Final Report - Antonio Tomao

Cost Action: FP 1203 "European Non-Wood Forest Products Network"

STSM title: **Study of the effect of silvicultural practices on mushroom yield**.

Reference code: COST-STSM-ECOST-STSM-FP1203-120415-057057

STSM dates: from 18-04-2015 to 18-05-2015

Location: CTFC Centre Tecnològic Forestal de Catalunya, Solsona, Spain

Host: Dr Jose Antonio Bonet

Purpose of the STSM and description of the work carried out during the STSM

In the last decades multiple-use forestry has been more and more integrated into forest policy programs in order to maximize forest multi-functionality and ecosystem services, including non-wood products. In particular, mushroom production has recently become a very important socioeconomic resource, especially in woodlands where timber value is low, such as in most Mediterranean pine forests. This growing interest has stimulated a lot of studies trying to understand which factors influence production and if and to what extent forest management can be used to increase mushroom yield.

Models based on empirical studies are a useful tool to integrate mushroom production in the management of these areas. Based on these considerations specific purposes of the STSM are (i) analyze if and to what extent forest management (thinning) can influence composition of fungal communities; (ii) to develop predictive empirical models for mushroom production and diversity from data collected in *Pinus pinaster* stands. Thus, this STSM contributes to the Action FP1203, by increasing knowledge about NWFP (mushrooms) production and its modeling. In particular, STSM focusing on mushroom yield, contributes to Working Group 1 (Mushrooms and truffles) aims and scopes.

During the month of my STSM in the Centre Tecnològic Forestal de Catalunya (Solsona, Spain) the work consisted in a twofold activity: (i) the analysis of mushroom production and diversity data of thirty plots established between 2008 and 2009 in the Natural Park of Poblet (Tarragona, Northeastern Spain) in a 50-year-old plantation and (ii) field survey of stand variables (diameter increment) of permanent plots. In particular, 28 plots in Poblet, 12 in the Solsonés disctrict and 2 plots in the central Pyrenees were inventoried. Moreover, meteorological data (soil and air moisture and temperature) have been collected from permanent weather station in nine plots in the central Pyrenees.

1. Introduction

Fungi, as major component of biodiversity, are essential for forest ecosystems due to their leading role in decomposition and nutrient cycling. In fact, stability and resilience of the system are strongly supported by high fungal diversity. At the same time, mushrooms are an important economic resource for picking activity both for recreational use and market purposes.

Although factors affecting sporocarp production have been studied for many years (Vogt et al., 1992), many questions related to fungal ecosystem functioning and forest management practices able to increase mushroom yield and biodiversity are still open. Studies regarding the impact of silvicultural systems on diversity and community have recently grown in number (e.g. Bonet et al., 2012; Luoma et al., 2004; Pilz et al., 2006; Seiwa et al., 2012; Teste et al., 2012; Yamashita et al., 2014; Lin et al., 2015).

Thinning, for example, is nevertheless a disturbance to organisms in forest ecosystems and affects biodiversity and ecosystem function (Bengtsson et al., 2000). Combined with other factors such as vegetation, environmental factors (Tedersoo et al., 2011) or climate changes (Kauserud et al., 2008) it can lead to changes in fungal community (Lin et al., 2015). Studies like Bonet et al (2012) show the immediate effect of thinning in yield of *Lactarius* group *deliciosus*. Thinned stands were more productive than the unthinned ones, and the effect of thinning was higher during the first productive season after the thinning. Furthermore, Lin et al. (2015) found that forest thinning affects macrofungal species richness but not abundance and influences macrofungal community composition even if differences between thinning intensities were not significant.

However, the effect of silvicultural treatments, such as thinning, on mushroom yield are variable and even contradicting. Thus, more studies must be developed about this topic.

Understanding effects and trade-offs of silviculture practices on fungal communities can help in defining the better way to manage forests in a multifunctional framework, integrating production of timber and non wood products with biodiversity conservation. This resource planning by multifunctional managing is a complex task, but it could be the best way to improve incomes for forest owners and provide an increase in resilience of these ecosystems.

2. Material and methods

2.1 Study area

The study area (Figure 1) is located within the Natural Park of National Interest (PNIN) in Poblet (Tarragona -Catalonia-). PNIN extends to 2,276 ha ranging between 400 and 1,201 m.a.s.l. The climate is Mediterranean with continental traces: precipitation of about 600 mm and annual mean temperature of 13.2ºC. Soil is siliceous with franc-sandy textures.

Figure 1. Location of study area, Natural Park of National Interest (PNIN) in Poblet (Tarragona -Catalonia-)

15 inventory plots (10m x 10m) were established in 2008 in a 50 year-old Pinus pinaster stand: and13 plots of the same size located in larger 1600 m^2 (40 m x 40 m) thinned areas - in order to reduce edge effect - treated in July and August 2009. Felled trees were taken away manually to reduce a possible effect of the treatment on the soil. *Quercus* spp. shoots were also removed. All the plots were fenced in order to prevent mushroom harvesting.

All fungal sporocarps (cap diameter > 1 mm) have been collected weekly during fruiting season (end of summer - autumn) between September 2009 and December 2014. Total number of sporocarps has been counted and fresh and dry weight were measured.

Stand variables have been also collected within each of these plots: diameter at breast height of all trees and total height. Based on these data, density, basal area, mean diameter, dominant and mean height were calculated.

Annual precipitation and temperatures and monthly means from August to November were obtained by the meteorological station of Poblet.

2.2 Data analysis

Shannon Wiener (H' = 0 $\rightarrow\infty$) and Pielou's (E = 0 $\rightarrow$1) indices (Magurran, 2004) were calculated. Fruitbody biomass was used as indicator of total biomass (Bonet et al., 2004) to estimate the diversity of the communities. Changes in fungal diversity and abundance over the course of the study were estimated by performing analysis of variance (ANOVA) using STATA v.13 software.

In particular, changes in composition (weight and abundance) of fungal functional groups (saprophyte and ectomycorrhizal) due to forest management practices have been analyzed.

Furthermore, three empirical models (GLS mixed effects panel models) were developed for shannon diversity index and saprophyte and ectomycorrhizal mushroom production using climatic variables, stand and topographical characteristics as predictors. This model has been chosen because the phenomena of interest is repeatedly measured on the same sample unit over time (longitudinal data). This analysis enabled us also to verify which variables are significantly correlated with mushrooms production.

3. Preliminary results

3.1 Analysis of effect of thinning on diversity of ectomycorrhizal and saprophytic communities production

Calculation of Shannon index enabled us to evaluate fungal diversity in Poblet permanent inventory plots. Shannon index values across years have been reported in Figure 2. While there is a high variability across years, due to different climatic conditions, no significant differences were found between thinned and unthinned plots for Shannon index ($p>0.1$). However relative Pielou's index was significantly lower in 2011 ($p<0.05$). Even if we take into account intensity of thinning (Figure 3 and Figure 4) we do not find any significant difference in diversity index value for the same year, except in 2013, when Pielou's index was significantly lower in the high intensity thinning treatement. Low absolute values for the year 2013 can be explained by the meteorological factors influencing fungal production: very low precipitations and high temperature determined low yield.

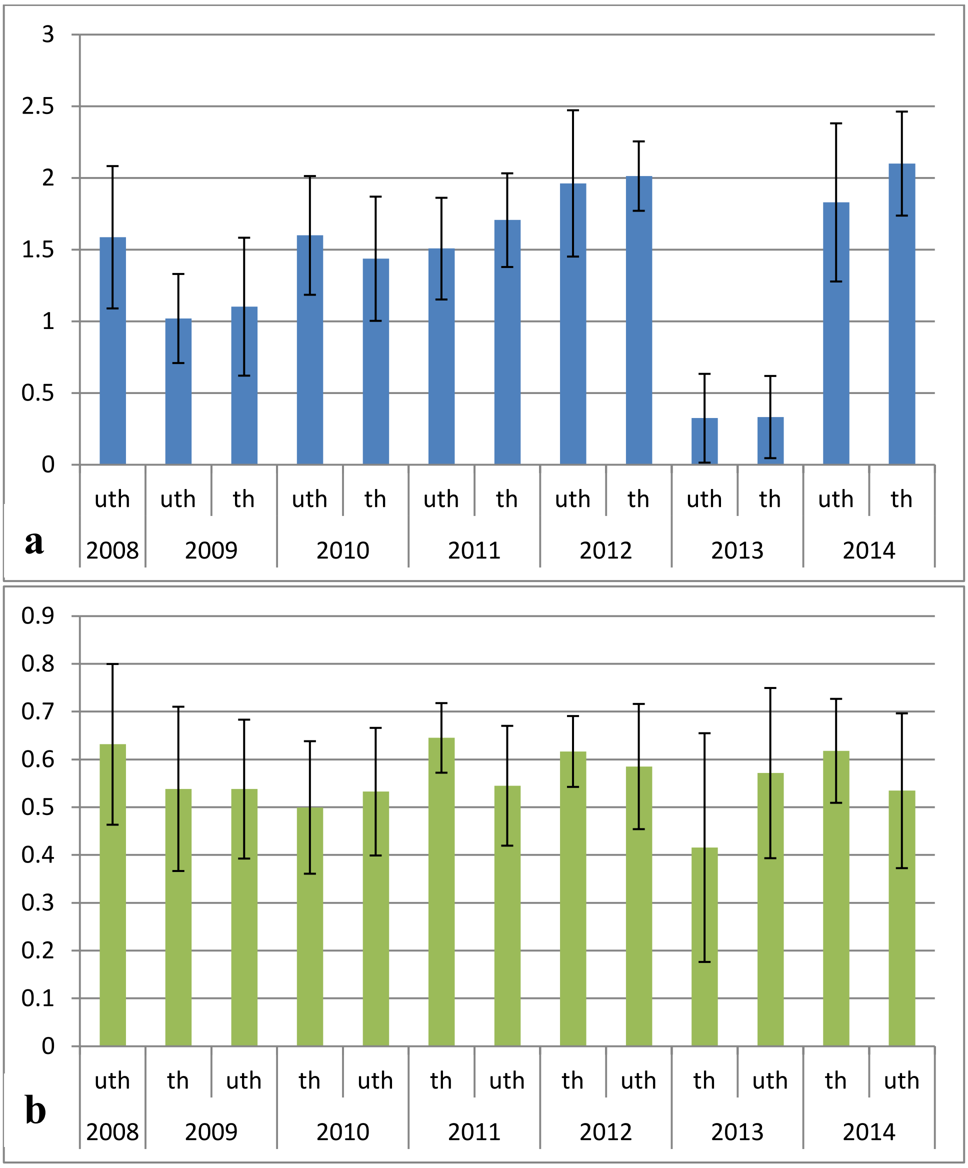

Figure 2. Shannon index (a) and Pielou's index (b) calculated for inventory plots in Poblet across years. *Uth* refers to unthinned plots and *th* to thinned ones. No significant differences were found between thinned and unthinned plots in the same year (p>0,05) except for relative index in 2011 (p<0,05).

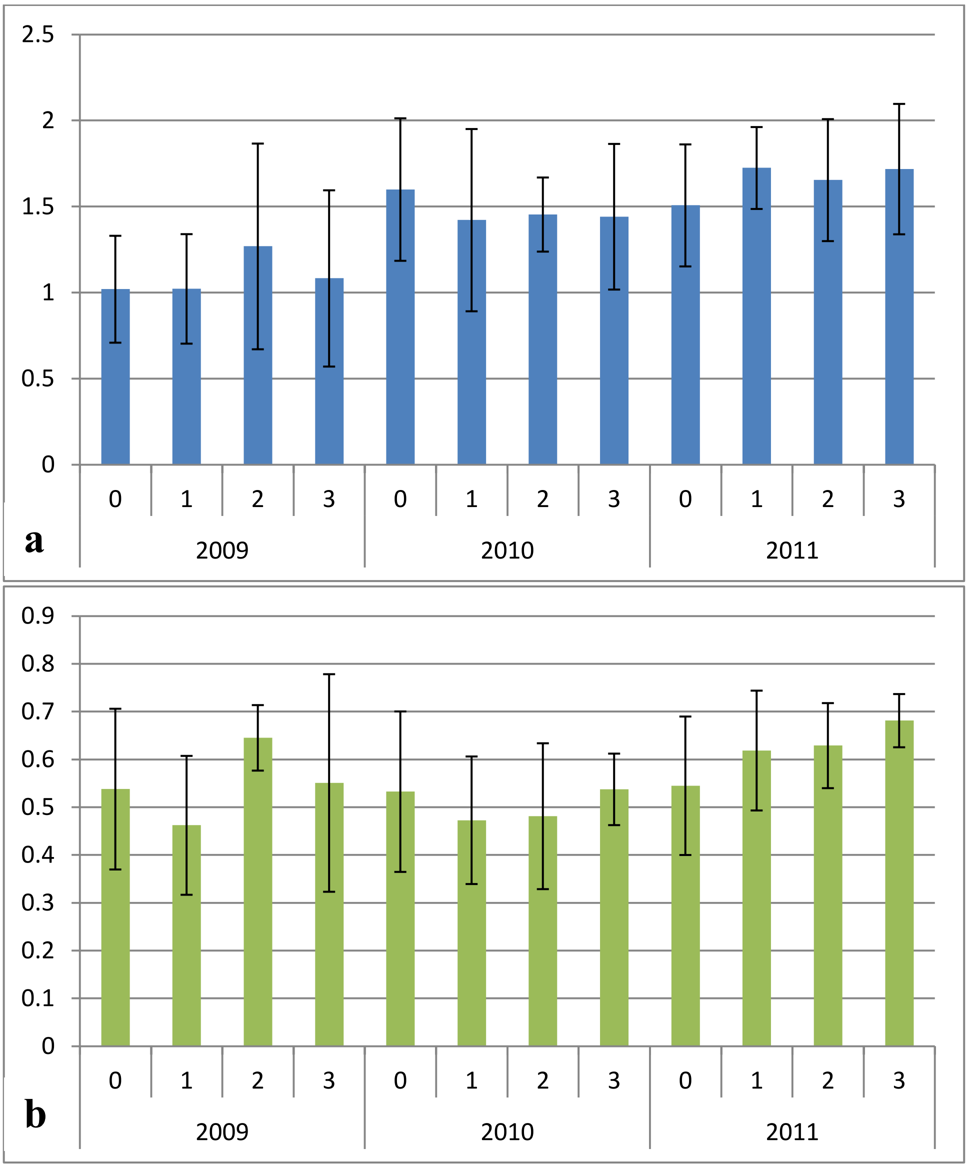

Figure 3. Immediate effect of forest management (years 2009-2011) on Shannon index (a) and Pielou's index (b). Four thinning intensity have been considered: 0) control; 1) 0-29% of removed basal area; 2) 30-49 of removed basal area; 3) more than 50% of removed basal area. No significant differences were found between thinned and unthinned plots and among thinning intensities in the same year (p>0,01).

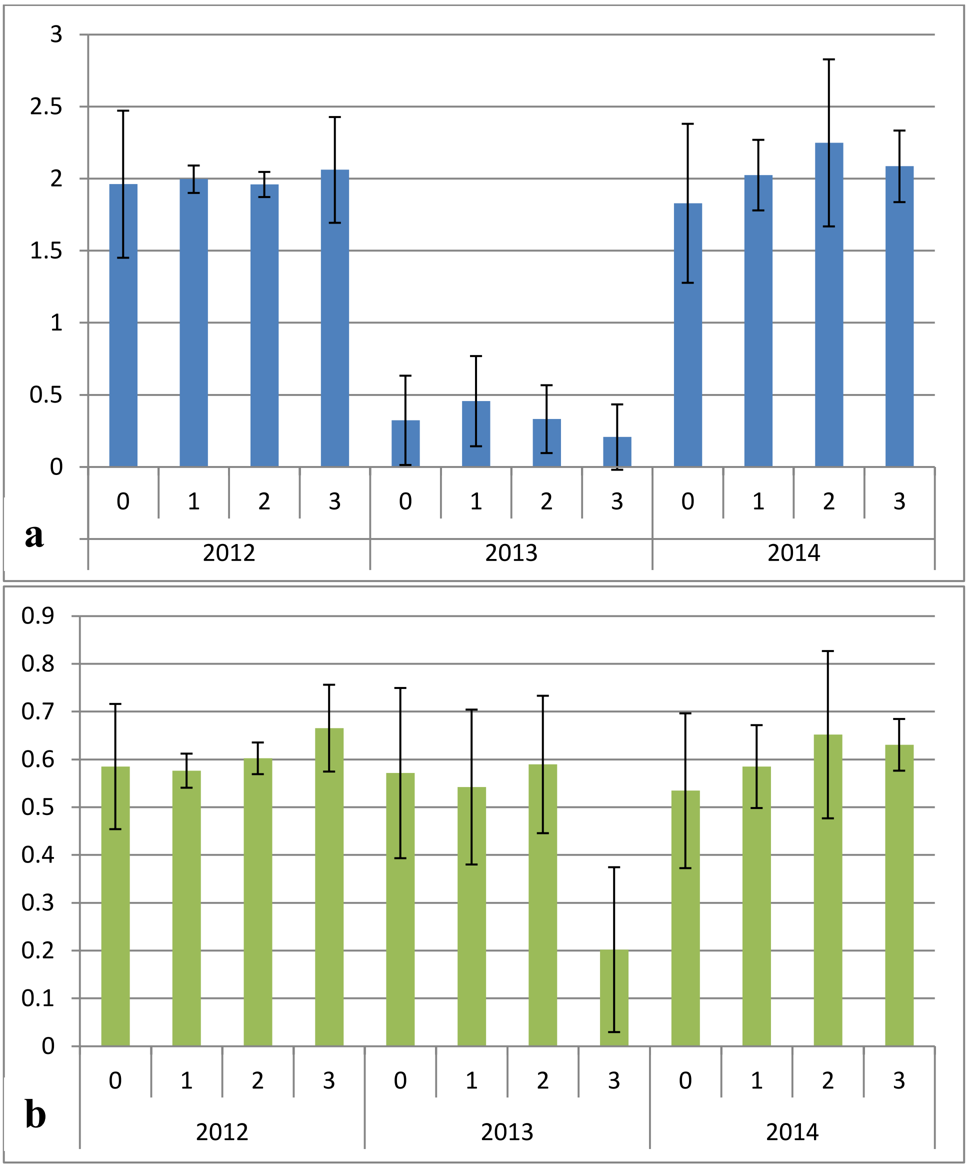

Figure 4. Mid-term effect of forest management (years 2012-2014) on Shannon index (a) and relative index (b). Four thinning intensity have been considered: 0) control; 1) 0-29% of removed basal area; 2) 30-49 of removed basal area; 3) more than 50% of removed basal area. No significant differences were found between thinned and unthinned plots and among thinning intensities in the same year (p>0,05), except for the year 2013, when E index was significantly lower (p<0,05) in the highest thinning intensity.

In order to verify if forest management has effects on fungal community both saprophytic and mycorrhizal species abundance has been analyzed. After seven-year monitoring period, 40.071 sporocarps have been collected, with a fresh weight of 171,63Kg and a dry weight of 23,33kg. These collections included 359 different fungal species and an additional 24 taxa identified to genus.

177 are saprotrophic or parasitic species, 138 ectomycorrhizal species. 7 taxa were identified as ectomycorrhizal by genus. Moreover, 63 species or genus which ectomycorrhizal status is unclear were collected.

In this work two main groups of ectomycorrhizal and saprotrophic species were considered. Abundance in terms of number of sporocarps across years is reported in Figure 5. ANOVA one-way test has been performed to evaluate differences among thinned and unthinned plots.

In terms of number of sporocarps no significant difference has been found between managed and unmanaged plots. However, in 2011, 2013 and 2014 saprotrophic species experienced an higher abundance compared to ectomycorrhizal. On the other hand, ectomycorrhizal abundance is higher in the first two years after thinning.

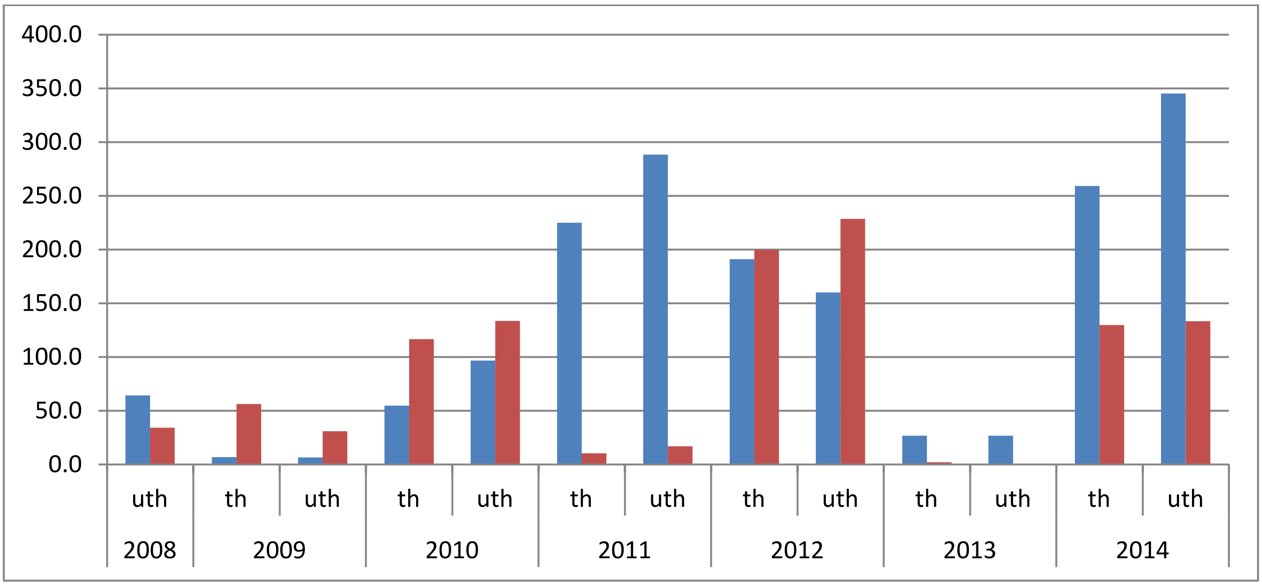

Figure 5. Average number of sporocarps across years for saprophytic (blue) and ectomycorrhizal (red) species.

Comparing dry weight of these two ecological groups (Figure 6) we can see that a clear difference can be found between production of ectomycorrhizal and saprophytic species. Dry weight is, in fact, significantly higher than saprophytic in 2009, 2010 and 2014. Moreover, a significant difference ($p<0,05$) in dry weight of ectomycorrhizal fungi between control and managed plots is highlighted in 2009. This result confirms the immediate effect of thinning found by Bonet et al. (2012).

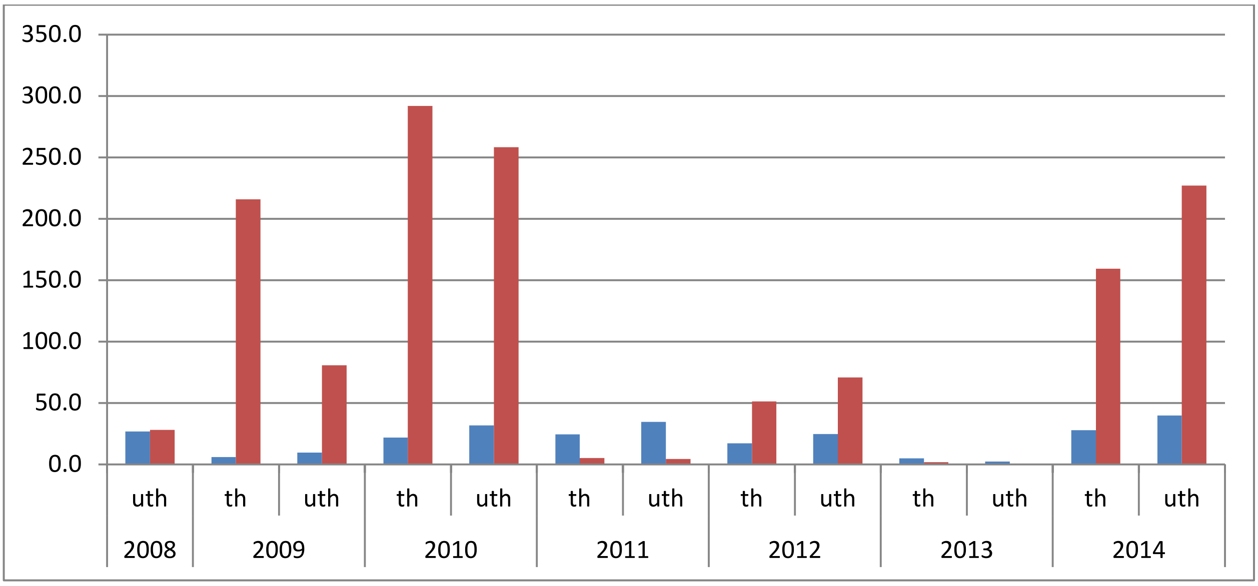

Figure 6. Dry weight of sporocarps across years for saprophytic (blue) and ectomycorrhizal (red) species.

Considering also intensity of thinning the same analysis has been performed. Results are reported in Table 1 and Table 2. While no significant differences have been found in terms of number of sporocarps and dry weight (p>0,05) for saprophytic species, a significant enhance of ectomycorrhizal sporocarp production (p<0,05) has been observed in low intensity thinned plots in the first year after thinning. This effect is more evident in terms of dry weight (p<0,01).

year	intensity of thinning	average number of sporocarps	CV	average dry weight [g]	CV
2009	0	6.4	0.6	9.6	1.4
	1	9.2	0.8	11.5	1.2
	2	3.7	1.1	2.8	0.8
	3	6.4	1.3	1.9	1.2
2010	0	77.1	0.7	27.1	1.1
	1	59.8	0.9	34.3	0.4
	2	89.0	0.3	25.3	0.4
	3	28.6	0.8	7.3	0.6
2011	0	258.9	0.6	29.9	1.1
	1	210.4	0.5	27.8	0.8
	2	290.3	0.3	28.4	0.8
	3	200.2	0.7	18.8	0.8
2012	0	174.3	0.4	21.1	0.6
	1	179.0	0.5	17.6	0.3
	2	167.7	0.6	18.0	0.1
	3	216.6	0.5	16.2	0.5
2013	0	26.7	0.8	3.5	0.7
	1	23.0	0.9	2.3	1.3
	2	33.3	0.2	3.8	0.7
	3	26.6	0.9	8.0	1.0
2014	0	305.2	0.7	34.1	0.9
	1	207.2	0.1	26.2	0.8
	2	331.7	0.4	46.5	0.4
	3	267.4	0.8	18.2	0.7

Table 1. Number of sporocarps and dry weight of saprophytic species. Four thinning intensity have been considered: 0) control; 1) 0-29% of removed basal area; 2) 30-49 of removed basal area; 3) more than 50% of removed basal area. No significant differences were found between thinned and unthinned plots and among thinning intensities in the same year (p>0,05).

year	intensity of thinning	average number of sporocarps	CV	average dry weight [g]	CV
2009	0	30.9	0.8	80.6	1.1
	1	90.8*	0.7	331.0**	0.7
	2	18.7	0.5	96.5	0.3
	3	43.8	0.6	172.0	0.7
2010	0	125.7	0.7	273.9	0.5
	1	159.2	0.5	428.6	0.5
	2	104.7	0.2	267.0	0.2
	3	81.2	0.6	169.9	0.7
2011	0	13.8	1.5	4.8	1.4
	1	14.2	0.9	9.6	0.7
	2	6.0	0.7	3.5	0.9
	3	8.8	0.5	1.6	1.2
2012	0	215.0	0.9	61.7	1.0
	1	338.2	1.1	95.2	0.7
	2	98.7	0.2	24.6	0.1
	3	121.2	0.7	23.3	0.5
2013	0	1.1	1.5	0.9	0.5
	1	4.8	2.0	4.7	2.0
	2	0.0	-	0.0	-
	3	0.0	-	0.0	-
2014	0	131.5	0.7	195.6	1.0
	1	182.8	0.5	207.5	0.6
	2	85.0	0.1	198.3	0.7
	3	103.0	0.7	87.5	0.5

Table 2. Number of sporocarps and dry weight of ectomycorrhizal species. Four thinning intensity have been considered: 0) control; 1) 0-29% of removed basal area; 2) 30-49 of removed basal area; 3) more than 50% of removed basal area. Significant differences with $p<0,05$ (*) and $p<0,01$ (**) have been reported.

Analysis of predictors of fungal diversity and yield

In order to better understand influence of silvicultural treatements and other environmental variables on fungal communities GLS random effects panel models were developed. Results for shannon index are shown in Table 3. Thinning intensity was included as a categorical variable and mean temperature from august to november were excluded because of collinearity.

Shannon index model (overall R-squared= 0.67) highlights how meteorological variables such as precipitation in August, October and November are strong predictors of fungal diversity, together with slope.

	Coefficent	Standard Error	Statistical significance
thinning intensity			
0	ref.	ref.	
1	0.14	0.09	
2	0.17	0.1	
3	0.02	0.09	
meteorological variables			
total annual precipitation	0.001	0.0008	
august precipitation	0.07	0.03	*
september precipitation	-0.01	0.01	
october precipitation	0.006	0.0006	**
november precipitation	0.0035	0.001	*
mean annual temperature	-0.17	0.09	
topographical variables			
altitude	-0.0002	0.0002	
aspect	0.0003	0.0002	
slope	-0.013	0.005	*

Table 3. GLS random effects panel model for shannon index. Significance is reported for p value lower than 0.05 (*) and than 0.01 (**).

GLS random effects panel models for dry weight of saprophyte (overall R-squared= 0.28) and ectomycorrhizal mushrooms (overall R-squared= 0.52) are reported in Table 4 and Table 5. Significant predictors for saprophyte production include october and november precipitation. High thinning intensity can also be considered a predictor with a negative effect on saprophyte yield.

On the other hand, ectomycorrhizal total dry weight can be successfully predicted using low intensity of thinning -very high positive effect-, november cumulated precipitation, annual total precipitation and mean temperature. Among topographical variables aspect is a significative predictor with a slight negative effect.

	Coefficent	Standard Error	Statistical significance
thinning intensity			
0	ref.	ref.	
1	-6.42	4.72	
2	-4.99	5.64	
3	-11.13	4.57	*
meteorological variables			
total annual precipitation	-0.06	0.04	
august precipitation	0.1	1.65	
september precipitation	0.27	0.62	
october precipitation	0.08	0.04	*
november precipitation	0.23	0.07	**
mean annual temperature	-4.82	4.85	
topographical variables			
altitude	0.01	0.01	
aspect	-0.02	0.012	
slope	0.45	0.28	

Table 4. GLS random effects panel model for production of saprophyte species (dry weight). Significance is reported for p value lower than 0.05 (*) and than 0.01 (**).

	Coefficent	Standard Error	Statistical significance
thinning intensity			
0	ref.	ref.	
1	56.1	24.45	*
2	1.76	29.24	
3	-28.8	23.69	
meteorological variables			
total annual precipitation	-0.64	0.21	**
august precipitation	8.57	8.55	
september precipitation	-0.21	3.2	
october precipitation	-0.07	0.19	
november precipitation	0.76	0.39	*
mean annual temperature	-64.64	25.17	**
topographical variables			
altitude	-0.11	0.07	
aspect	-0.26	0.07	**
slope	2.22	1.45	

Table 5. GLS random effects panel model for production of ectomycorrhizal species (dry weight). Significance is reported for p value lower than 0.05 (*) and than 0.01 (**).

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Future collaboration with host institution

This STSM will allow to the two institutions (the University of Tuscia of Viterbo and Centre Tecnològic Forestal de Catalunya) to start collaborating in order to improve their knowledge about effect of forest management on fungal yield and diversity. This could lead also to the establishment of a first network useful for building future research projects at Mediterranean level.

Foreseen publications/articles resulting or to result from the STSM

Work and results of this STSM will be published in a peer-reviewed scientific journal. This article has already been started and it will be submitted to a journal in the next few months.