: Wildfire, mycorrhizal fungi, Maritime pine, bioassay

1. Introduction

Wildfires constitute the main source of disturbance in Mediterranean forest ecosystems (Gassibe et al., 2011), strongly disturbing soil fungi and plant communities (Carney and Bastias, 2007; Dahlberg et al., 2001; Rincón and Pueyo, 2010). This comes both as a direct result of the high temperatures reached during a forest fire, but also indirectly through changes to soil features, like a pH increase, enhanced hydrophobicity or changes of available nutrients (Certini, 2005).

Ectomycorrhizal fungi (ECM) play an important role in the colonization of new areas of land or in the restoration of the vegetative community following a disturbance (Claridge et al., 2009; Martínez-de-Aragón et al., 2001; Rincón and Pueyo, 2010). Indeed, survival of tree seedlings strongly depends on the rapid formation of an efficient root system, determined by the development of mycorrhizal symbiosis but also by favourable ecological conditions (Jackson et al., 2007; Palfner et al., 2008). ECM help their plant symbionts by improving water and nutrient uptake from the soil, carbohydrate distribution and growth substance production and avoiding water losses and root desiccation (Rincón et al., 2007). All this contributes to prevent environmental stress caused by drought (Scattolin et al., 2014), pathogenic agents (Martín-Pinto et al., 2006a) or heavy metal pollutions.

Then, how does fire affect the ability of soil fungi to establish new mycorrhizal symbiosis with emerging seedlings helps us to understand forest recovery after fire. ECM inoculum may survive in infected roots, as sclerotia, or as resistant spores (Baar et al., 1999; Horton et al., 1998), likely coming from deep soil horizons (Claridge et al., 2009). Abundant research has shown that ECM can be particularly active soon after wildfire (Claridge et al., 2009; Hart et al., 2005; Jiménez-Esquilín et al., 2007). For example, a study in a *Pinus muricata* forest in California (Baar et al., 1999) showed that ectomycorrhizae formed within six months after a stand-replacing wild fire.

But however present and active in forest soils after fire; ECM fungal communities do suffer consequences from forest fires. Many studies report large shifts in species abundances, decreases in diversity, large losses in ECM sporocarp production, and even complete loss of ectomycorrhizal species after severe fire (Gassibe et al., 2011; Grogan et al., 2000; Martín-Pinto et al., 2006b; Treseder et al., 2004). Furthermore Dahlberg et al. (2001) observed in a Scandinavian boreal forest, that different fire severities affected mycorrhizal flora after 4 months of prescribed burns, killing them all in high intensity burning treatments, while that in slight burned treatments part of the existing mycorrhizae were kept in the mineral soil. Thus, soil horizon also mediates the impact of fires on soil fungi (Bastias et al., 2006; Jiménez-Esquilín et al., 2007; Kipfer et al., 2010; Ponzetti et al., 2007). This is evident as heat is greater closest to the surface, and it is within the top few centimeters of soil where the majority of fungal communities are located (Cairney and Bastias 2007**;** Dahlberg et al., 2001; Kipfer et al., 2010).

In summary, after a forest fire, the extent of the decrease in species richness (Hernández-Rodríguez et al., 2013; Smith et al., 2005) and relative abundance (Jonsson et al., 1999) and its further impact on forest regeneration depend on many factors such as variations in the intensity/severity of the fire, time elapsed after fire, the frequency of burning and soil depth (Cairney and Bastias, 2007; Turrión et al., 2012). However, while the direct effect of fire is mainly destructive for existing fungal communities, at the same time fire also provides large inputs of new, mainly competition-free resources and substrates for fungi and plants to use (Penttilä and Kotiranta, 1996).

Pinus pinaster is a common widespread conifer species in Western Mediterranean countries, whose forests typically suffer from frequent forest fires. Varying fire regimes have even selected for different fire-related trait across *P. pinaster* populations, like bark thickness, serotiny (Tapias et al., 2004) and precocity (Santos-del-Blanco et al., 2012). *P. pinaster* is also an obligate mutualist with ectomycorrhizal fungi and normal growth does not occur without them (Smith and Read, 1997; Read, 1998). Given its ecological relevance and intimate relationship between forest fires and fungi, *P. pinaster* has been the focus of several studies on how does fire affect fungal communities (Gassibe et al., 2011; Martín-Pinto et al., 2006b; Rincón et al., 2010).

A devastating crown-fire occurred in Central Spain in 2008 providing the opportunity to increase further this knowledge. Here, we looked at how vegetative traits of *P. pinaster* seedlings differ when they are grown in soils exposed to different fire severity, having as a consequence different biochemical properties and likely different potential for ECM infection. We performed a bioassay experiment under controlled conditions and measured frequency of ECM infected roots at two different depths and seedling vegetative traits. Considering the potentially beneficial effects of ECM on pine seedlings and how fire severity and soil depth affect ECM propagules, our hypothesis was that ECM-infection would be more abundant in undisturbed soils and at deeper soils layers, having this a beneficial effect on seedling growth.

2. Materials and methods

2.1. Study site

Our study was performed in Central Spain, in the Autonomous Community of Castilla y León, the third European region in size and one of the most strongly damaged by wildfires. According to the European Forest Fire Information System (EFFIS), 1996 fires occurred in the region during 2008 affecting 152.64 km^2 (Quintano et al., 2011). The fire season occurs during the period of June-September, corresponding to the warm to hot and dry summer, typical of Mediterranean climate.

The study was carried out in a Mediterranean ecosystem dominated by *Pinus pinaster* plantations established by the Spanish Forest Services in previously deforested areas (440901-443169 longitude-UTM, 4592 704-4590583 latitude-UTM, 750-880 m.a.s.l.). Here, a large wildfire burned 1200 ha of forest and canopies in August 2008, time at which pine trees were about 40 years old. This site has a supra-Mediterranean climate with 3 months of dry season in the summer, a mean annual rainfall of 480-500 mm and mean temperatures ranging from 8 to 13 °C. The warmest month is July and the coldest January. These data were provided by the closest meteorological station (Linares del Arroyo 41º 31´ 40´´ N, 3º 32´ 72´´ W) located 15 km from the study area.

This area is composed of Paleozoic metamorphic rocks, dominated by Ordovician and Silurian shales (Barrenechea and Rodas, 1992). The soil is classified as Inceptisol suborder Xerept (Alvarez et al., 1993).

In the forest understory, sparse individuals of *Cytisus scoparius* (L.) Link, *Quercus faginea* Lam and *Quercus ilex* (L.) subsp. *ballota* (Desf.) Samp. where found.

The main fungal species found by us in previous surveys at this location were *Collybia butyracea*, *Inocybe sp*., *Astraeus hygrometricus*, *Rhizopogon luteolus*, *Hebeloma mesophaeum*, *Laccaria laccata*, *Baeospora myosura*, *Tricholoma scalpturatum*, *Hemimycena sp*., *Collybia sp*., *Mycena pura*, *Mycena pura subsp. lutea*, *Hygrophorus gliocyclus*, *Cystoderma amianthinum*, *and Cortinarius cinnamomeus-luteus* at sites not affected by fire. While *Galerina sp*., *Pholiota carbonaria*, *Omphalina sp*., *Gerronema sp* were found in places moderately affected by fire and only pyrophitic *Pholiota carbonaria* was found at those sites fully impacted by the fire.

2.2. Sampling

Fire severity was classified following Rincón and Pueyo (2010) criteria: control site was established in an adjacent *P. pinaster* forest unaffected by fire at least in the preceding 40 years (dominant trees of approximately 40-cm diameter); moderate fire severity site where those with all pine crowns and upper barks burned, and high fire severity sites those with pines, canopy and understorey litter totally burned and the entire humic soil organic layer consumed. In mid-June 2009, a total of 15 intact soil blocks (22 x 22x 20 cm), five per site, were extracted randomly with a metal cube with a sharpened edge at the high severity fire (HSF), moderate severity fire (MSF) and adjacent unburned (UB) sites. Soil blocks where then placed into square plastic containers of the same dimensions and taken to the greenhouse facilities of the Forest Engineering Faculty, Palencia Campus, University of Valladolid. Nine soil samples, three from each site, were also extracted using a cylindrical (2 cm radius, 20 cm deep, 250 cm^3) soil borer (Taylor, 2002) for soil analysis at Agrarian Technologic Center (ITAGRA), located in the same campus.

Glasshouses where naturally lit, with controlled temperature (15-20°C) and humidity, maintaining the substrate at field capacity. Twenty five *P. pinaster* seeds were used per container. All seeds were surface sterilized with hydrogen peroxide 30% for 30 min and washed tree times with sterile water. Seeds were sown into each container and tap-watered daily. No nutrients were added. Four months after sowing, five randomly selected seedlings were extracted from each container.

2.3 Mycorrhizal status and morphometric measurements

Each plant was rinsed with tap water in a plastic tray and gently shaken to soften and remove adhering soil. Tap root length and shoot length were measured in millimeters with a ruler (Palfner et al., 2008). Dry biomass of shoots and roots were determined after drying the plant material at 70ºC for 48 h. in a drying oven (Sousa et al., 2011). Root/shoot ratio was derived from these measurements (Palfner et al., 2008).

Roots were divided into two depth segments, from 0-10 cm and to 10-20 cm (Anderson et al., 2007). All root tips were classified morphologically and anatomically as mycorrhizal vs. non-mycorrhizal or “non vital” (Agerer, 1987-2002; 1991; de Román and de Miguel, 2005; Scattolin et al., 2008), using a Leica M3 dissecting microscope and a 15x magnification stereomicroscope. The percentage of total mycorrhization in each soil sample and depth level (%M) was calculated by dividing the number of mycorrhizal root tips into the total number of root tips (Brundrett et al., 1996).

For practical reasons we did not identify ectomycorrhizal root tips to the species level, since molecular and morphology based techniques are used for that matter and require extensive database (Dickie et al., 2006), but will be carried out on the next experiment to asses which are the existing ECM species most fit for seedling outplanting in a post fire scenario .

2.4. Statistical analysis

Data were subjected to a Repeated Measures ANOVA and means were compared by LSD Fisher Tests ($P < 0.05$). STATISTICA ’08 Edition software (StatSoft Inc., 1984-2008) was used for the analysis. A Principal Component Analysis (PCA) was performed based on Pearson product-moment correlation coefficient after a DCA (Detrended Correspondent Analysis), in which the longest gradient length was < 3.0. Fire severity parameters were classified as 1 (UB), 2 (MSF) and 3 (HSF). Only those variables showing a relatively strong relationship to the first two PCA axes (vector length greater than 0.5 units) are shown.

3. Results

3.1. Plant development

Mortality rates were low and did not affect more than three plants per container. Seedlings grown in high severity fire soils (HSF) had heavier and longer shoots ($P < 0.01$ and $P=0.030$, respectively) and longer roots ($P=0.004$) than those grown in moderate severity fire (MSF) and unburned (UB) substrates (Fig. 1). In turn, root length from MSF sites was also longer than that from UB sites ($P=0.042$). Root/root ratio was highest for HSF and MSF sites and lowest for UB samples (HSF vs MSF $P=0.798$; HSF vs UB $P=0.0000$; MSF vs UB $P=0.0001$) (Fig.1).

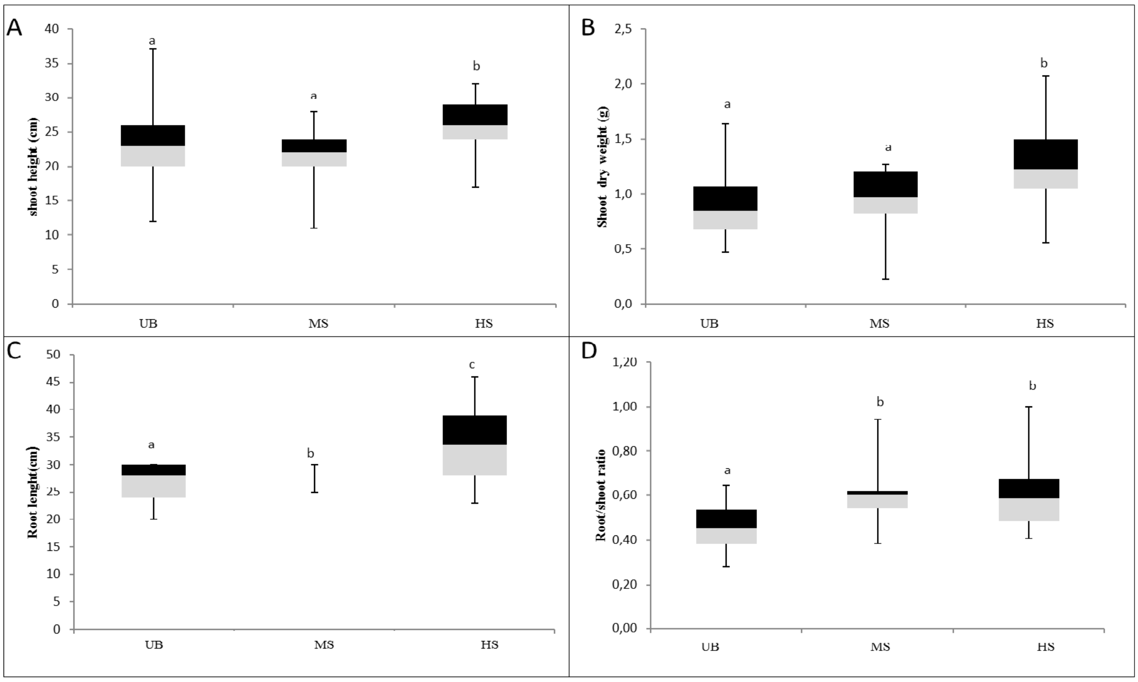

Fig.1. Morphometric parameters of P. pinaster seedlings grown in a bioassay with forest soils taken from Unburned (UB), Moderate severity fire (MSF) and High severity fire (HFS) sites after a forest fire in Central Spain (Honrubia de la Cuesta, Segovia); A shoot height; B shoot dry weight; C tap root length; D root/shoot ratio; box plots showing mean values (n=10) and 5th and 95th percentile. Box plots marked with different letters differed significantly according to LSD Fisher test ($P < 0.05$).

3.2 Mycorrhization

The proportion of total mycorrhization in the seedlings from both post-fire treatments was significantly lower than that from UB soils (HSF, $P=0.006$; MSF $P=0.005$). In relation to depth, higher levels of mycorrhization ($P<0.01$) were observed in the upper (0-10cm) layer compared to the lower one (>10 cm) for all three sites (Fig. 2). At the upper layer, we observed a higher colonization rate in UB sites when comparing them to the MSF ($P=0.012$) and HSF ($P=0.044$) sites respectively (Fig. 2). The same trend was followed by colonization in deeper layers, but in this case no differences were observed between seedlings from MSF soils compared to the other two sites.

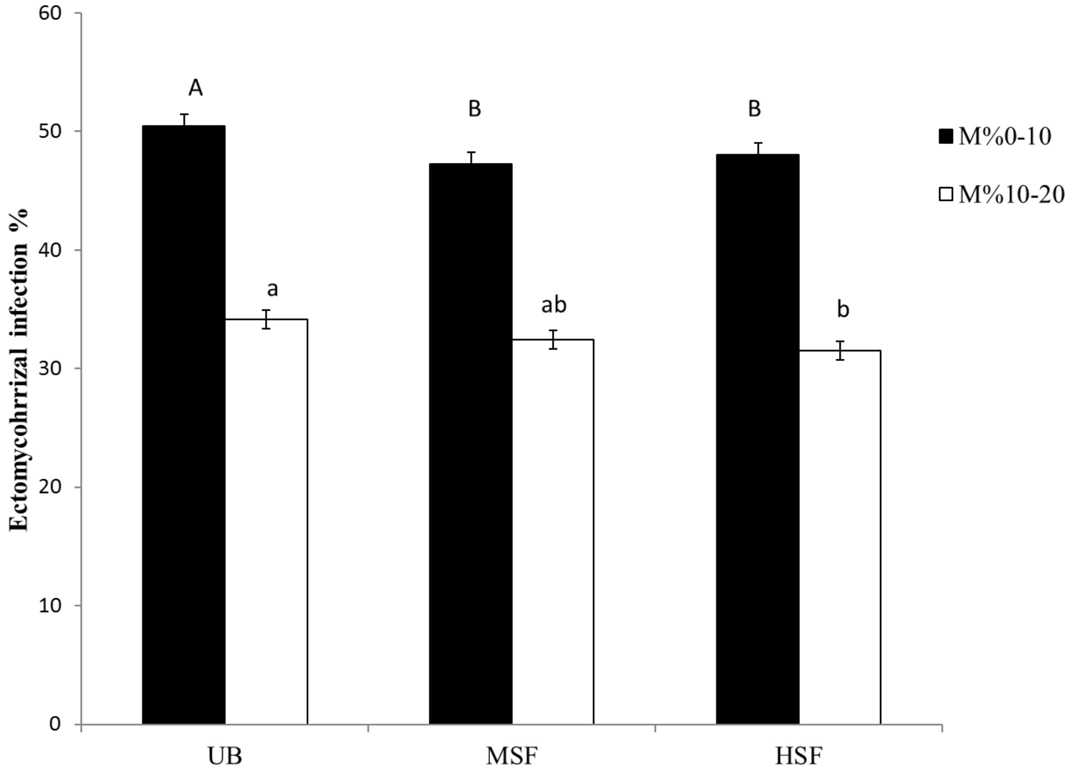

Fig. 2. Percentage of natural ectomycorrhization of P. pinaster seedlings according to vertical distribution for Unburned (UB), Moderate severity fire (MSF) and High severity fire (HFS) bioassay treatments at 0-10 cm depth (black bars) and 10-20 cm (open bars) from forest soils taken after a forest fire in Central Spain (Honrubia de la Cuesta,Segovia). Columns marked with different letters differed significantly according to LSD Fisher test ($P < 0.05$).

3.3. Relationships among plant development, fungal colonization and soil chemistry

The PCA performed on the soil characteristics and seedling vegetative variables of the three treatments showed that samples from HFS and MFS sites were grouped in the positive area of Axis 1 whereas UB ones are located in the negative area (Fig. 3). The two axes explained 68% of the variation present in the samples (50% axis 1 and 19% axis 2). Unburned soil samples appear associated with higher values of organic matter, N, Ca and cation exchange capacity (CEC), but negatively to pH, Mg, Na and Conductivity. The opposite is true for both burned (HFS and MFS) sites (Table 1).

Table 1: Chemistry parameters of soils sampled in 2009 in a Pinus pinaster forest in Central Spain, one year after a wildfire. Unburned, soils not affected by fire; MFS, soils moderalety affected by fire; HFS, soils highly affected by fire. (n=3,±SE)

SITE	**Unburned**	**MFS**	**HFS**	***p***
N (%)	0.106±0.004a	0.053±0.004b	0.050±0.004b	0.001
P (mg/kg)	4.40±1.30a	6.06±1.30b	7.66±1.30c	0.028
K(mg/kg)	115.0±6.75a	168.0±6.75b	115.3±6.75a	0.002
pH	6.04±0.12a	7.30±0.12c	6.83±0.12b	0.001
Organic matter(%)	1.37±0.11a	0.91±0.11b	1.05±0.11ab	0.069
Na (meq/100g)	0.016±0.004a	0.020±0.004a	0.020±0.004a	0.787
Mg(meq/100g)	0.87±0.05a	0.60±0.05ab	0.59±0.05b	0.008
Ca (meq/100g)	4.08±0.38a	3.07±0,38b	2.65±0.38b	0.088
Conductivity(mS/cm)	0.04±0.02a	0.06±0.02a	0.06±0.02a	0.576
CEC µg/g	11.35±0.69a	8.23±0.69b	6.88±0.69b	0.010

Regarding seedling vegetative variables, the PCA revealed a positive correlation between shoot biomass of *P. pinaster* seedlings with P and fire severity Axis 1 also seems reflect a correlation between higher levels of mycorrhization (total, 0-10 cm and 10-20 cm) with the unburned sites.

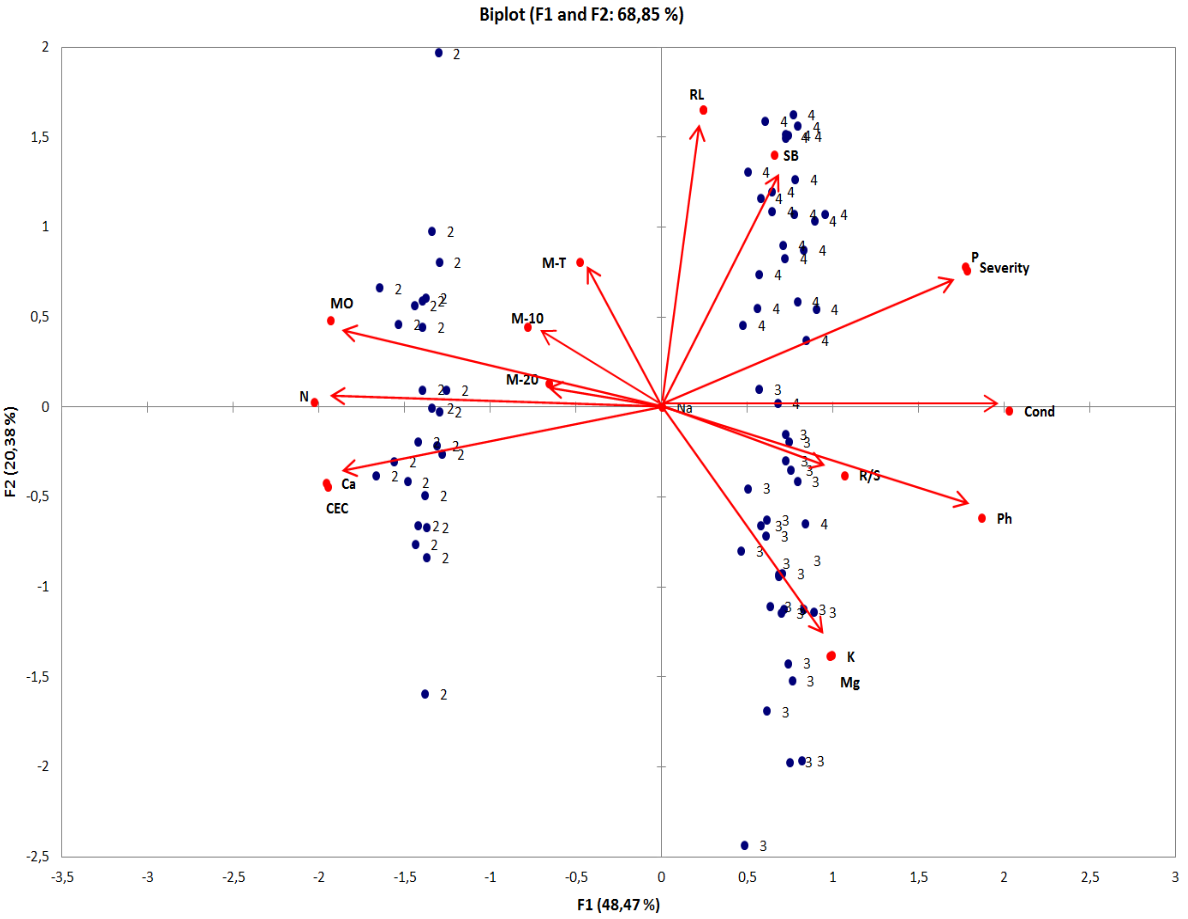

Fig.3. Ordination diagram of Principal Component Analysis (PCA) including treatments: 2: Unburned (UB), 3: Moderate severity fire (MSF) and 4: High severity fire (HFS) of soil properties for samples from the studied location (Honrubia de la Cuesta), ectomycorrhization and morphometric parameters (SH: shoot height in cm; SB: shoot biomass in gr; RL: root length in cm; R/S: root to shoot ratio).

4. Discussion

Considering the potentially beneficial effects of ECM on pine seedlings and how fire severity and soil depth affect ECM propagules, our hypothesis was that ECM-infection would be more abundant in undisturbed soils and at deeper soils layers, having this a beneficial effect on seedling growth.

Our results revealed a higher mycorrhization rate for seedlings grown in unburnt substrate and at upper soil layers for all sites. However, our initial hypothesis was only partially supported as plants grown in high severity fire soil attained the largest size despite having lower levels of mycorrhization. Here we discuss these results in the light of mycorrhization, soil chemistry and their interactions.

4.1. Plant development

Seedlings in the high severity fire soil showed higher shoot height, root length and root/shoot ratio and more than 10% of the shoot biomass of those growing in unburned soil. Similar biometric results have been found by Pausas et al. (2003) in *Pinus halepensis* seedlings in eastern Iberian Peninsula under three fire severity classes, where seedlings from sites most affected by fire grew significantly more. Those differences may lie in the changes in soil chemistry caused by the different fire severities and leading to higher fertility (see below) (Pausas et al., 2003).

4.2 Fungal mycorrhization and plant development

Maximum ECM colonization was found in soils from unburnt sites, although it did not vary significantly among high and moderate severity fire sites. Our results are in agreement with a large body of research on Mediterranean dry forests (Buscardo et al., 2010; de Román and Miguel, 2005; Hernández-Rodríguez et al., 2013; Martín-Pinto et al., 2006b) with some studies conducted also with *P. pinaster* (Buscardo et al., 2011; Gassibe et al., 2011; Rincón and Pueyo, 2010; Rincón et al., 2014; Sousa et al., 2011). Overall, there is a clear connection between undisturbed habitats and higher mycorrhization rates. Regarding the influence of soil depth on mycorrhization rate, our results revealed that upper layers had the highest mycorrhization rates. This is likely due to the fact that the mycelium of ECM fungi in undisturbed ecosystems is usually most abundant in the superficial organic soil layers (Neville et al., 2002; Visser et al., 1995; Wallander et al., 2004), although some studies show that fire disturbance might influence or even reverse this pattern (Dahlberg et al., 2001). For example, Bastias et al. (2006) found different fungal different community structure in the upper 10 cm layer of soils variously affected by fire, but no obvious trend was observed for soil from a depth of 10–20 cm.

In this sense, it is known that fire primarily affects soil organic horizons where a high proportion of ECM fungi live (Rincón et al., 2014). As fire directly heats superficial soil layers the soil mycelia colonization capabilities are diminished (Izzo et al., 2006; Torres and Honrubia, 1997). Despite intense heating in the upper few centimeters of soil (Neary et al., 1999), potential inoculum can remain on roots or directly in the soil as ectomycorrhizal resistant propagules e.g. sclerotia (Kipfer et al., 2011; Rincón and Pueyo, 2010), from which pioneer fungal species develop during the early post-fire season in the soil as post fire fungi (Claridge et al., 2009; Visser et al., 1995). This is relevant as the role of post-fire ectomycorrhizal fungi is essential to the post-fire recovery of forests as they develop soon after wildfire (Claridge et al., 2009).

Considering mycorrhization rate alone, our *P. pinaster* seedlings attained smaller sizes in those soils where mycorrhization rates were highest i.e. unburned soils. Nonetheless, given that potential mycorrhizal inoculum was confounded with soil chemistry, direct conclusions cannot be drawn. Inclusion in our study of seedlings grown on sterilized soils from the three studied sites, would have allowed measuring the effect of soils chemistry alone on plant growth. Notwithstanding, a negative effect of mycorrhization on plant growth rates under nursery conditions has been previously reported (Le Tacon et al., 1992; Stenström et al., 1985, 1990), even though the general consensus is that mycorrhization does enhance plant growth (Sousa et al., 2012). Interestingly, this consensus weakens for those cases where mycorrhization is low (below 50%) (Flykt et al., 2008; Marx et al., 1982) as also seen in our results regarding total mycorrhization (<40%) in the burned treatments.

4.3.1. Soil nutrients and plant development

The main differences between sites were a lower N, K and organic matter content and a higher pH and P content in burnt soils. The decrease in N availability after fire may not be exclusively explained by fire severity, but also by volatilization and mineralization by heating the top soil (Pausas et al., 2003). Reversely, the P content lost by volatilization is usually low and the amount of P available to plants is dependent on ash deposition (Raison et al., 1985; Pausas et al., 2003). Fires can cause a short-term fertilizing effect (Turrión et al., 2012), and in our study, we found a high correlation between seedling size and higher amounts of P (Fig. 3), suggesting a causal relationship between them although third variables cannot be ruled out (Gray and Dighton, 2009; Pausas et al., 2002). Furthermore, *P. pinaster* is a pyrophitic species typically adapted to post-fire regeneration. Under that scenario it is not surprising to find enhanced vegetative development for seedlings grown in soils from burnt sites (Fernandes and Rigolot, 2007).

4.3.2. Soil nutrients and fungal colonization

In our study, the percentage of ECM colonization and P soil content were negatively correlated, while organic matter and N were positively correlated. However, causal relationships between both soil chemistry and mycorrhization rates might be not causal given likely differences in inoculum content between sites and also considering the short time span of our assay. Our results are in concordance with Dickie et al. (2006) in unburned bioassay soils with *P. sylvestris* where seedling infection was best described by humus percent N (total), soil Ca, soil percent clay, and soil cation exchange capacity at 0- to 20-cm depth.

4.3. Relationships among plant development, fungal colonization and soil chemistry

Considering the short term plant development evolution of 4 months, our results are in concordance with Sousa et al. (2011), in which N was not a factor that limited plant growth in ECM inoculated *P. pinaster* seedlings tested in burned soils with a larger content of P in contrast to total N, which decreased by the effect of fire. Furthermore, other authors noticed that fungal communities can be adapted to more nitrogen-rich sites (Kranabetter et al., 2009; Toljander et al., 2006), which could be the case in the unburned treatment where a larger mycorrhizal colonization was observed. While that in a post fire scenario, *P. pinaster* may prefer fungi that provide greater inputs of phosphorus but little or no N (Bent et al., 2011), when there has been a reduction on N content, thus benefiting the pyrophitic nature of this species as a host, which may rely on this nutrient above others in a post fire soil. Since a higher P content may increase plant vigor (Conjeaud et al., 1996), it can also depress the mycorrhizal association (Grant et al., 2005) as observed in our results.

Regardless the limitations that bioassay studies present, they are a useful tool when studying ectomycorrhizal infectivity and may be comparable to some field situations (Izzo et al., 2006). In our study, mycorrhizal inoculum potential appeared to be affected by soil disturbance caused by fire (Buscardo et al., 2010; Sousa et al., 2011), while plant growth increased along a fire severity gradient. This was likely due to post fire nutrient deliverance in the forest soil such as P (Fierro et al., 2007) rather than ECM colonization. Although we could not separate fire severity and ECM colonization effects, the latter also may have influenced the uptake of P (De Lucia et al., 1997), but in a lesser degree due to the fire effect (Turrión et al., 2010).

Integration of laboratory and field approaches in which environmental factors as soil depth, erosion and vegetation cover (Buscardo et al., 2011) play a key role are needed to asses ECM functioning in ecosystems dominated by Mediterranean pyrophitic species such as *P. pinaster*, therefore aiming to achieve a successful restoration of Mediterranean forest areas affected by wildfire.

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