**Can tree rings be used to predict fungi production?**

**A study case in Catalonian forests, NE Spain**

COST Action: FP1203–European Non Wood Forest Products

STSM Applicant: Irantzu Primicia, Czech University of Life Sciences, Prague, Czech Republic.

STSM Period: From 27/04/2015 to 27/05/2015

STSM Reference: ECOST-STSM-FP1203-080415-059892

Host: Dr. Jesús Julio Camarero, Instituto Pirenaico de Ecología (IPE- CSIC), Zaragoza, Spain.

## PURPOSE OF THE STSM

The view of forests as a simple source of woody products is nowadays mostly outdated and forest policies aim to achieve a sustainable and multi-purpose management of forest ecosystems, including protective, environmental, social and cultural ecosystem services (MCPFE, 1993). Non-wood forest products such as fungi provide important recreational and commercial activities in European forested areas and represent an important income for rural societies (Food and Agriculture Organization of the United Nations, 1995).

Fungi production depends on several factors such as climate variability, forest tree species, site quality, and the market value of the mushroom species (Alexander et al., 2002). Previous empirical models have highlighted the importance of site (e.g. altitude, slope, aspect), stand conditions (tree composition, basal area, tree density) and management on mushroom yield and diversity in pine forests from USA (Diez et al., 2013), Switzerland (Boddy et al., 2014), and Catalonia (NE Spain Bonet et al., 2010; de Miguel et al., 2014). Predictive models for mushroom yield would allow forecasting fungal production, an essential tool to optimize the multiple uses of forests.

The understanding of the relationships between mushroom yield and climate conditions is necessary to estimate mushroom production under warmer climatic scenarios. Climate plays a major role on mushroom production since a decrease in soil water availability after summer, usually related to drier and warmer climatic conditions, delays and reduces fungi production, even though the effects are highly variable among fungi species (Diez et al., 2013). Additionally, when investigating mushroom production in relation to climate variables, clear trends are not always observed, probably because of differences between ecological groups, ecosystems and data available (Boddy et al., 2014).

The study of tree-ring features (width, density, wood anatomy) for ecological purposes (dendroecology) is a powerful tool to understand how coupled are long-term climatic conditions, radial growth (stem wood production) and mushroom production (Büntgen and Egli, 2014). Here we propose using dendroecology to investigate the long-term relationships between climate, secondary growth, a proxy of carbon availability to trees since wood formation has a low allocation priority compared to shoot development, and mushroom production. Additionally, wood anatomical features (e.g., lumen area, wall thickness) can encode additional ecological information, which can now be recovered thank to recent technical advances (von Arx and Carrer, 2014). For instance, a more ample tracheid transversal lumen area usually reflects increased potential hydraulic conductivity and enhanced productivity in conifers (Eilmann et al., 2006). During the STSM, the relationships between basal area increment, a variable here used to quantify annual secondary growth, and other seasonal tree-ring features (earlywood and latewood width) and climate conditions on mushroom production at long time scales will be investigated. Additionally, it is hypothesized that latewood features related to wood anatomy (e.g. lumen area) would encode climatic information related to the production of mushrooms which usually peaks in autumn when the late latewood is formed.

## MATERIAL AND METHODS

### Data collection: mushroom yield assessments

Mushroom yields were weekly assessed in 107 plots located in forests of several pine species across Catalonia (NE Spain) for the period 1997-2014 (de Miguel et al., 2014). Mushroom production and species richness were inventoried every week during autumn (September–December) and the collection included all epigeous ectomycorrhizal species as well as non-ectomycorrhizal edible species. All sporocarps collected were identified at the species level whenever possible, even though some samples could only be identified to the genus, subgenus, section, or subsection levels. Most of the plots are pure and mixed pine stands. Only monospecific pine forests (*Pinus sylvestris*, *Pinus nigra*, *Pinus halepensis* and *Pinus pinaster*) located in two areas (Solsonès and Prades) have been considered for the current study based on the available series of mushroom production and the different climatic conditions of these regions (Table 1). The Solsonès region is subjected to continental and sub-Mediterranean conditions, whereas Prades region experiences a stronger Mediterranean influences. The sampled Prades stands were plantations performed in the 1960s. Physiographic and stand attributes such as slope, aspect, elevation, tree density and basal area were also recorded for each plot. Additional information on the sampling methodology can be found in Bonet et al. (2004, 2010).

**Table 1.** Summary of the plot characteristics data and mushroom yield (fresh mash) in Catalonian pine forests, NE Spain. Values of stand variables are means (ranges are given between parentheses).

| Region | Pine species | Period (No. years) | Tree density (No. trees $ha^{-1}$) | Stand basal area ($m^2$ $ha^{-1}$) | Altitude (m a.s.l.) | Aspect (º) | Slope (%) | Mushroom yield (kg $ha^{-1}$ $yr^{-1}$) | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | Mycorrhizal | Saprotrophic | Total |
| Solsonès | Scots pine (PS) | 1997-2001 (5) 2007-2014 (8) | 1453 (552 - 3893) | 21.1 (11.1 - 30.2) | 1033 (854 - 1502) | 156 (63 - 282) | 24 (17 - 33) | 79.1 (0 - 286.5) | 2.4 (0 - 20.4) | 81.5 (0.2 - 307) |
| | Black pine (PN) | 1997-2001 (5) 2007-2014 (8) | 1613 (1100 - 2292) | 26.2 (15.3 - 41.7) | 773 (630 - 1040) | 188 (14 - 317) | 11 (5 - 19) | 105.1 (0 - 472.7) | 2.6 (0 - 21.4) | 107.8 (0 - 474.7) |
| | Aleppo pine (PH) | 1997-2001 (5) 2007-2014 (8) | 1905 (281 - 3883) | 24.2 (16.4 - 31) | 613 (530 - 661) | 243 (170 - 292) | 16 (10 - 34) | 38 (0 - 281.2) | 4.2 (0 - 63.6) | 42.2 (0 - 293.2) |
| Prades | Scots pine (PS) | 2008-2014 (7) | 1066 (541 - 1592) | 49.7 (48.3 - 51.1) | 853 (841 - 864) | 325 (310 - 340) | 13 (8 - 18) | 228.5 (0 - 551.2) | 21.4 (0 - 87.3) | 249.9 (2 - 638.5) |
| | Maritime pine (PP) | 2008-2014 (7) | 1088 (446 - 2552) | 40.6 (21 - 80.3) | 807 (594 - 1013) | 154 (10 - 360) | 13 (3 - 22) | 79.1 (0 - 450.7) | 17.3 (0 - 81) | 96.4 (0.2 - 481.6) |

Species abbreviations: PS: *P. sylvestris*, PN: *P. nigra*, PH: *P. halepensis*, PP: *P. pinaster*

**Dendrochronological methods**

Near each plot where mushroom production was annually assessed, 10-15 dominant trees were randomly selected for sampling in late 2014 and early 2015. Two radial cores were extracted at 1.3 m above the ground level per tree using a Pressler increment borer.

***Work carried out during the STSM:***

**Sample processing**

During the STSM, the cores were air-dried, mounted on wood boards, and polished with sandpaper grits until rings were clearly visible. The wood samples were visually cross-dated. Then, earlywood and latewood widths were separately measured to the nearest 0.001 mm using a stereomicroscope and a LintabTM sliding-stage measuring device in conjunction with TSAP-WinTM software (F. Rinn, Heidelberg, Germany). Earlywood and latewood were visually distinguished based on the different lumen area and cell-wall thickness of the tracheids forming each type of wood. In some sites (e.g. *P. pinaster* plots in Prades), intra-annual density fluctuations appeared in the latewood. Cross-dating was verified using the COFECHA program (Holmes, 1983). Annual basal area increment (BAI) was calculated from the raw ring-widths by assuming a circular stem shape and using the *dplr* package (Bunn, 2010) in the R software (R Core Team, 2015). We obtained chronologies of BAI, earlywood and latewood widths for each plot by averaging the values for each year across the trees sampled within each plot. We used BAI instead of tree-ring width because the former variable reflects better tree aging and enlarging processes related to tree functioning such as sapwood production and crown transpiration (Biondi and Qeadan, 2008).

In one plot per tree species and locality ($n$ = 5 sites), 5 cores from 5 trees successfully cross-dated were used for wood-anatomical analyses ($n$ = 25 trees). Wood samples were prepared and processed following the standard protocol for wood anatomical analysis according to von Arx and Carrer (2014). The cores were cut into small (3–5 cm) pieces and thin sections (ca. 20-30 µm thick) were obtained with a freezing microtome (Anglia Scientific AS 200). The sections were stained with safranin and astra blue and then permanently fixed with Eukitt©. Images of the stained sections were acquired with a digital camera (Nikon Digital Sight DS-5M) mounted on a light microscope (Nikon Eclipse80i; Nikon, Tokyo, Japan). Images will be afterwards analyzed using ROXAS v1.6 obtaining the following data for each ring: number of tracheids, transversal lumen area and cell wall thickness of all tracheids. Anatomical analyses will be performed for the last 20 years of growth considering separately earlywood and latewood tracheids.

## Climate data

Monthly climatic variables (minimum, maximum and mean temperatures, and total precipitation) were obtained from 1970 from the "Atles Climàtic Digital de Catalunya" by selecting the 0.25º grids including the sampling sites (Ninyerola et al., 2003). The potential evapotranspiration rate was estimated following Hargreaves and Samani (1982) and using monthly minimum and maximum temperature using the *SPEI* package (Vicente-Serrano et al., 2010; Beguería et al., 2014) in the R software.

## Statistical analyses

The relationships between mean annual mushroom production (mycorrhizal, saprothrophic and total fungi) quantified as fresh mash (in kg $ha^{-1}$ $year^{-1}$) and stand structure data were analyzed using linear models (for tree density, basal area, elevation and slope) or analysis of variance (for aspect). The mean BAI series and the earlywood and latewood mean width series obtained for each plot were correlated against monthly mean temperature and precipitation using bootstrapped Pearson correlation coefficients on a 13-month window from September of the year prior to tree growth until October of the year of tree-ring formation. The statistical significance of the correlations was tested using the 95% percentile range method (Dixon, 2001).

To assess the effects of the stand characteristics on the relationships between basal area increment and fungi production, we performed linear regression analyses to determine the slope and intercept for each plot followed by a meta-analysis of the regression coefficients. To investigate the relationships between the BAI and climate conditions on mushroom production we will use structural equation modelling. The statistical analyses were conducted using the R statistical software (R Core Team, 2015).

# MAIN RESULTS

## Mushroom production

Mushroom yield was highly variable among years (CV = 83.1 %) and plots (CV = 68.6 %), and mean annual production of mycorrhizal fungi accounted for over than 90 % of the total fungi yield (Fig. 1). The highest mushroom yields were recorded in the Prades sites, especially in the Scots pine plots (250 kg $ha^{-1}$ $yr^{-1}$). Years 2000, 2008, 2010, and particularly, 2014 were characterized by a high production of fungi, whilst years 1997, 2009 and 2013 were characterized by low values of fungi fresh mass in some sites.

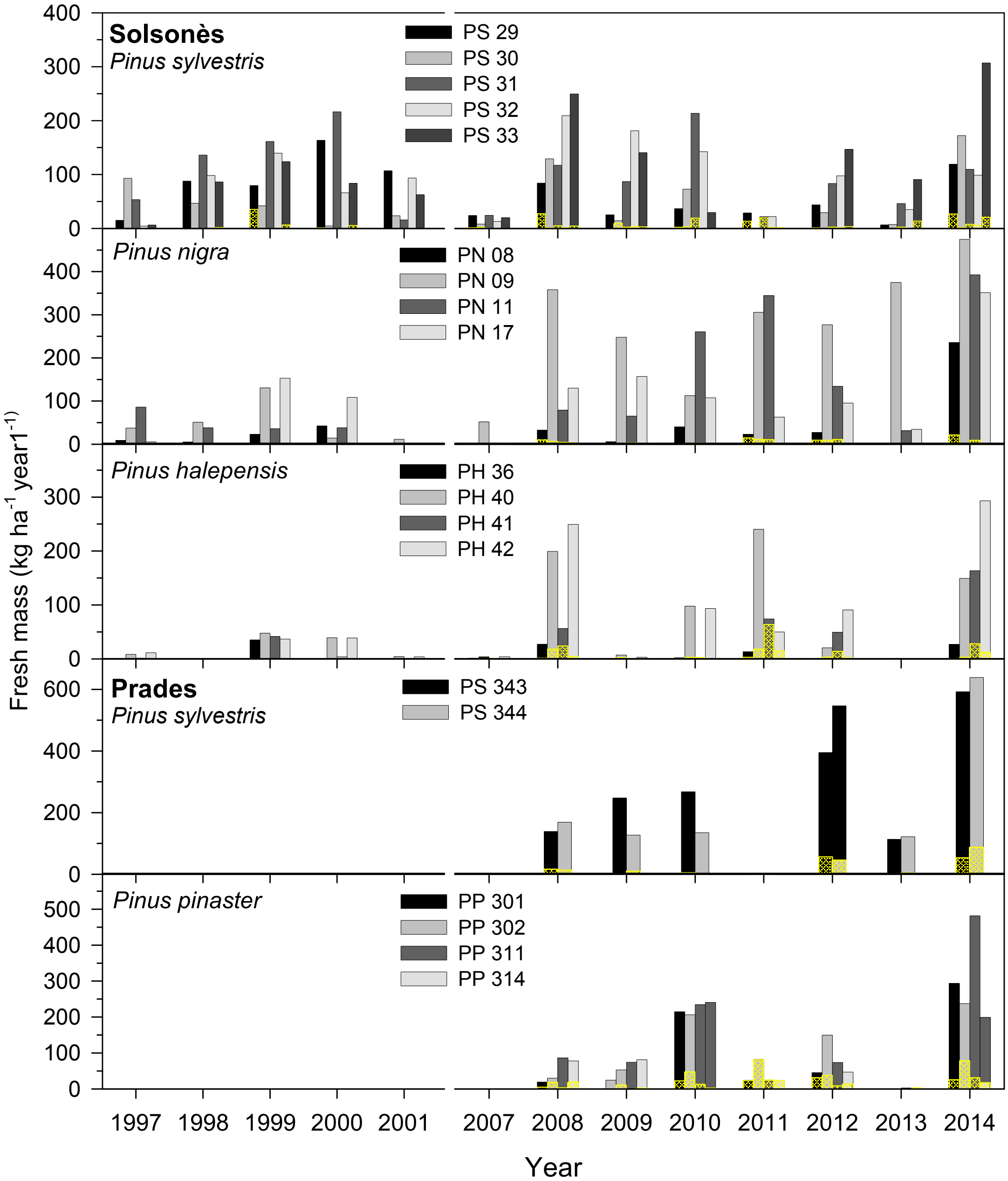


**Figure 1.** Mean annual mushroom yield data recorded in five pine species from the two study regions. Bars in gray represent mycorrhizal fungi, bars in yellow represent saprotrophic fungi. Pine species abbreviations are as in Table 1.

The mean annual production of both mycorrhizal and saprotrophic fungi was positively related to plot basal area ($P < 0.05$, Fig. 2), whereas the mean annual yield of saprotrophic fungi decreased with plot slope ($P < 0.1$). Although mushroom yield increased in northern-oriented slopes, no significant differences in mushroom production were found between slope aspects.

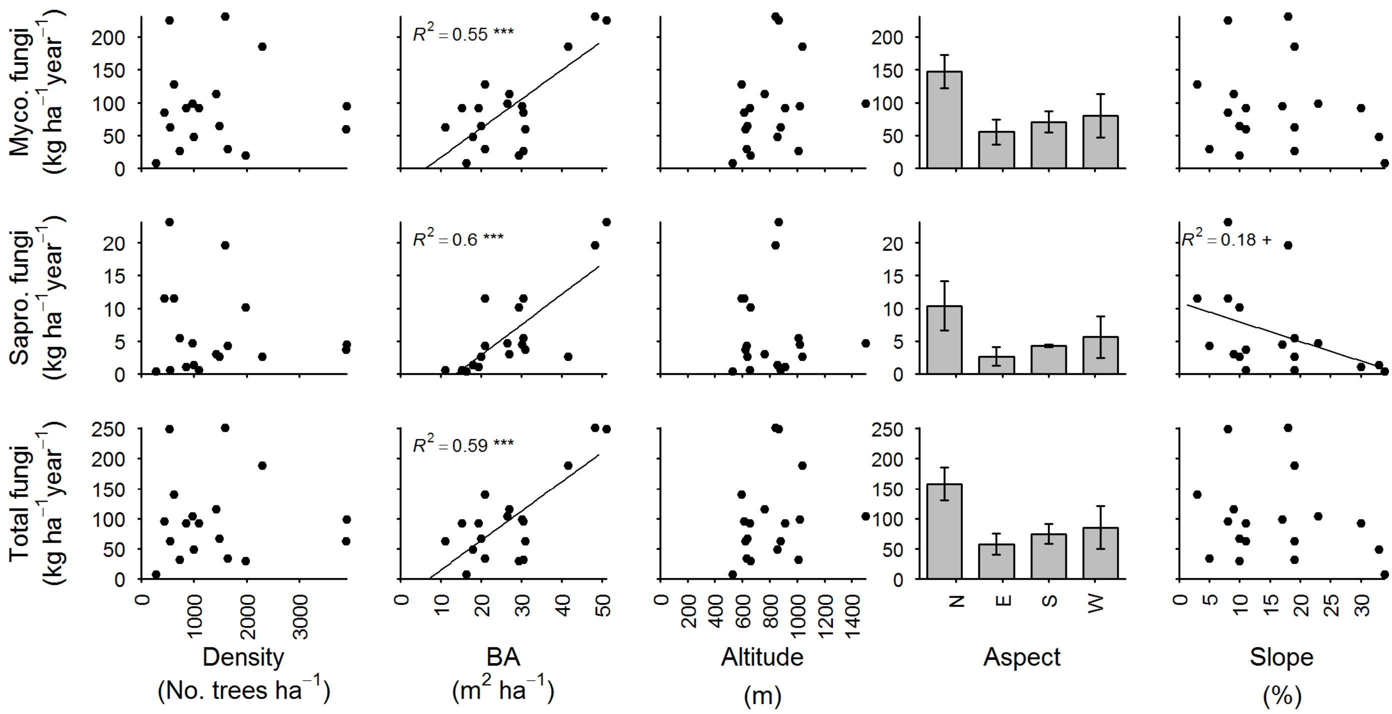


**Figure 2.** Relationships between mean annual mushroom yield and plot characteristics (tree density; BA, stand basal area; altitude; aspect and slope). Only significant relationships are shown. Significance levels: + $P < 0.1$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

## Growth response to climate

The main characteristics of sampled trees within each plot are described in Table 2.

**Table 2.** Descriptive statistics (age, diameter at breast height- DBH, basal area increment- BAI, and correlation with the mean series of each forest) for the tree-ring width chronologies of each study plot. Values are means ± SD.

| Region | Pine species | Plot | Age | DBH(cm) | BAI for the period 1990-2015 ($cm^2$) | Correlation with Master |
|---|---|---|---|---|---|---|
| Solsonès | Scots pine (PS) | PS029 | 70 ± 7 | 11.8 ± 2.9 | 4.5 ± 1.7 | 0.54 |
| | | PS030 | 73 ± 9 | 12.4 ± 1.9 | 2.5 ± 1.3 | 0.53 |
| | | PS031 | 33 ± 4 | 11.4 ± 3.2 | 2.6 ± 0.9 | 0.81 |
| | | PS032 | 63 ± 7 | 15.9 ± 2.8 | 3.9 ± 2.6 | 0.61 |
| | | PS033 | 42 ± 13 | 17.9 ± 7.7 | 5.6 ± 2.9 | 0.67 |
| | Black pine (PN) | PN008 | 78 ± 6 | 15.5 ± 2.7 | 3.6 ± 1.4 | 0.71 |
| | | PN009 | 48 ± 3 | 11.6 ± 2.1 | 2.8 ± 1.3 | 0.73 |
| | | PN011 | 76 ± 5 | 14 ± 2.2 | 2.7 ± 1 | 0.67 |
| | | PN017 | 93 ± 26 | 14.6 ± 4.2 | 2.7 ± 1.3 | 0.71 |
| | Aleppo pine (PH) | PH036 | 78 ± 17 | 18.9 ± 5.6 | 5.2 ± 3.6 | 0.72 |
| | | PH040 | 76 ± 13 | 13.2 ± 2.2 | 3.9 ± 1.6 | 0.63 |
| | | PH041 | 64 ± 21 | 14.7 ± 3 | 3.6 ± 1.4 | 0.63 |
| | | PH042 | 87 ± 13 | 15.3 ± 3 | 3.8 ± 1.6 | 0.67 |
| Prades | Scots pine (PS) | PS343 | 53 ± 3 | 16.6 ± 2.7 | 8.7 ± 5.6 | 0.71 |
| | | PS344 | 116 ± 24 | 20 ± 4.8 | 4.1 ± 3 | 0.57 |
| | Maritime pine (PP) | PP301 | 41 ± 3 | 17.3 ± 2.8 | 5.3 ± 2.2 | 0.66 |
| | | PP302 | 40 ± 2 | 16.6 ± 2.5 | 8.2 ± 2.8 | 0.79 |
| | | PP311 | 35 ± 6 | 17.1 ± 2.3 | 8.2 ± 3.9 | 0.76 |
| | | PP314 | 64 ± 7 | 20.2 ± 3.1 | 5.4 ± 1.7 | 0.62 |

The basal area increment (BAI) was influenced by temperature mainly in the Scots pine stands from the Prades region, where high maximum temperatures in January, February, March, May and July lead to BAI reduction (Fig. 3). Humid conditions in previous December and current September – October were positively related to BAI in the Solsonès region, while in Prades, BAI was positively influenced by high precipitation from July to September, particularly in the case of Scots pine stands.

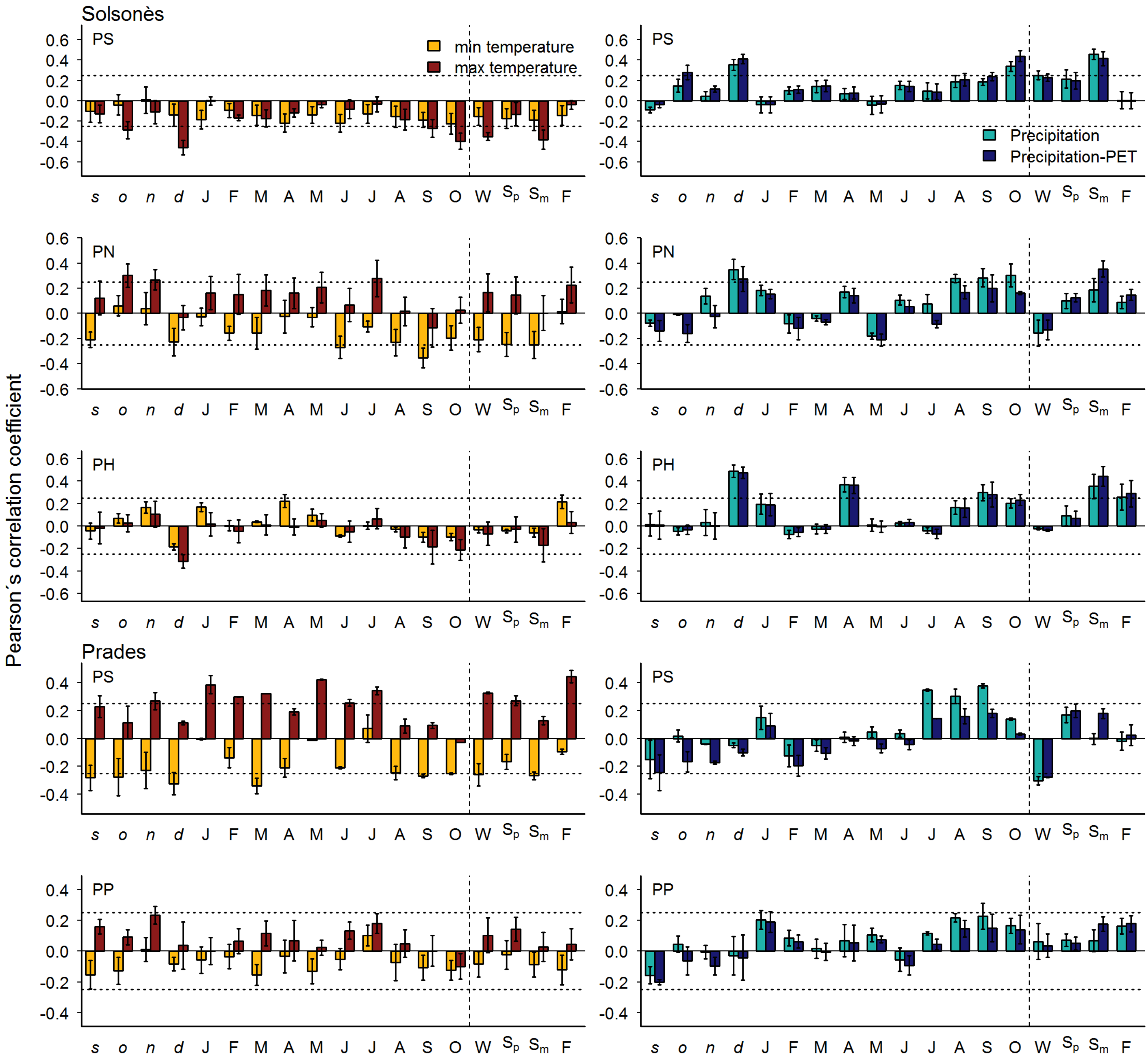


**Figure 3.** Pearson correlation coefficients between basal area increment and minimum and maximum temperature, precipitation, and precipitation minus potential evapotranspiration rate (PET) variables. Months abbreviated by lowercase or uppercase letters correspond to months from the previous and current years, respectively. Letters after the vertical line correspond to seasonal means or sum in the case of precipitation: "W", winter, from December of previous year to February of year of tree ring formation; "Sp", spring, from March to May; "Sm", summer, from June to August; "F"; fall, from September to November. Horizontal dashed lines represent $P < 0.05$. Species abbreviations are as in Table 1.

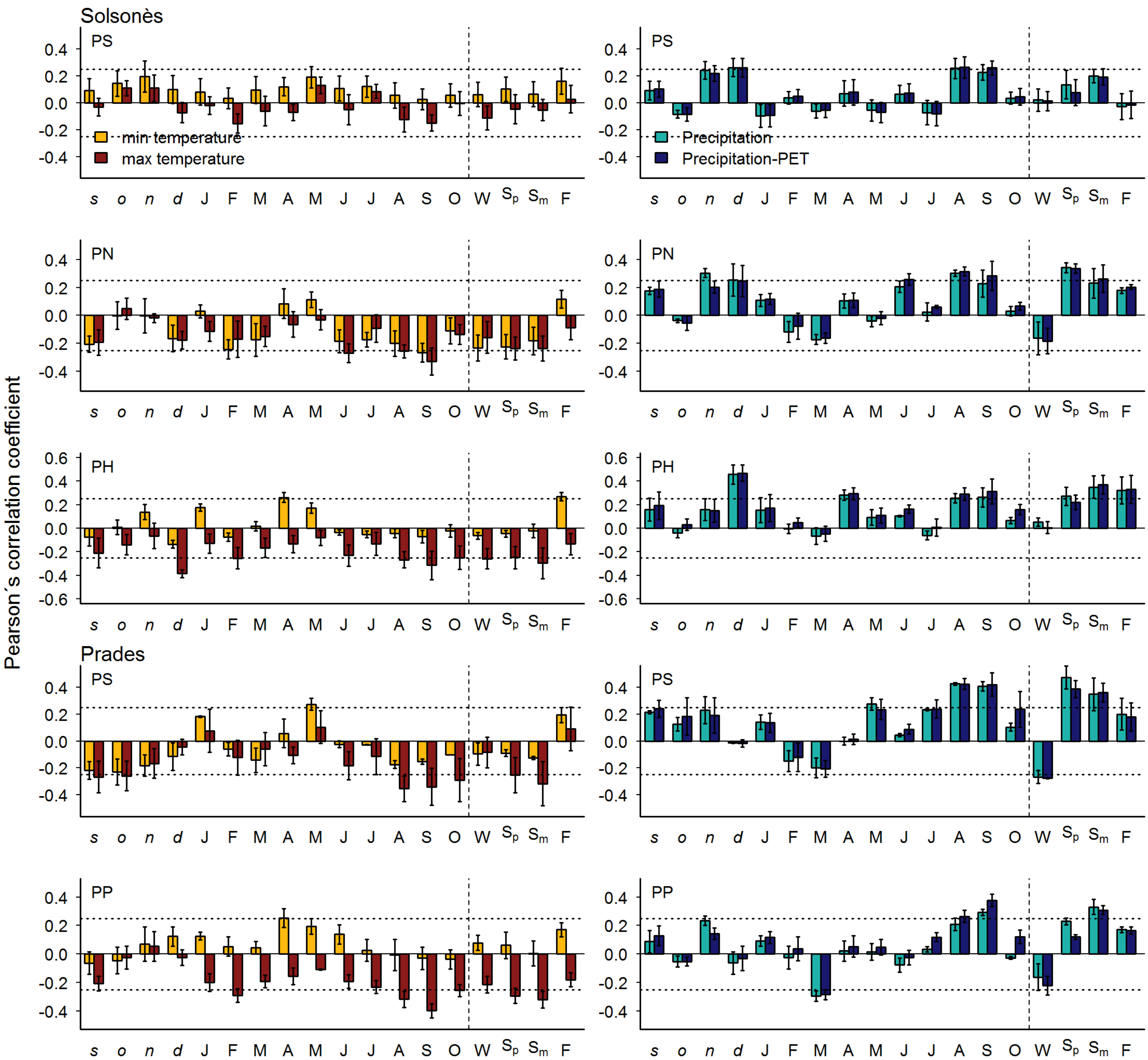


**Figure 4.** Pearson correlation coefficients calculated between earlywood width and minimum and maximum temperature, precipitation, and precipitation minus potential evapotranspiration rate (PET) variables. Horizontal dashed lines represent $P < 0.05$. Rest of explanations are as in Figure 3.

The earlywood production was more sensitive to climate than latewood formation was (Figs. 4 and 5). Overall, earlywood width of the study species decreased with higher maximum temperatures from June to September of the year of tree ring formation, and it increased with higher precipitation values in previous November and December, and also with wet conditions during current August and September. However, a great variability of the response of earlywood width to climate among tree species and regions was observed. For instance, the Prades Scots pine trees responded more negatively to warmer maximum temperatures in terms of earlywood production that the Solsonès Scots pine trees. The latewood width decreased with warmer maximum temperatures in current September and it was enhanced by precipitation in the same month in all tree species (Fig. 4). The variability of the response of latewood width to climate among tree species was higher than in the case of earlywood.

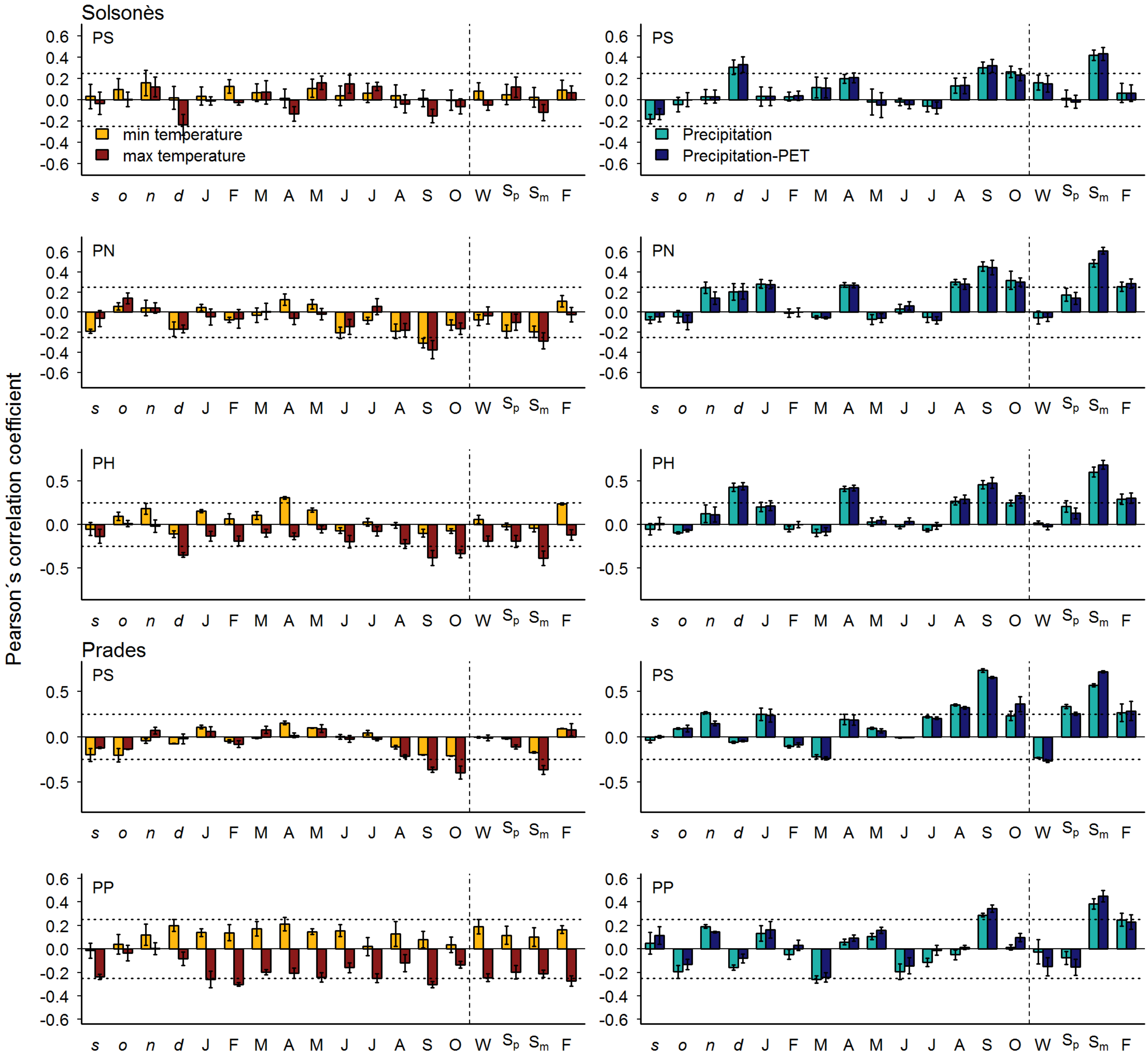


**Figure 5.** Pearson correlation coefficients calculated between latewood width and minimum and maximum temperature, precipitation, and precipitation minus potential evapotranspiration rate (PET) variables. Horizontal dashed lines represent $P < 0.05$. Rest of explanations are as in Figure 3.

**Relationships between mushroom production and tree growth**

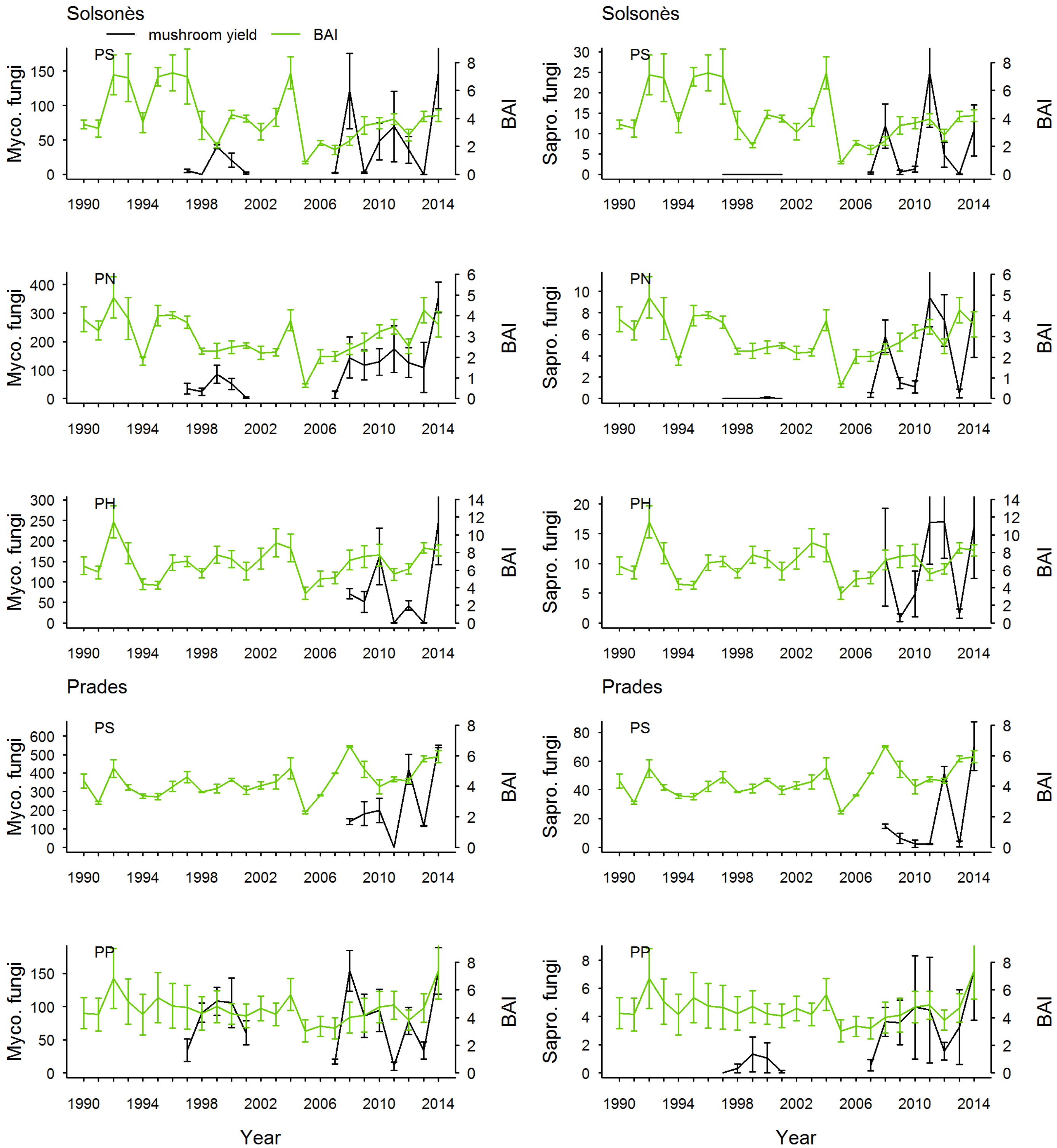


**Figure 6.** Temporal trends of mean annual mycorrhizal and saprotrophic fungi production (fresh mass in kg ha$^{-1}$ year$^{-1}$) and basal area increment (BAI, cm$^2$ year$^{-1}$). Species abbreviations are as in Table 1. Values are means ± SE.

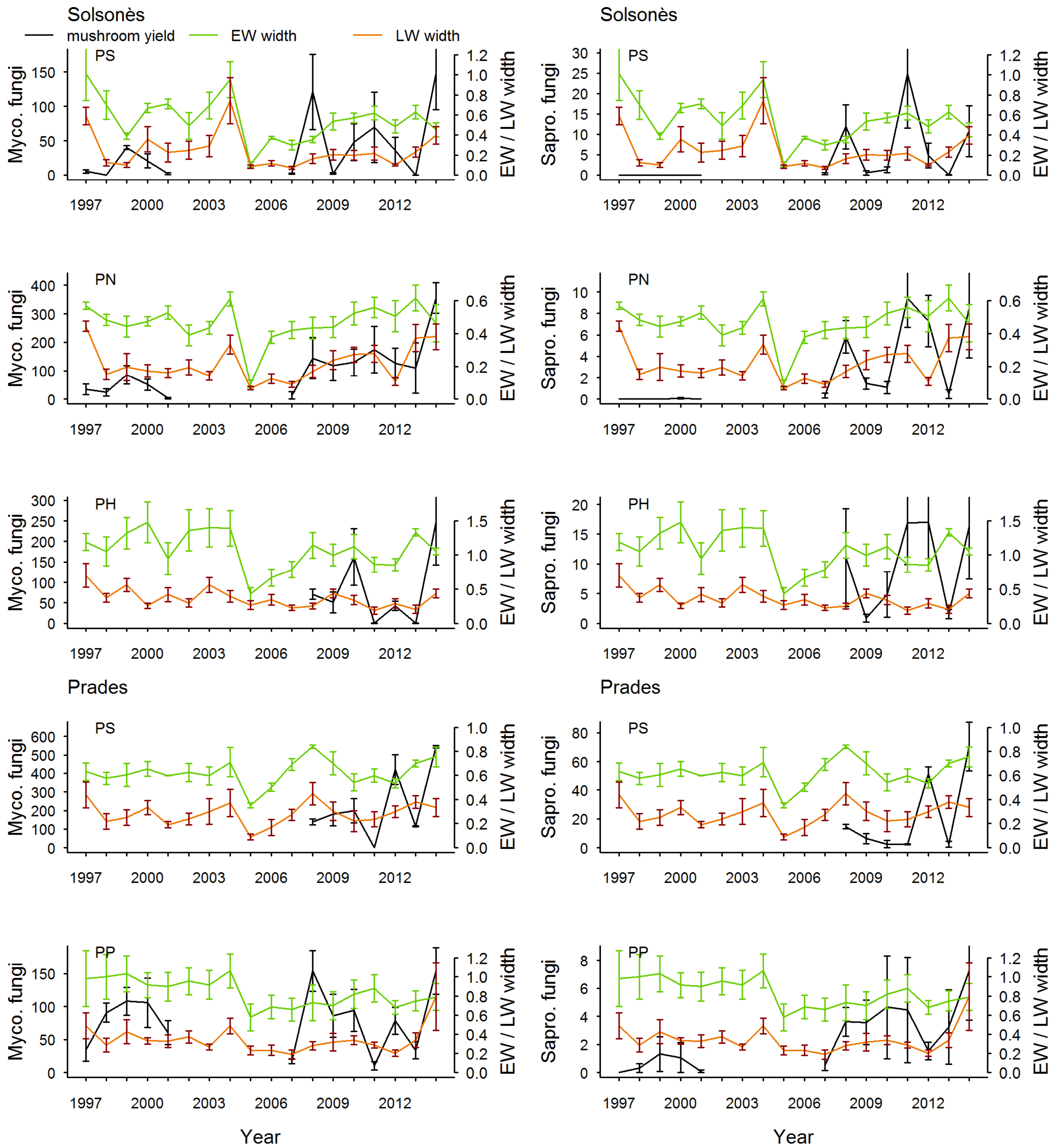


**Figure 7.** Temporal trends of mean annual mycrorrhizal and saprotrophic fungi production (fresh mass in kg ha$^{-1}$ year$^{-1}$) and earlywood and latewood widths (in mm). Species abbreviations are as in Table 1. Values are means ± SE.

Generally, no similar temporal trends were observed between the production of mushrooms and BAI (Fig. 6) or earlywood and latewood widths (Fig. 7). However, mycorrhizal fungi yield followed a similar temporal trend than latewood production in black pine stands, while the temporal variability of saprotrophic fungi production appeared to be coupled with latewood production in the maritime pine stands.

There was a great variability in the relationships observed between plot mean BAI, earlywood and latewood production, and mean annual mushroom yield for their common periods 1997-2001 and 2007-2014 (Fig. 8).

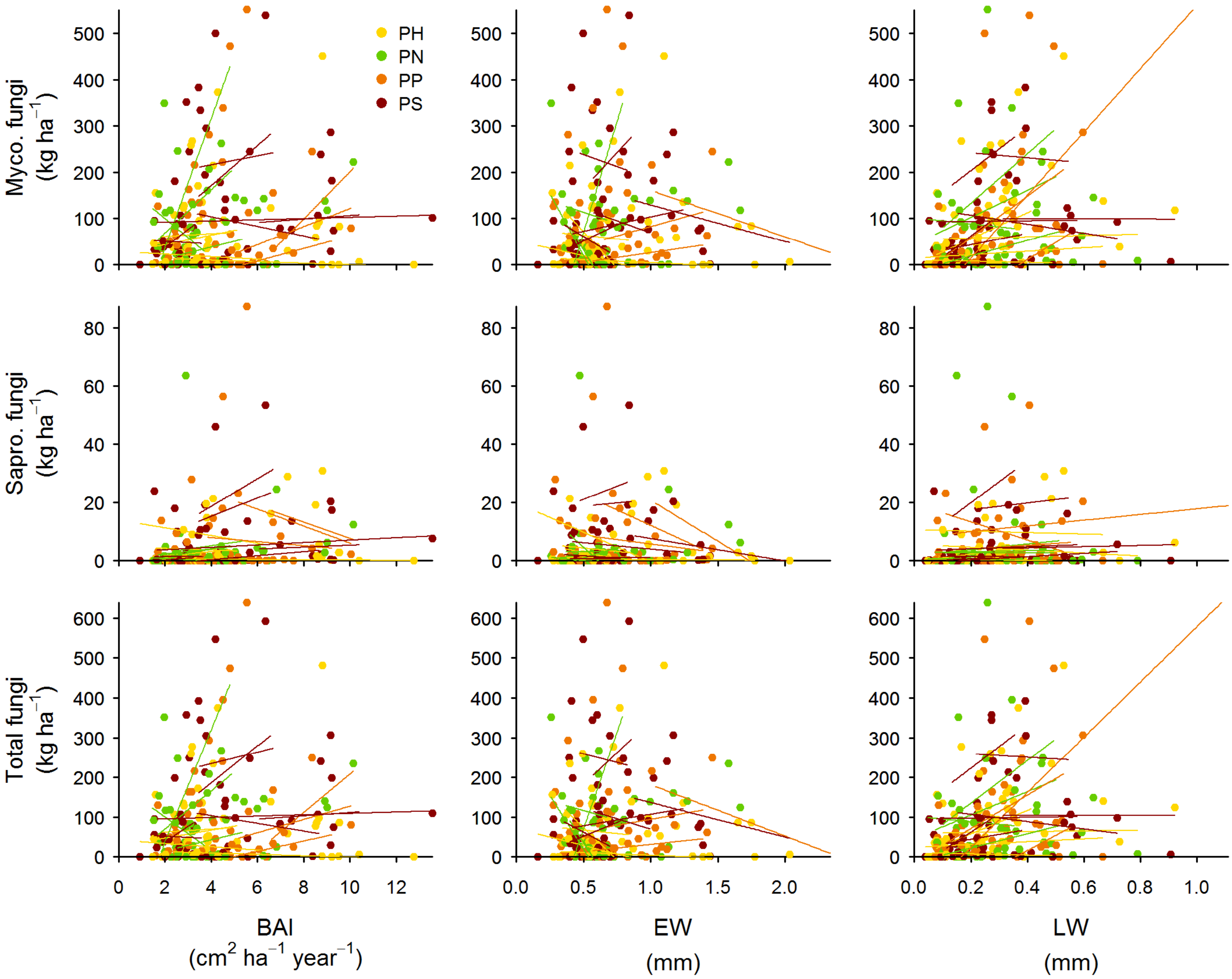


**Figure 8.** Linear relationships fitted to basal area increment (BAI, in $cm^2$ $yr^{-1}$) and mean annual mushroom yield for each sampled plot. Species abbreviations are as in Table 1.

The relationships (slopes of the linear models) between plot BAI and mean annual mushroom production was influenced by the plot basal area, the tree species and region for mycorrhizal and saprotrophic fungi, and the interaction between basal area and tree species in the case of mycorrhizal fungi (Table 3). The relationships between mean annual earlywood width and the production of mycorrhizal fungi also depended on the plot basal area, region, and the interaction between basal area and species.

**Table 3.** Statistical parameters obtained for the linear models fitted to the slope between mean basal area increment, earlywood and latewood width, and fungi production (Fig. 8).

| | | **Mycorrhizal fungi** | | | | | | **Saprotrophic fungi** | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | BAI | | EW | | LW | | BAI | | EW | | LW | |
| | Df | *F* value | *P* value | *F* value | *P* value | *F* value | *P* value | *F* value | *P* value | *F* value | *P* value | *F* value | *P* value |
| BA | 1 | 26.6 | **0.001** | 15.9 | **0.003** | 0.6 | 0.448 | 25.8 | **0.001** | 3.7 | 0.087 | 2.3 | 0.162 |
| Species | 3 | 7.5 | **0.010** | 0.1 | 0.964 | 3.5 | 0.064 | 14.3 | **0.001** | 1.5 | 0.291 | 1.0 | 0.431 |
| Region | 1 | 9.8 | **0.014** | 9.8 | **0.012** | 0.0 | 0.861 | 8.9 | **0.017** | 0.0 | 0.974 | 5.0 | 0.053 |
| BA *x* Species | 3 | 29.5 | **0.000** | 8.3 | **0.006** | 2.8 | 0.102 | 1.3 | 0.348 | 1.2 | 0.364 | 0.4 | 0.783 |
| BA *x* Region | 1 | 2.8 | 0.134 | | | | | 2.1 | 0.184 | | | | |
| Residuals | 8 | | | | | | | | | | | | |

## FUTURE COLLABORATION WITH HOST INSTITUTION

The STSM represents the onset of collaboration on the application of dendrochronological methods to understand the relationships between climatic conditions, radial growth, wood anatomy and mushroom production at the long-term. Future collaboration comprises further sample and data processing, finishing the analyses of wood-anatomical data which are very time consuming and writing reports and scientific publications. Additional collaborations include similar approaches applied to European comparisons using several country datasets from a N-S gradient encompassing mushroom and tree-ring data from Finland, Switzerland and Spain.

## FORESEEN PUBLICATIONS TO RESULT FROM THE STSM

Work and results from the STSM will be published in a peer-reviewed scientific journal of the ecology or forestry fields due to the novelty of the research. We aim to publish two papers: a first one will be focused on relationships between tree growth and climate variables on mushroom production at the long term and a second one, in which we will investigate if mushroom yield could be predicted by wood anatomical features as related to climate variability.

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GOBIERNO DE ESPAÑA MINISTERIO DE ECONOMÍA Y COMPETITIVIDAD

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27 May 2015

JESÚS JULIO CAMARERO MARTÍNEZ, researcher at the INSTITUTO PIRENAICO DE ECOLOGÍA (IPE-CSIC)

This is to confirm that Ms. Irantzu Primicia has successfully completed the Short-Term Scientific Mission (STSM) in the framework of the EU COST Action FP1203 "European Non-Wood Forest Products (NWFPs) Network", from 27 April to 27 May 2015, in the Instituto Pirenaico de Ecología (IPE, CSIC), located at Zaragoza (Spain).

Sincerely,

Signed: J.J. Camarero

Zaragoza, 27 May 2015