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**MÁSTER ERASMUS MUNDUS: MEDITERRANEAN FORESTRY AND NATURAL  
RESOURCES MANAGEMENT (MEDFOR)**

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

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## Resumen

En el presente trabajo se estudió y describió la productividad, diversidad y composición de especies en comunidades fúngicas de zonas Mediterráneas dominadas por *Pinus pinaster*. El objetivo fue determinar cómo las comunidades fúngicas se vieron afectadas por las diferentes condiciones edafoclimáticas que caracterizaron tres diferentes hábitats. Los esporocarpos fueron recolectados e identificados a partir de transectos de 100 m<sup>2</sup> durante las estaciones de otoño de 2006 a 2012. Los datos analizados se originaron a partir de tres sitios diferentes caracterizados como tratamientos de 1) suelo silíceo, 2) duna continental y 3) suelo calcáreo. La producción y riqueza totales de hongos fueron mucho más altas en los bosques silíceos y calcáreos que en los de duna interior. Los resultados fueron similares en cuanto al análisis de la producción en hongos micorrícicos y para el índice de diversidad de hongos saprófitos.

Por otra parte, la composición de taxones fúngicos se correlacionó principalmente con variables climáticas tales como precipitación y temperatura. Por otra parte el contenido de nitrógeno y potasio del suelo afectaron de manera significativa la distribución de los taxones pertenecientes a los dos grupos funcionales (saprófitos y micorrícicos). Los resultados mostraron un amplio rango ecológico para grupos taxonómicos como *Lycoperdum perlatum*, *Russula torulosa* y especies del género *Galerina* y *Mycena*. Por otra parte, algunas especies mostraron una gran especificidad de acuerdo a las condiciones ambientales. Por lo tanto, todas las especies incluidas en los géneros *Macrolepiota* se recogieron en los suelos calcáreos mientras que *Laccaria bicolor* y *L. Laccata* se asociaron exclusivamente a parcelas silíceas donde las precipitaciones son mayores y el contenido de nitrógeno es más alto. Los resultados mostraron una notable alta producción y diversidad de hongos asociada a *P. pinaster* en condiciones mediterráneas extremas, pudiendo representar implicaciones ecológicas y económicas que los gestores forestales deberían tener en cuenta para dar mayor valor a estos bosques.

**Palabras clave:** producción fúngica, diversidad, composición fúngica, variables ambientales, ecosistemas mediterráneos.

**Abstract**

The aim of this work was to study and describe fungal communities regarding their productivity, diversity and species composition in Mediterranean areas covered by *Pinus pinaster*. The objective was to determine how fungal communities were affected by the different edaphoclimatic conditions that characterized three different habitats. Sporocarps were collected and identified from 100 m<sup>2</sup> transects during the autumn seasons from 2006 to 2012. Data analysed were originated from three different sites characterised by a siliceous soil treatment, an inner dune and a calcareous soil type. Total fungal production and richness were much higher in the siliceous and calcareous forests than in the inner dune type. The results were similar when analysing production of mycorrhizal taxa and diversity index for saprotrophic fungi.

On the other hand, the fungal taxa composition was mainly correlated with climatic variables such as precipitation and temperature. Moreover nitrogen and potassium soil contents significantly affect the distribution of taxa for both saprophytic and mycorrhizal functional groups. The results showed a broad range of ecological taxa such as *Lycoperdum perlatum*, *Russula torulosa* and species within genus *Galerina* and *Mycena*. Moreover, other species showed a great specificity according to environmental conditions. Thus, all the species included within *Macrolepiota* genera were collected in the calcareous soils whereas *Laccaria laccata* and *L. bicolor* were exclusively associated with higher precipitations and contents of nitrogenous in the siliceous plots. The results showed a noticeable high fungal production and diversity associated to *P. pinaster* in extreme Mediterranean conditions which could represent ecological and economical implications to be taken into account by forest managers in order to add value to these forests.

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

## 1. Introduction

*Pinus pinaster* Ait. forests are within the most relevant representatives of Mediterranean areas and this species reach great economic importance (Fernandes and Botelho, 2004). It occupies almost 1,700,000 ha, from which 0.6 million are supposedly resulting from direct planting (Alía *et al.*, 1996) being the second most important tree species by surface area in Spain (Rodríguez *et al.*, 2008). Its peninsular distribution is patchy and comprises a broad spectrum of substrates (limestone, granite, schist, marly limestone, peridotite), topographies and climates, including from montane sub-humid to Mediterranean semi-arid with summer drought (Nicolás and Gandullo, 1967; López-Sáez *et al.*, 2010). *P. pinaster* can form closed forests that are either single-species or mixed with different evergreen or deciduous trees and a varied range of understory species (Blanco *et al.*, 1997). This species 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). 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 on colonizing and establishing on disturbed soils and former agricultural fields may be attributed in part to its compatibility with a wide range of its fungal symbionts (Carson *et al.*, 2010; Gassibe *et al.*, 2011; Oria-de-Rueda *et al.*, 2010). For that matter artificial and natural stands of *P. pinaster* have the potential to support a significant fungal production and diversity (Oria-de-Rueda *et al.*, 2010). Some previous studies have studied fungi associated with this Mediterranean species (Fernández-Toirán *et al.*, 2006; Martín-Pinto *et al.*, 2006a; Oria-de-Rueda *et al.*, 2010; Gassibe *et al.*, 2011, Bonet *et al.*, 2012). This is important since fungi can play an essential ecological and economical role in these communities. In this sense, mycorrhizal associations have significant effects on nutrient and water uptake, growth and plant survival (Courty *et al.*, 2010; Brundrett, 2009), improving soil aeration and porosity (Fernández-Toirán *et al.*, 2006), resistance against pathogens (Martín-Pinto *et al.*, 2006b), and also as food source to many organisms in the food chain (Sato *et al.*, 2012). On other hand, saprotrophic fungi play a key role in Mediterranean forests since they guarantee dead matter transformation and, therefore, the recycling of nutrients in the ecosystems (Ferris *et al.*, 2000). Formation of sporocarps is influenced by various factors, such as host specificity (Molina *et al.*, 1992), physiological conditions and nutritional status of the mycelium (Murat *et al.*, 2008) and environmental factors (Bonet *et al.*, 2004). In this sense, climatic variables are directly related to the 60 % to 80 % of 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 (Pinna et al., 2010; Egli, 2010; Bonet et al., 2010). Rainfall and temperature are generally recognized as important factors, but quantifications have hardly been made (Straatsma et al., 2011). On the other hand, fungal species composition are strongly determined by soil chemical properties (Straatsma et al., 2011), specially in the case of saprophytic fungi due to their lack of mutualism 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 play a relevant role for mycorrhizal and saprotrophic groups (Richard et al., 2004).

Apart from their ecological importance, sporocarps of both mycorrhizal and saprotrophic species can be edible and much of the recent interest on fungi resides on their direct economic value (Cai et al., 2011). During the last decade, there has been a sharp increase in the demand for edible fungi and a significant rise in the marketing and distribution of these products (Pettenella et al., 2007), becoming an important source of rural income, therefore timber and mushroom production combined are an example of joint production (Diaz-Balteiro and Romero, 2008).

Fungi have become strategic in the conservation and management of Mediterranean forest systems (Martínez-Peña et al., 2012). A deeper knowledge of the fungal communities associated to *P. pinaster* is essential to find an adequate sustainable forest management focused on forests production and conservation (Carson et al., 2010). Hence, the goal of this study was to assess the potential fungal community associated to *P. pinaster* forests in Mediterranean areas. Our specific aims were; (i) to analyze the diversity and production of fungal fruiting bodies, according to functional groups, in three *P. pinaster* forests under different edaphoclimatic conditions and ii) to assess the influence of edaphoclimatic variables on taxa composition.

## **2. Materials and methods**

### **2.1 Study Plots**

The analysis was carried out in three Mediterranean ecosystems dominated 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, three zones can be distinguished according to ecological and edaphoclimatic differences:

Site 1. Celadilla del Río (Palencia): Located between at 985 m above the sea level in the most elevated plateau in the north of the province. This site is characterized by siliceous soils (Umbrisol) with a mean annual rainfall of 622 mm and mean annual

temperature of 10 °C. The stand has a homogenous structure with a mean density of 363 trees /ha and basal area of 20 m<sup>2</sup>/ha. The vegetation in the understory is composed by *Agrostis castellana* Bois. and Reut, *Avenula sulcata*(Gay ex Bobs.) , *Calluna vulgaris* (L.) Hull, *Erica cirinea* L., *Erica vagans* L., *Halimium umbellatum* (L.) Spach. Subsp. *Viscum* (Willk.) O. Bolós and Vigo, *Hylocomium splendens* (Hedw.) Br.Eur., *Lavandula stoechas pedunculata* L., *Thymus mastichina* (L.) L., *Thymus mastichina*(L.) L., *Thymus zygis* Loefl.ex L.

Site 2. Montemayor de Pililla (Valladolid): These are high plateau lands located at 890 m above the sea level in southeast of the province This site is characterized by calcareous soils (Calcareous cambisol) with a mean annual rainfall of 500 mm and mean annual temperature of 12 °C. The stand has a homogenous structure with a mean density of 153 trees /ha and basal area of 8,5 m<sup>2</sup>/ha. Its understory vegetation is composed mainly by calcareous and sandy species: *Cistus laurifolius* L., *Arrhenatherum elatius* (L.) Beauv, *Ornithopus compressus* L., *Ornithopus compressus* L., *Micropyrum tenellum* (L.) Link, *Vicia laxiflora* Brot., *Andryala ragusina* L., *Ononis spinosa* L., *Leuzea conífera* (L.) DC., *Juniperus thurifera* L. ,*Tuberaria guttata* (L.) Fourr. *Corynephorus canescens* (L.) Beauv., *Centaurea castellana* Boiss. et Reuter , *Medicago sativa* L.

Site 3. Tudela del Duero (Valladolid): Located at 700 m above the sea level This site is characterised by sandy soils, with a mean annual rainfall of 430 mm and a mean annual temperature of 12, 5° C . The stand has a homogenous structure with a mean density of 130 trees /ha and basal area of 7,2 m<sup>2</sup>/ha . Its understory vegetation is composed mainly by sandy species: *Lavandula stoechas pedunculata* L., *Vulpia myuros* (L.) C.C. Gmelin, *Lupinus angustifolius* L., *Helichrysum italicum serotinum* (Roth) G. Don fil., *Sedum amplexicaule* DC., *Ornithopus compressus* L., plus *P. pinea* trees scattered in the area.

All the studied stands can be considered as even-aged stands of 50–60 years. In order to avoid different light conditions which can influence on fungal production, canopy cover in the studied stands was always between 70% and 80%.

## **2.2. Climatic and soil data**

Climatic data for the period 2006-2012 were provided by the closest meteorological stations (Table 1). Mean monthly potential evapotranspiration (PE), was calculated by the empirical method of Thornthwaite and Mather (1955) for the latitude of the three meteorological stations. Soil samples in each plot were taken once to represent a gradient between minimum and maximum productivity under the three treatments in 2014 in mid-May before the fruiting season, six soil samples were extracted after carefully removing the overlying litter and humus in each plot using a cylindrical (2 cm

radius, 20cm deep, 250 cm<sup>3</sup>) soil borer (Taylor, 2002). Samples were taken next to the angles and in the center of the squares plots with a minimum distance of 30 cm apart from any tree trunk (De la Varga *et al.*, 2012) and taken to ITAGRA Laboratory for soil analysis to represent a gradient between minimum and maximum productivity (Table1).

**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	0.010c
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	25.33a	15.24b	20.48c
T(°C)	10.63a	12.11b	12.45b
TMAX(°C)	16.70a	19.01b	18.38b
TMIN(°C)	4.53a	5.88b	6.51b
PREC(mm)	561.18a	374.62b	373.24b
PET(mm)	640.40a	695.60b	629.80a
PAN(mm)	206.30a	151.80b	147.24b
PAS(mm)	50.34a	36.40a	39.84a
TAN(°C)	12.85a	14.79b	14.54b

### 2.3. Sampling

Data for the study were collected during autumn sampling campaigns between October and December 2003–2012, when cold temperatures stop the emergences of sporocarps (Bonet *et al.*, 2012) of nine randomly selected *P. pinaster* stands. The study consisted of three treatments; siliceous (Palencia), sandy (Tudela del Duero) and calcareous (Montemayor de Pililla). Three plots of 2 x 50 m per each treatment were established in accordance with previous studies conducted by Oria-de-Rueda *et al.* (2010) and Smith *et al.* (2002). During the fruiting period, all the mushrooms (both ectomycorrhizal and saprophytic) with a cap diameter wider than 1 cm were collected weekly on Fridays to reduce error due to mushroom removals by recreational weekend collectors (Bonet *et al.*, 2004) and taken to the laboratory at 4 °C and processed within 24 h after collection for identification, fresh and dry weight measurements (Bonet *et al.*, 2012).

#### 2.4. 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.* (2006b).

Sporocarps were dried in air-vented ovens at 35 °C and were dry weighed in order to obtain comparable biomass data. For statistical purposes, data were grouped into the following categories: saprotrophic/mycorrhizal, and edible/inedible.

#### 2.5. Production, diversity and richness calculations and statistical analysis

Statistical analyses were conducted by data groups according geographical and soil conditions: a Palencia group from 2006 to 2012 (45-55 year-old forest stands coming from a reforestation) in siliceous soils, and a Valladolid group (45–55 year-old natural stands) divided in two treatments (sandy and calcareous soils). Henceforth wild mushroom production in Mediterranean areas has a strong heterogeneity, mainly because of the great variations in the yearly precipitation (Agreda *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 (Agreda *et al.*, 2014), therefore some logarithmic transformation were carried out .

Fresh weights, edibility and life strategies of the three 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.

Shannon's  $H'$  diversity index (Shannon and Weaver, 1949), based on dry weight of the fruiting bodies (Dahlberg, 1991) was calculated. An analysis of richness (S) (Martínez-Ruíz *et al.*, 2001; Straatsma and Krisai-Greilhuber, 2003) was also done. These variables were calculated using the following formula where coefficient  $p_i$  indicates the relative importance of each fungal species and S is the total number of species found:

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

S = number of species

Species composition was analyzed using ordination techniques on fungal dry weight data. Firstly, data of dry weight of mycorrhizal and saprotrophic taxa were subjected to



a detrended correspondence analysis (DCA) (Ter Braak and Prentice, 1988). Since the length of the extracted gradient was bigger than 3 SD units in both analyses (4.185 and 5.569 respectively), canonical correspondence analysis (CCA) (Ter Braak, 1986) was used to assess the effect of environmental variables in fungal dry weight. Two CCA tests were conducted, separately with mycorrhizal and saprotrophic taxa, in order to analyze whether there were differences in the ecological behavior of these two groups. Forward selection was used to select significant explanatory variables and only those significant at the  $P < 0.05$  level were included in the models. Monte Carlo permutation tests (499 permutations) were performed to study the signification of the models. These analyses were conducted using CANOCO for Windows version 4.5 Software (Ter Braak and Šmilauer, 2002). CCA results were displayed by ordination diagrams drawn with Cano Draw 4.1. Software.

### **3. Results**

#### **3.1. Richness, diversity and sporocarps production**

*P. pinaster* Mediterranean forests showed to provide adequate ecological conditions for fungal production and richness. In this sense, a total of 194 taxa were found in the 9 sampled plots (Table 2). According to Oria-de-Rueda *et al.* (2010), 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: *Agaricus* sp., *Agrocybe* sp., *Clitocybe* sp., *Collybia* sp., *Conocybe* sp., *Coprinus* sp., *Cortinarius* sp., *Cystoderma* sp., *Entoloma* sp., *Flammulaster* sp., *Galerina* sp., *Hebeloma* sp., *Inocybe* sp., *Lepiota* sp., *Marasmius* sp., *Mycena* sp., *Omphalina* sp., *Psathyrella* sp., *Rhizopogon* sp., *Russula* sp., *Tricholoma* sp and *Tubaria* sp.

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. From the total taxa list, 79 can be identified as mycorrhizal and 115 as saprotrophic fungi. Fifty of the total taxa found were edible.

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

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

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

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

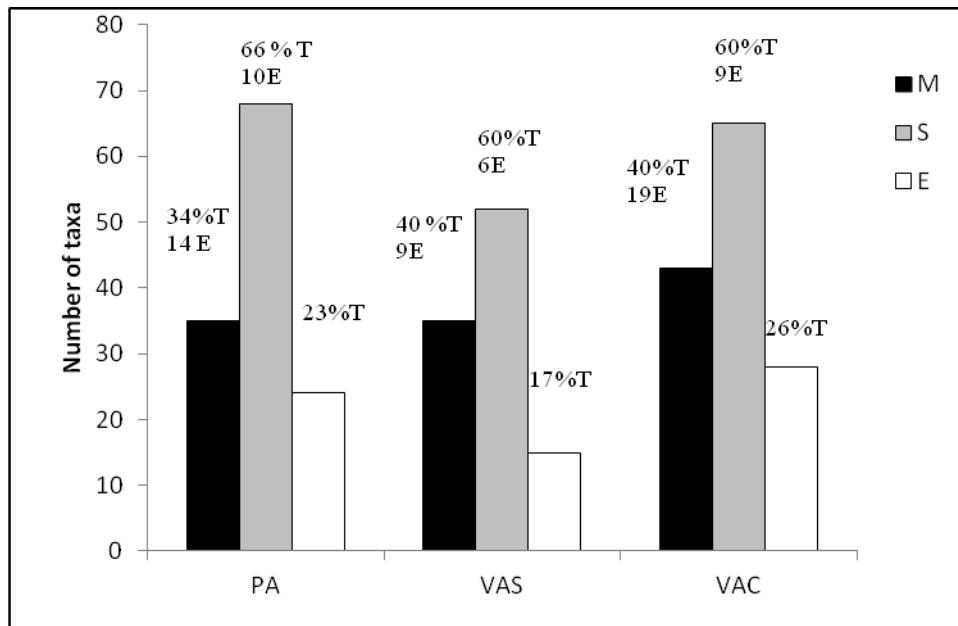
<i>Mycena galericulata</i> (Scop.) Gray	Myga	+		+	S	
<i>Mycena inclinata</i> (Fr.) Quél.	Myin	+			S	
<i>Mycena leptcephala</i> (Pers.) Gillet	Myle	+	+	+	S	
<i>Mycena polygramma</i> (Bull.) Gray	Mypo	+	+	+	S	
<i>Mycena pseudopicta</i> (J.E. Lange) Kühner	Myps		+	+	S	
<i>Mycena pura</i> (Pers.) P. Kumm.	Mypu	+	+	+	S	
<i>Mycena pura f. lutea</i> (Gillet) Arnolds	Mypurl			+	S	
<i>Mycena rosella</i> (Fr.) P. Kumm.	Myro	+		+	S	
<i>Mycena seynesii</i> Quél.	Myse	+	+	+	S	
<i>Mycena silvae-nigrae</i> Maas Geest. & Schwöbel	Mysil	+			S	
<i>Mycena sp.</i> (Pers.) Roussel	Mysp	+	+	+	S	
<i>Mycena ustalis</i> Aronsen & Maas Geest.	Myus			+	S	
<i>Mycena viridimarginata</i> P. Karst.	Myvi	+			S	
<i>Mycena vulgaris</i> (Pers.) P. Kumm.	Myvu	+			S	
<i>Mycetinis scorodoni</i> (Fr.) A.W. Wilson & Desjardin	Mysc	+		+	S	
<i>Omphalina pyxidata</i> (Bull.) Quél.	Ompy		+	+	S	
<i>Omphalina sp.</i> Quél.	Omsp		+		S	
<i>Phanerochaete sanguinea</i> (Fr.) Pouzar	Phsa	+			S	
<i>Psathyrella hydrophila</i> (Bull Ex Merat) Mre.	Pshy	+			S	
<i>Psathyrella ammophila</i> (Fr.) Quél.	Pssp			+	S	
<i>Psilocybe luteonitens</i> (Fr.) Park.-Rhodes	Pslu			+	S	
<i>Rhizopogon luteolus</i> Fr. & Nordholm	Rilu		+	+	MY	E
<i>Rhizopogon sp.</i> Fr.	Rhsp		+		MY	E
<i>Rhodocollybia butyracea</i> (Bull.) Lennox	Rhbv	+	+	+	S	E
<i>Rhodocollybia butyracea f. asema</i> (Fr.) Antonín, Halling & Noordel.	Rhbuf	+			S	E
<i>Rickenella mellea</i> (Singer & Clem.) Lamoure	Rime	+			MY	
<i>Russula adusta</i> (Pers.) Fr.	Ruad	+		+	MY	
<i>Russula cessans</i> A. Pearson	Ruce	+		+	MY	
<i>Russula delica</i> Fr	Rude			+	MY	
<i>Russula heterophylla</i> (Fr.) Fr.	Ruhe			+	MY	E
<i>Russula integra</i> (L.) Fr.	Ruin	+		+	MY	E
<i>Russula olivacea</i> Pers.	Ruol	+		+	MY	E
<i>Russula romellii</i> Maire	Ruro			+	MY	E
<i>Russula sanguinea</i> Fr.	Rusa	+			MY	
<i>Russula sardonias</i> Fr.	Rusar	+			MY	
<i>Russula sp.</i> Pers.	Rusp	+	+	+	MY	
<i>Russula torulosa</i> Bres.	Ruto	+	+	+	MY	
<i>Russula xerampelina</i> (Schaeff.) Fr.	Ruxe	+			MY	
<i>Stropharia coronilla</i> (Bull. ex DC.) Quél.	Stco			+	S	E
<i>Suillus bellinii</i> (Inzenga) Watling	Sube			+	MY	E
<i>Suillus granulatus</i> (L.) Roussel	Sugr	+			MY	E
<i>Suillus luteus</i> (L.) Roussel	Sulu	+			MY	E
<i>Tapinella atrotomentosa</i> (Batsch) Šutara	Taat	+			S	
<i>Tapinella panuoides</i> (Batsch) E.-J. Gilbert	Tapa	+			S	
<i>Tephrocycbe rancida</i> (Fr.) Donk	Tera		+		MY	

<i>Thelephora caryophyllea</i> (Schaeff.) Pers.	Thca			+	MY
<i>Thelephora terrestris</i> Ehrh.	Thte			+	MY
<i>Tremella mesenterica</i> Schaeff.	Trme	+			S
<i>Tricholoma equestre</i> (L.) P. Kumm.	Treq	+	+		MY
<i>Tricholoma focale</i> (Fr.) Ricken	Trfo			+	MY
<i>Tricholoma gausapatum</i> (Fr.) Quél.	Trga		+		MY E
<i>Tricholoma myomyces</i> (Pers.) J.E. Lange	Trmy		+	+	MY E
<i>Tricholoma portentosum</i> (Fr.) Quél.	Trpo	+			MY E
<i>Tricholoma sculpturatum</i> (Fr.) Quél.	Trsc		+		MY E
<i>Tricholoma sp.</i> (Fr.) Staude	Trsp			+	MY
<i>Tricholoma terreum</i> (Schaeff.) P. Kumm.	Trte		+	+	MY E
<i>Tricholomopsis rutilans</i> (Schaeff.) Singer	Trru	+			S
<i>Tubaria sp.</i> (W.G. Sm.) Gillet	Tusp	+	+	+	S
<i>Xylaria hypoxylon</i> (L.) Grev.	Xyhy			+	S

MY: mycorrhizal; S: saprotrophic; E: edible

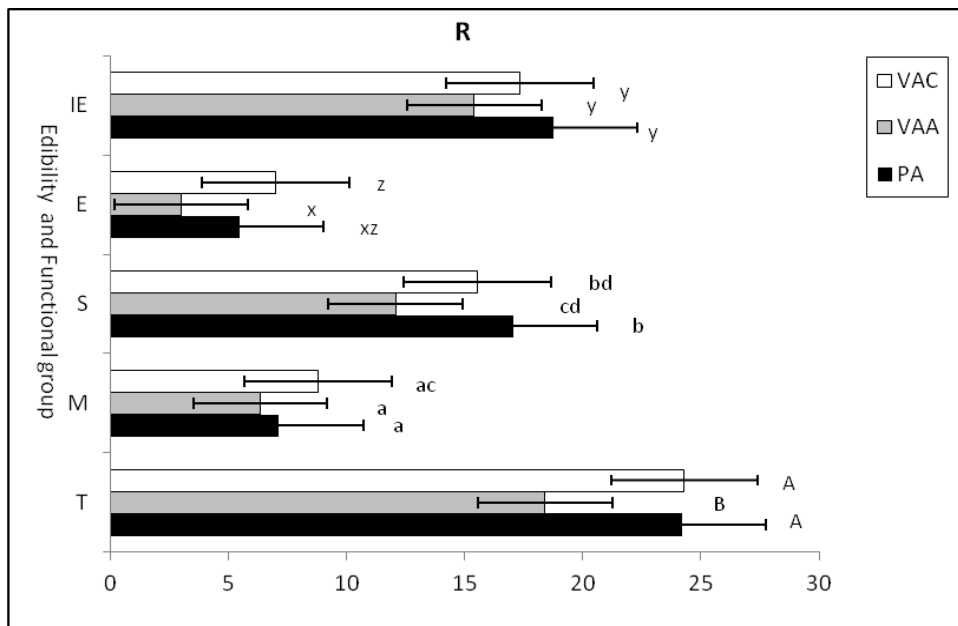
Sporocarps collected from the three Palencia plots, were classified into 103 different taxa. From the total taxa found, 35 can be classified as mycorrhizal and 68 as saprotrophic fungi. Twenty four of total collected taxa were classified as edible fungi (Fig 1).

On the other hand, 87 taxa occurred in VAS plots (40 % mycorrhizal and 60 % saprotrophic) of which 15 are edible and six are marketed. 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. Edible fungi represented 26% (28 taxa) and five of them are marketed (Fig 1).

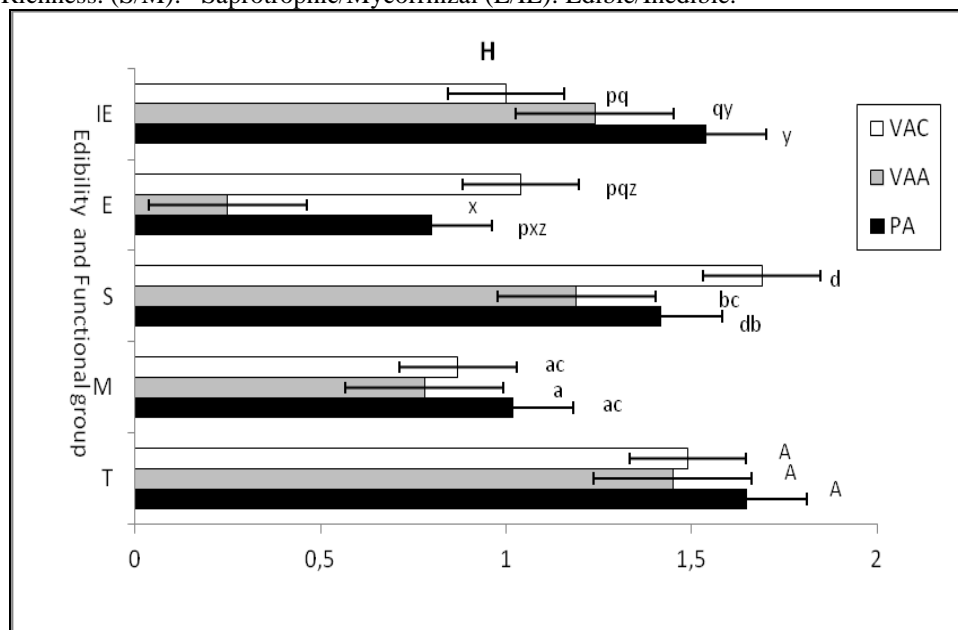


**Fig. 1.** Number and % of taxa found in the studied forest stands depending on vital strategy (mycorrhizal saprotrophic). (Tt): number of total taxa. (Et): number of edible taxa.

Fungal richness was significantly different when comparing mean values found in the three sites. Thus, values in PA and VAC were significantly higher than those observed in VAS plots (Fig 3a;  $P_{PA-VAS}=0.0292$ ;  $P_{VAC-VAS}=0.0026$ ). Similar results between PA and VAC plots were observed analysing richness and Shannon's  $H'$  Diversity index for saprotrophic and edible species, since no differences were found. Within the three locations values for saprophytic 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;  $P>0.05$ ).



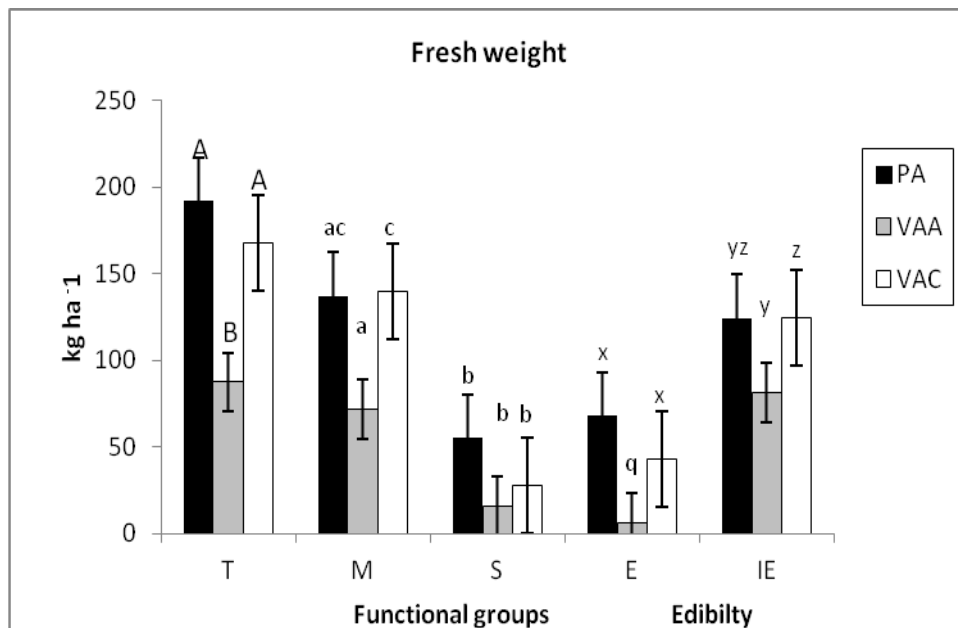
**Fig. 2a.** Richness variable analysed depending on functional groups and edibility. (S): Richness. (S/M): Saprotrophic/Mycorrhizal (E/IE): Edible/Inedible.



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

Noticeable total production of 447.31 kg fw ha<sup>-1</sup> (39.41 kg ha<sup>-1</sup> dry weight) was collected over the 5-year study period, where 192.7 kg fw ha<sup>-1</sup> was from PA treatment, 167.66 kg fw ha<sup>-1</sup> from VAC, decreasing to 87.32 kg fw ha<sup>-1</sup> in VAS treatment (Fig 3). While no differences were found comparing production among treatments for mycorrhizal species, lowest values were found for saprotrophic fungi in VAS, significantly different than those found in PA plots (Fig 3;  $P=0.0033$ ).

Regarding edible species, yields were significantly different among the three studied forests. Highest production was found in PA and VAC Plots (67.79 and 43.29 kg fw ha<sup>-1</sup>). While significant lower values (6.39 kg fw ha<sup>-1</sup>) were collected in VAS (Fig 3;  $P_{PA-VAS}=0.0001$ ;  $P_{VAC-VAS}=0.0000$ ). This same trend was observed regarding to edible mycorrhizal species, decreasing from PA and VAC plots (31.28 and 29.20 kg fw ha<sup>-1</sup>) to VAS ones (2.33 kg fw ha<sup>-1</sup>) (Fig 2a;  $P_{PA-VAS}=0.0070$ ;  $P_{VAC-VAS}=0.0111$ ). Attending to edible saprophytic species, highest values were again found in PA plots 36.7 kg fw ha<sup>-1</sup> and the lowest production was picked in VAS treatment (4.28 kg fw ha<sup>-1</sup>) (Fig 3;  $P=0.0431$ ).



**Fig.3.** Production of carpophores according to functional groups and edibility. (kg fw ha<sup>-1</sup>). M: Mycorrhizal, S: Saprotrophic, E: Edible, IE: Inedible. Values with the same letter are not significantly different.

### 3.2. Taxa composition

According to taxa composition, Jaccard similarity coefficients showed that VAS and VAC were found to be the most similar with 52 taxa in common. In relation with the



mycorrhizal and saprotrophic taxa, 18 and 34 were appreciated in common respectively, showing again a higher coefficient between VAS and VAC (Table 3).

**Table 3**

Jaccard similarity coefficients (lightface) and the numbers of taxa in common (boldfaces) among habitats

Total	PA	VAS	VAC
PA		0.251	0.250
VAS	<b>38</b>		0.368
VAC	<b>42</b>	<b>52</b>	
Mycorrhizal	PA	VAS	VAC
PA		0.150	0.184
VAS	<b>9</b>		0.310
VAC	<b>12</b>	<b>18</b>	
Saprotrophic	PA	VAS	VAC
PA		0.302	0.291
VAS	<b>29</b>		0.409
VAC	<b>30</b>	<b>34</b>	
Edible	PA	VAS	VAC
PA		0.090	0.157
VAS	<b>3</b>		0.294
VAC	<b>6</b>	<b>10</b>	

Fungal community assemblies along three locations in a five years evolution period (data not shown) 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; 4b). The projection of the taxa points onto any axis indicates the position of taxa presence along an environmental factor (Ter Braak, 1986). A total of 11 edaphoclimatic variables ( $P < 0.05$ ) were significant in the ordination of mycorrhizal (8) 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 saprophytic taxa compositions. For mycorrhizal taxa, axis 1 is positively correlated with the significant edaphic variables such as N, P and K. Also mean precipitation is correlated with this axis. On the other hand, this axis is positively correlated with temperature conditions, including mean temperature but also temperatures for autumn fungal production period. Axis 2 is correlated with Potential Evapotranspiration (PE), but an inverse correlation is observed for precipitation values during fructification period such as PAS and PAN (Table 5, Fig. 4a). For mycorrhizal taxa, similar trends were observed

for Axis 1. However, Axis 2 is positively correlated with PAS and PAN (P=0.002 in both cases).

**Table 4**

Summary of Monte Carlo permutation test for the canonical correspondence analysis of fungal taxa presence and edaphoclimatic factors according to functional groups

Functional group	Variables	F-ratio	P-value
Mycorrhizal	N	2.86	0.002
	PAN	2.14	0.002
	ETP	1.98	0.004
	K	1.73	0.006
	Prec	1.57	0.010
	TAN	1.40	0.020
	P	1.34	0.054
	PAS	1.29	0.080
Saprotrophic	PAN	2.91	0.002
	N	2.74	0.002
	K	2.30	0.002
	Prec	2.08	0.002
	TMIN	1.65	0.020
	T	1.68	0.052
	TMAX	2.43	0.002
	TAN	1.76	0.002
	PAS	1.69	0.002
	ETP	1.61	0.028

**Table 5**

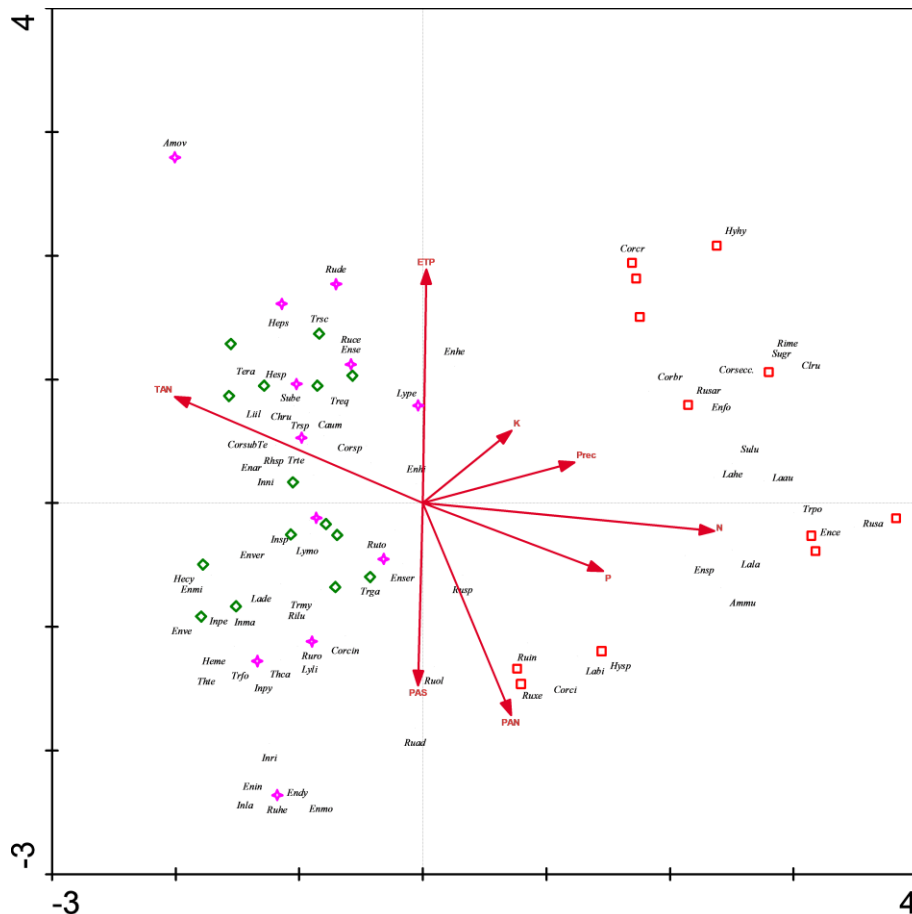
Summary of canonical correspondence analysis of fungal taxa presence and environmental factors according to functional groups in *P. pinaster* plots.

Functional group	Mycorrhizal		Saprotrophic	
	1	2	1	2
Axes				
Eigenvalues :	0.594	0.418	0.564	0.549
Species-environment correlations :	0.967	0.938	0.951	0.968
Cumulative percentage variance				
of species data :	7.1	12.1	6.7	13.2
of species-environment relation:	23.1	39.3	16.4	32.4

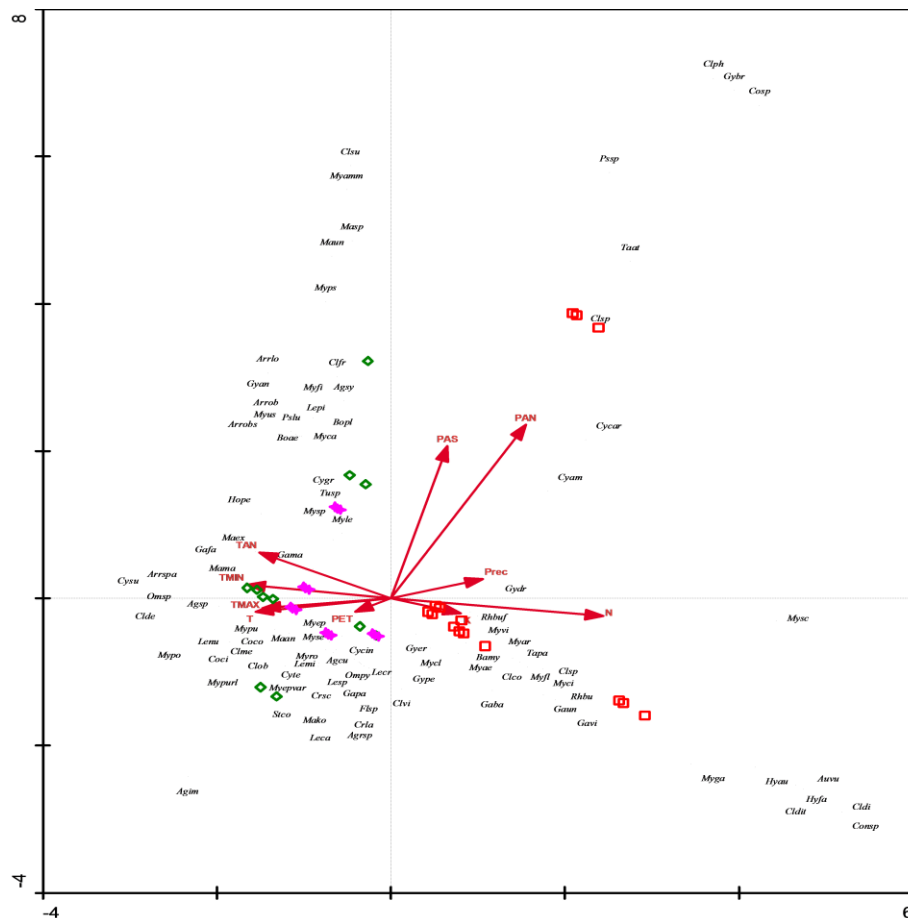
CCAs based on both fungal functional groups species composition, expressed that there are clear differences between fungal communities of *P. pinaster* sites (Figs. 4a, 4b). Axis 1 separates PA plots from VAC and VAS sites, and Axis 2 shows further

differences between the three treatments, especially between VA and VAC sites where there is a relative species overlap. VAS species tend to concentrate in middle while VAC species are dispersed towards the extreme of Axis 2 for both functional groups (Figs. 4a, 4b). Axis 1 explains 7.1 and 6.4 % of the variation in the data (mycorrhizal and saprophytic taxa respectively), and the two Axes together explain an accumulative variance for the interaction between taxa and environment of 23.1 and 16.4 % for mycorrhizal and saprotrophic groups respectively.

Regarding mycorrhizal species, VAN and VAC species are positively affected by temperatures from August to November (TAN) on behalf of the Axis 1, while PA is affected by the precipitations (Prec and PAN) and edaphic variables such as N, P and K. On the other hand, Axis 2 indicates an inverse correlation between Potential Evapotranspiration and PAS (Fig. 4a). On the other hand, saprophytic species in PA treatment are positively correlated to precipitations, N and K, while the VAC and VAS treatments are influenced by the temperatures and ET (Fig. 4b).



**Fig. 4a.** Canonical correspondence analysis ordination triplots showing fungal 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= Prec (August + September + October+ November) accumulated precipitation; PAS = (August + September) accumulated precipitation; ETP= annual mean ETP according to Thornthwaite (1955).N=Nitrogen; K=Potassium; P=Phosphorus. Fungal taxa are identified by the code shown in Table 2.



**Fig. 4b.** Canonical correspondence analysis ordination triplots showing fungal 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= Prec (August + September + October + November) accumulated precipitation; PAS = (August + September) accumulated precipitation; ETP= annual mean ETP according to Thornthwaite (1955).N=Nitrogen; K=Potassium. Fungal taxa are identified by the code shown in Table 2.

## 4. Discussion

### 4.1. Richness, diversity and sporocarps production

General data obtained in the present study coming from a mid-term survey of a 5 year period in three different edaphoclimatic sites are interesting since fungal diversity and production are considered useful tools in order to describe the general biodiversity in ecosystems (Oria-de-Rueda *et al.*, 2010) dominated by *P. pinaster*.

A total of 194 fungal taxa collected manifest a very high fungal richness in these ecosystems comparing to other conifer forests. In this sense, Bruns *et al.* (2002) found lower richness results 15-35 fungal species were reported for *P. contorta* and *P. muricata* stands. A total richness of 119 taxa was recorded for *Pinus sylvestris* forests in a long term inventory in the inner northeast zone of the Iberian Peninsula (Martínez-Peña *et al.*, 2012). For this same species this figure is similar to those found by Bonet

et al. (2004) with 164 taxa in central Pyrenees and Martínez de Aragón et al. (2007) who found 46 taxa in pine forests of the pre-Pyrenees mountains. Also, lower richness was reported associated with *P. Sylvestris* in northwest of Spain, when 43 taxa were collected (Oria de Rueda et al., 2010). Also *P. halepensis*, a typical Mediterranean conifer species present in Iberian Peninsula showed a lower richness than that found in *P. pinaster* stands. Thus, Oria-de-Rueda (2010) and Martínez de Aragón et al. (2007) found 16 and 35 taxa respectively.

The high richness values found for *P. pinaster* forests can be explained by the elevated levels of genetic variability in this host due to an important genotype by environment interaction that has enabled local adaptation to ecological conditions (Alfá *et al.*, 1996). This ecological adaptation causes a large ecological plasticity, and this species grows in a great range of soil and climatic conditions (Rodríguez-García et al., 2010). *P. pinaster* can be specially adapted to high temperatures and summer-time droughts but it is also present in areas characterized by soft temperatures and high precipitation values. In these 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 associated (Gassibe et al., 2011; Oria-de-Rueda et al., 2010; Martín-Pinto et al., 2006). Furthermore, our results showed a higher richness than that 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. Also, Oria-de-Rueda et al. (2010) found lower richness values recording 49 taxa in a 50-year old *Pinus pinaster* reforestation growing on degraded and eroded soils in northwestern Spain. Richness observed in our study was also higher than the one reported by Martín-Pinto et al. (2006), who recorded 39 taxa in a Mediterranean ecosystem dominated by *Pinus pinaster*. This data were also higher than those showed by Gassibe et al. (2011). In that study 115 fungal were recorded in a *Pinus pinaster* forest located in poor, degraded and stony soils with few disperse individuals of *Cistus ladanifer*. The high richness values reported in this work comparing to other *P. pinaster* studies can be explained since the three locations included in the present study cover a wide range of ecological variables from extreme Medierranean conditions present in VAS and VAC plots and pre Eurosiberian ecosystems (PA).

Regarding proportion of functional groups, we found an average ratio 1:2 for mycorrhizal and saprophytic taxa. In the three sites higher richness and diversity of saprotrophic compared to mycorrhizal fungi was observed. This fact could be due to

the presence of high amounts of organic matter in the forests, since decomposition rates are particularly low in these ecosystems where no coincidence of high precipitations and high temperatures is observed. Low decomposition rates are frequently found in *Pinus* stands (Seen-Irlet and Bieri, 1999), in contrast with the high amount of decomposed organic matter that is found in other deciduous forest located in more humid areas (Oria-de-Rueda *et al.*, 2010). This fact was accentuated in the PA and VAC plots since the environmental conditions are even more favourable for saprophytic taxa respect to VAS plots. Our results showed significantly higher values for Organic Matter contain and lower ones for Potential Evapotranspiration (PE) were found in PA and VAC plots (Table 1), and organic matter is likely to influence fungal communities through its impact on soil-moisture content and water-holding capacity, with poor aeration effectively halting spread of non-rhizomorphic mycelium (Dowson *et al.*, 1988). Hence, a build-up of litter would be likely to favour the rapid spread of many wood saprotrophs (Ferris *et al.*, 2000).

Moreover, despite mean precipitation is not different between VAS and VAC places, saprophytic richness was higher in VAC. In this sense, the values for Potential Evapotranspiration (PE) in VAS were significantly higher than in VAC affecting soil humidity and this fact could explain the differences regarding saprophytic richness between these two sites. However, there were no differences for the Shannon diversity index, between these two locations. This may indicate that although the number of saprophytic taxa was different, these species did not fruit uniformly. Low uniformity values resulted in a low Shannon diversity index despite the high richness observed (Hernández-Rodríguez *et al.*, 2013). On the other hand, no differences were found comparing mycorrhizal richness among sites. This fact is explained since this group of taxa is mainly affected by host species and the stand age (Bonet *et al.*, 2012) and, in our study, the three studied forests had similar age.

Regarding sporocarps yield, high total average fresh weight production (447.31 kg fw ha<sup>-1</sup>) was found. Similar values were previously referenced by Oria-de-Rueda *et al.* (2010), with an average plot yield of 476.3 kg fw ha<sup>-1</sup>. However, lower values of 332.7 kg fw ha<sup>-1</sup> had been found by Martín-Pinto *et al.* (2006) and 209.95 kg fw ha<sup>-1</sup> by Gassibe *et al.* (2011) under similar age and stand conditions. Our yield results were also higher than those found by Agreda *et al.* (2014) who reported 330 kg fw ha<sup>-1</sup> for different *P. pinaster* age classes in Mediterranean ecosystems in northwestern Spain. Despite these studies are referenced to the same host species, the high productions found in the present study may be attributed to the very different edaphoclimatic conditions which characterize the three sampled forest stands (Table 1). Many

previous studies link mushroom fructification to habitat characteristics (Bonet *et al.*, 2010) and climate conditions, mainly soil moisture and temperature (Martínez de Aragón *et al.* 2007, Barroetaveña *et al.*, 2008, Pinna *et al.*, 2010). Thus, in our case *P. pinaster* is able to form fungal associations with a large number of different mushroom taxa adapted to the broad ecological conditions that occurs in this study.

The results for sporocarps production were similar according to total, mycorrhizal and saprophytic functional groups. Significantly higher values were found in both PA and VAC plots than those in VAS forest.

The lower values for VAS treatment can be explained since this substrate is considered as a continental dune with highly particular soil properties. The sand surface is lacking of the protecting layer of mosses and lichens that the other two sites contain while VAS is influenced by drought, strong wind and moving sand, representing a harsh environment for establishment of a primary mycelium, making it difficult for the spores to settle and germinate (Høiland, 2012). In general this is considered as sandy soil with low holding capacity and fertility and characterized by significantly lower C/N ratio, Nitrogen and Organic Matter percentages. The lowest C/N ratio of the organic layer would explain major mycelia growth as noted by D'Agostini *et al.* (2011). Also, increased nitrogen can influence formation of mycorrhizas, production and distribution of extra radical mycelium in the soil, and sporocarps formation (Trudell *et al.*, 2004). Furthermore, other authors pointed that fungal communities can be adapted to more nitrogen-rich sites (Kranabetter *et al.*, 2009; Toljander *et al.*, 2006).

Moreover, soil moisture is an important factor in sporocarps production by macro fungi, 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 plots could explain higher yield values, while evapotranspiration differences between VAC and VAS plots suggest lower soil humidity and fungal productions in VAS soils, since a significant higher evapotranspiration affects reducing soil moisture (Trudell, 2004) which negatively influences fungal production.

Yield results according to functional groups showed the same trend in the three studied locations where mycorrhizal higher 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). Therefore, our result can be due to the fact that *P. pinaster* is considered as a fast-growing species

producing quickly high amounts of biomass. This is in relation with mycorrhizal taxa production since it depends directly on tree production.

Edible mushroom production can provide supplementary economical incomes for population as complement to those obtained from wood resources in forestry areas. In this sense according to edibility, a total of 117 kg fw ha<sup>-1</sup> were collected. This result was similar to the obtained in *P. sylvestris* forests in north-eastern Spain (Martinez -Peña *et al.*, 2012), where this host specie is considered highly productive. However, in the present study lower yields were reported in comparison to some of our previous results (Oria-de-Rueda *et al.*, 2010; Martín-Pinto *et al.*, 2006).

#### **4.2. Taxa composition**

High similarity was found between VAS and VAC treatments for total species, functionality and edibility and they showed a strong dissimilarity in macromycetes respect to PA plots. The main environmental variables affecting this result were link to climatic data. Thus, temperature (Tmean, Tmax., Tmin. and TAN) and precipitation (Pmean, PAN and PAS) were similar within Valladolid treatments whereas they were significantly different to those in Palencia plots (PA). As noted in previous studies, climatic variables can explain the 60 % to 80 % of the mass of mushrooms produced (Pinna *et al.*, 2010; Bonet *et al.*, 2012). 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 by calculation of Jaccard similarity coefficients, and few species were found in common between the sites. Furthermore, according to Jaccard similarity index, the Canonical Correspondence Analysis also showed a significant effect of the climatic variables on fungal communities. Thus, the results found support those of Humphrey *et al.* (2000) who reported that climatic variables influenced fungal community composition. The influence of precipitation and temperature has been previously studied. In this sense, O'Dell *et al.* (1999) observed an increase in species richness with higher precipitations. Also, other studies related fungal yield to climatic variables (Martínez de Aragón *et al.*, 2007; Bonet *et al.*, 2012; Gassibe *et al.*, 2013). In the present study, climate variables such us T, Tmin, Tmax, TAN, PE, 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, 2010). This is in accordance with the results observed where differences in fungal taxa composition were also appreciated correlated to superficial soil nutrients such us N and K. An increase trend of these variables is found towards PA plots. This may be a consequence of the management practices since PA



forest stand was located in crop land area which a higher fertility. Therefore, the inputs of organic matter are reflected in soil nutrient availability, particularly in N. In contrast, both VAC and VAS plots are sited 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 Nantel 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 evidences a large degree of community specialization along the soil quality gradient (Reverchon *et al.*, 2010; 2012).

Regarding mycorrhizal taxa, CCA 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 (Carney *et al.*, 2011; Wallander, 2000).

Some mycorrhizal genera such as *Cortinarius*, *Inocybe*, *Entoloma*, *Russula* and *Tricholoma* included a higher number of species. This is in accordance with previous results which reported several species within these genera associated with similar pine stands (Agreda *et al.*, 2014; Oria-de-Rueda *et al.*, 2010; Gassibe *et al.*, 2013). *Cortinarius*, *Entoloma* and *Russula* showed a great ecological range since they were notably present in the three studied forests under significantly different edaphoclimatic conditions. In this sense, *Cortinarius* and *Russula* genera were frequently found in four different Irish forests characterized by particular soil factors (O'Hanlon and Harington, 2011). A comparable tendency for the dominance of Russulaceae species was previously observed by Azul *et al.* (2010) in southern Portugal. This result was also reported in other Mediterranean areas (Bergemann and Garbelotto, 2006; Courty *et al.* 2008; Richard *et al.* 2004, 2005). This fact may be explained since this family comprises a large range of species which are able to fruit along very diverse ecological requirements. The taxonomical diversity and cosmopolitan distribution within Russulaceae further impose 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.*, 2010). It is noted that although *Entoloma* species are highly frequent, they most commonly fruit in summer, and therefore their fruiting is less constant (Heilmann-Clausen and Vesterholt, 2008).

Even more, at species level, *Lycoperdum perlatum* and *Russula torulosa* were found in common and able to form symbiotic associations with *P. pinaster* in the three locations. *L. perlatum* showed to be present en recent studies carried out under different

ecological environments (Azul *et al.*, 2010; Agreda *et al.*, 2014). *Russula torulosa* was also found by Bonet (2004) and Gassibe *et al.* (2011) in diverse edaphoclimatic conditions.

While *Suillus bellini* occurred linked to VA plots, where xerophitic conditions are present, *S. Granulatus* and *S. Luteus* were exclusively collected in PA plots, linked to higher precipitation and lower temperature conditions. *S. granulatus* is positively correlated to ETP and negatively to TAN, following the findings by Martínez-de-Aragón *et al.* (2007) who found a negative correlation between production for these species based on temperatures and positive relation with potential evapotranspiration during September. In the case of *S. luteus*, our results showed a positive correlation between P. annual with its distribution and negatively correlated to TAN, also similar to that reported by Martínez-de-Aragón *et al.* (2007). These authors referenced this taxa to water deficits and precipitations from September to November for Mediterranean conditions.

According to CCA results, *Laccaria laccata* and *L. bicolor* were found positively correlated to N, P as well to annual precipitation. Similar results were obtained by Azul *et al.* (2010) where *L. laccata* was closely related to undisturbed and fertile soils. Reverchon *et al.* (2012) at Mexican pine site, showed positive correlations towards P and N nutrients for both species. These results contradict those obtained by Buée *et al.* (2011), who stated the negative impact of fertilisation on *Laccaria bicolor*, negatively in coniferous stands in France.

All the species within *Inocybe* genus were exclusively collected in Valladolid plots. When analysing CCA Axis 2, a positively correlation between these species and PAS variable could be observed. This result is according with the same found by Martínez-de-Aragón *et al.* (2007) when studied some species within this genus. In that case, a direct correlation with September precipitation was noted. A similar trend was observed for *Hebeloma* genus. In this case, while *H. psammophilum* and *Hebeloma sp.* were found to be correlated to TAN, *H. mesophaeum* and *H. cylindrosporum* were linked to PAS. Similar correlations for species within this genus were previously found (Martínez-de-Aragón *et al.*, 2007).

Some species from *Tricholoma* genus showed contrary trends. Thus, while *Tricholoma portentosum* can be considered as a hygrophilous species that fructified in more humid years (Hernández-Rodríguez *et al.*, 2013), *Tricholoma terreum* is a xerophilous species typical of Mediterranean pine forests.

On other hand, our results on saprophytic taxa distribution revealed that they are positively correlated with high precipitation values, present in PA plots. Taxa from VAC

and VAS plots are highly correlated to rising temperatures whereas differences between these two locations could be caused by Potential Evapotranspiration, originating more extreme xerophytic conditions in VAS location. Also soil conditions such as N and K affect taxa composition. Higher nitrogen contents observed in PA plots are related to the most nitrophyllous species. In this ecosystem, 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 saprophytic taxa by genera, we found several genera such as *Mycena*, *Galerina* and *Cystoderma* which appeared in the three studied locations. The most represented genus was *Mycena* including 28 species. This genus is able to fruit under very different ecological conditions (Moore *et al.*, 2008). Also, *Galerina* was present in the three studied locations which correspond with that found by Heilman and Vesterholt (2008), who defined it as a genus with broader ecological amplitude. Moreover, the presence of *Cystoderma* species could be explained by the higher availability of substrata in older stands, where conditions of temperature and moisture are more adequate for fungal fruiting (Fernández-Toirán *et al.*, 2006; Hernandez-Rodríguez *et al.*, 2013).

However, other genera appeared exclusively in one of the locations associated to specific ecological conditions. In this sense, all the *Agaricus* species found in our study were exclusively collected in VAS, according to previous works in which several *Agaricus* species were found in an inland dune ecosystem in south Iberian Peninsula (Ortega and Esteve-Raventós, 2005). Also, some of these species were collected in the sandy desert areas of Almeria (Southern Spain), which is the most arid region in Europe (Calonge and Oria de Rueda, 1987), and in sandy dunes from England (Rotheroe, 2001).

Also, species within *Macrolepiota* genus exclusively appeared in VAC plots. The Mediterranean ecology of the three collected species can explain this fact (Ortega and Esteve-Raventós, 2005). Thus *M. excoriata*, *M. konradi* and *M. mastoidea* are characterized by fruiting during in autumn season in lighted positions in forests, and prefer calcareous sandy soils (Rotheroe, 2001). It was also noticeable that we could find in the Mediterranean studied areas some species that until now were exclusively picked in coastal sandy dunes. In this sense, mycorrhizal (*Gyroporus ammophilus* and *G. cyanescens* var. *lacteus*) and saprophytic (*Conocybe dunensis*) taxa were collected. Regarding the hygrophilus species that have been found in Palencia due to its more humid characteristics such as *Tricholoma portentosum*, *Tricholomopsis rutilans*, *Suillus*

*luteus*, *Suillus granulatus*, *Hygrophorus hypothejus*, *Hygrophoropsis aurantiaca*, *Entoloma cetratum*, *Entoloma formosum*, *Tapinella panuoides*, *Tapinella atrotomentosa*, *Russula xerampelina*, *Russula sardonina*, *Russula sanguinea*, *Rickenella mellea*, *Psathyrella hydrophylla*, *Phanerochaete sanguinea*, *Mycena sylvae-nigra*, *Mycena flavoalba*, *Lactarius aurantiacus*, *Hypholoma fasciculare*, *Cystoderma amianthinum*, *Cortinarius brunneus*, *Cortinarius croceus* and *Auriscalpium vulgare*, can be contrasted to the most xerophitic species found exclusively in the calcareous Valladolid plots, *Xylaria hypoxylon* and *Amanita ovoidea*, regarding the latest one, is a very peculiar result due to its association to *P. pinaster* in Mediterranean areas.

The results reported here showed the important economical and ecological role played by both *P. pinaster* natural and reforestation stands. It is noteworthy the high fungal production and the great fungal community that can be found associated to these forests, partially due to the great plasticity of this forest species. Thus, an adequate management of these *Pinus* stands including mycosilviculture would also result since they can be considered the most productive related to fungal yield and diversity in Mediterranean ecosystems. Therefore, management of these areas including both production and biodiversity purposes should take into account considerations regarding fungal communities and the influence of different ecological conditions on fungal production and diversity.

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