Rainfall homogenizes while fruiting increases diversity of spore deposition in Mediterranean conditions

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Abstract

There is a lack of knowledge regarding the main factors modulating fungal spore deposition in forest ecosystems. We described the local spatio-temporal dynamics of spore composition along a single fruiting season its relation with fruiting body emergence and rainfall events. Passive spore traps were sampled weekly during autumn and analyzed by metabarcoding of the ITS2 region in combination with qPCR. There were larger compositional changes of deposited spores across sampling weeks than amongst sampling plots. Spore diversity and abundance correlated with mushroom emergence and weekly rainfall. Spore compositional changes were related to rainfall, with lower spatial compositional heterogeneity across plots during weeks with higher rainfall. Soil saprotrophs and amongst them, puffball species, showed the strongest positive correlation with rainfall across fungal guilds. Deposited spore composition highly changed already at a fine-scale temporal scale, whereas we show that mushroom emergence and rainfall may be important factors driving airborne spore deposition.

Keywords: *Fungal diversity, Atmospheric diversity, qPCR, DNA barcoding, spore traps, dispersion.*

1. Introduction

Understanding soil fungal community assemblages is paramount to predict soil nutrient dynamics and plant-soil feedbacks. A general framework suggests that both competition between fungi and colonization processes structure fungal assemblages, sustaining hyper-diverse communities (Smith et al., 2018). Competition processes in fungi are largely determined by host, substrate and habitat availability, but fungal communities are also modulated by climate (Castaño et al., 2018; Hartmann et al., 2017), soil parameters (Rincón et al., 2015) or disturbances such as tree harvesting (Kohout et al., 2018) or fire (Clemmensen et al., 2013). However, the high spatial stochasticity found in soil fungal communities suggests that dispersal processes may be extremely important in determining community assemblages (Bahram et al., 2016). Stochastic and predictable processes driving spore dispersal may affect fungal colonization outcomes (Peay et al., 2012; Peay and Bruns, 2014), which may in turn cause a cascade effect on the belowground community build-up (Kennedy et al., 2009). Spore deposition may potentially be influenced by processes such as atmospheric conditions (e.g. wind, precipitation; Dam, 2013; Despres et al., 2012; Oliveira et al., 2009) or by inter-specific differences in spore dispersal abilities (Peay et al., 2012; Peay and Bruns, 2014). For instance, dispersal limitation observed in several fungal species (Galante et al., 2011) suggests that colonization in a given landscape may be highly determined by the surrounding local fruiting body communities, followed by a niche competition between successfully deposited fungal spores. Considering the ecological importance of all these processes, further research on spore dispersal and spore deposition needs to be carried out.

Fruiting bodies from basidiomycetes are known to produce billions of spores (Dahlberg and Stenlid, 1994; Kadowaki et al., 2010), which will deposit by gravity near the sporocarps (Galante et al., 2011; Peay et al., 2012; Peay and Bruns, 2014). A fraction of these spores may

be dispersed at long distances when reaching the turbulent layers of the atmosphere (Dressaire et al., 2016). In the turbulent layer, spores from many sources located over relatively large areas are mixed (Lacey and West, 2006; Nicolaisen et al., 2017). In contrast to short distance spore deposition, it is likely that long distance spore dispersal is driven by environmental factors (Kivlin et al., 2014; Nicolaisen et al., 2017). Among these environmental factors, rainfall may be a relevant process that potentially affect spore dispersal and deposition of airborne spores (Oliveira et al., 2009, Pakpour et al., 2015). Among other factors affecting spore deposition highlights wind that disperse away the spores or UV light that eliminate the spores from the atmosphere (Burch and Levetin, 2002). Rainfall effects on spore deposition may be especially relevant in drier climates such as Mediterranean, where mushroom emergence is seasonal and concentrated in autumn months together when rainfall is more abundant (Alday et al., 2017; Martinez de Aragón et al., 2007). Within this context, spore deposition at landscape level of some fungal species during autumn may be likely restricted to a few days, when rainfalls are produced, whereas for some other species dry deposition (gravity) may be predominant and therefore deposition occurs regardless of rain. In any case, the role of rainfall affecting spore deposition (i.e. spore community and diversity) compared with dry deposition is still unknown, mostly due to technical limitations related with species-typing of spore pools.

By using filter or funnel traps it is possible to profile and describe the spore or propagule community that would otherwise be deposited into the ground (Peay and Bruns, 2014). Molecular identification and composition profiling of these spores may be then achieved by using high-throughput DNA sequencing (HTS) techniques (Aguayo et al., 2018). HTS data has been shown to be adequate for quantitative purposes, as showed by qPCR using specific fungal species (Oliva et al., 2017). By profiling the spores in rainfall funnel traps, we previously observed that specific spore deposition of some species was correlated with

mushroom production, regardless of rainfall events (Castaño et al., 2017). This finding indicated that spores were released and deposited as fruiting bodies were formed. However, correlation between mushroom production and deposited spores did not match for several species, pointing out the existence of other factors involved in spore deposition. At the same time, in our previous study (Castaño et al., 2017) we did not work with the whole spore pool, being focused only on selected species that produced fruiting bodies at the studied plots. In addition, questions regarded how rainfall affects airbone spore deposition or spores compositional changes across plots and weeks were not targeted. However, in this study, we investigated the role of rain and mushroom emergence on spore deposition by concomitantly describing funnel trap captures and fruiting in a local scale Mediterranean forest during a single fruiting season.

We hypothesized that rainfall will homogenize spore deposition in a spatial scale by collecting spores from the turbulent layer. In this research, we have (i) tested whether compositional changes in deposited spores across weeks (deposited spore succession) will be higher than spatial changes, both considering the whole spore composition and splitting it in functional groups, and, (ii) describing how rainfall and mushroom emergence contributed to temporal changes at local scale in spore deposition (i.e. total abundance, composition and diversity).

2. Material and Methods

2.1 Study area

The study was carried out at the long-term experimental forest located in the Natural Area of Poblet (Northeast Spain, 41° 21’ 6.4728” latitude N and 1° 2’ 25.7496” longitude E). The site has a Mediterranean climate, with an average annual temperature of 11.8°C and annual

rainfall of 665.5 mm., with summer droughts usually lasting for 3 months. In this study, we used 8 randomly chosen plots (10 × 10 m) of an experimental set-up where fruiting bodies have been continuously monitored every fall since 2008 (Bonet et al., 2012). This set of plots was located in a forest area covering approximately 300 hectares (Fig. S1a). Forests are even-aged (60-years-old) *Pinus pinaster* trees with *Quercus ilex* as a co-dominant species. Spore trap and mushroom samples were obtained in these plots during a single autumn season. Sampling and identification of mushrooms at the study site is described in Castaño et al., (2017).

2.2 Spore trapping

Funnel traps were installed in the 8 plots, 30 cm above ground level. Traps consisted of 15 cm diameter plastic funnels attached to 1-liter dark jars, with a 50-µm nylon mesh fixed at the bottom of the funnel. Traps were installed one week after the first fruiting body was observed (October 14th, 2014) and remained in the plot until December 11th, 2014. Funnel traps were rinsed with ultrapure water (MilliQ) each week to collect any spores attached to the funnel. After that, the jars were replaced with new sterile jars. Spore suspension in the jar was filtered using sterile filter papers (90-mm diameter: Whatman no. 5) within 48 hours of collection. Filter papers were stored at –20ºC until further analysis.

2.3 Spore trap sample processing and DNA extraction

Filters containing spores from the funnel traps were cut in half and stored in separate 50-ml falcon tubes. A solution of 20-ml sodium dodecyl sulfate (SDS buffer) was added to each tube, and tubes were incubated at 65°C for 90 minutes. Tubes were then vortexed three times and filter from each tube was removed. Twenty milliliters of 2-propanol were added to the resulting solution and resulting solution was left overnight at room temperature. The solution was subjected to centrifugation (700 × r.p.m. for 10 min) and the resulting supernatant was

removed. The resulting pellet was resuspended by vortexing with 700 µl of SL2 lysis buffer (NucleoSpin® NSP soil DNA extraction kit, Macherey-Nagel, Duren, Germany) and transferred to a 2-ml tube. After the addition of SX Enhancer (NucleoSpin® NSP soil DNA extraction kit), the spore solution was homogenized in a FastPrep®-24 system (MP Biomedicals) at 5,000 × r.p.m for 30 s (twice) and total DNA was extracted following the instructions provided by the manufacturer.

2.4 qPCR using universal fungal primers

For quantification of the fungal spores, we used the universal fungal primers ITS1F (Gardes and Bruns, 1993) and 5.8S (Vilgalys and Hester, 1990), designed to amplify the ITS1 region located at the rRNA DNA. Quantitative PCR reactions were performed in triplicate including negative controls using 96-well transparent plates on an iCycler iQ thermal cycle (BioRad). Reactions contained 12.5 µL of SsoAdvanced Universal SYBR Green Supermix, 2.5 µL of bovine serum albumin (BSA: 5 ng/ µl), 0.25 µl of a 10 µM mixture of each primer, 5 µl of template (diluted extracted DNA 1:10) and sterile water to a final volume of 20 µl. Reaction conditions included 95ºC for 2 min, followed by 40 cycles of 95ºC for 15 s, annealing temperature for 30 s at 53ºC, and 72ºC for 30 s. Standard curves were prepared using a solution obtained from known number of spores of three fungal species (*Trichoderma* sp. (sticky spores), *Cryphonectria parasitica* (rainsplash spores) and *Cantharellus cibarius* (airborne spores)), which were extracted using the same protocol as filters. C_T values were converted to the number of fungal spores in each reaction using serial DNA dilutions of known amounts of spores, starting with 1×10^6 spores and ending with 100 spores. Based on this standard curves, results are expressed as number of spores × trap sample $^{-1}$. For all the reactions there was a linear relationship between the logarithm of the spore number and the

threshold cycle across the standard concentration range (R^2> 0.98) as well as efficiency values between 95%-105%.

2.5 Spore trap sample sequencing using Illumina MiSeq

Each spore trap sample was PCR-amplified using the fungal universal primers fITS7 and ITS4 (Ihrmark et al., 2012) which amplify the ITS2 region of the rDNA. Both primers were tagged with 8-bp sequences, differing in at least three positions. The number of PCR cycles was optimised for each sample, and most of the samples amplified well at 24–26 cycles. PCR amplifications of samples and both negative controls from DNA extraction and PCR were conducted in a 2720 Thermal Cycler (Life Technologies) in 50 µl. The final concentrations in the PCR reaction mixture were; 25 ng of template, 200 µM of each nucleotide, 2.75 mM $MgCl_2$, 200 nM of each primer, 0.025 U μl^{-1} polymerase (DreamTaq Green, Thermo Scientific, Waltham, MA) in 1× buffer PCR. The cycling conditions for PCR were: 5 min at 95°C, followed by 24–30 cycles of 30 s at 95°C, 30 s at 56°C, and 30 s at 72°C, and a final extension step at 72°C for 7 min before storage at 4°C. DNA from each sample was amplified in triplicate and amplicons were purified using an AMPure kit (Beckman Coulter Inc. Brea, CA) and quantified using a Qubit fluorometer (Life Technologies, Carlsbad, CA). Equal amounts of amplified DNA from each sample were pooled before sequencing. The final equimolar mix was finally purified using an EZNA Cycle Pure kit (Omega Bio-Tek, USA). Quality control of purified amplicons was carried out using a BioAnalyzer 2100 (Agilent Technologies, Santa Clara, CA) 7500 DNA chip. Libraries were prepared from ~10 ng of fragmented DNA using the ThruPLEX-FD Prep kit. The samples were sequenced using the Illumina MiSeq platform, with 300-bp paired-end read lengths, generating 13.4 million sequences.

2.6 Quality control and bioinformatic analysis

Quality control, filtering, and clustering were assessed using the SCATA pipeline (scata.mykopat.slu.se). Sequences were filtered to remove DNA sequences with a minimum base quality score of <10 at any position, an average quality score of <20, and a minimum sequence length of 200 bp, using the amplicon quality option. Sequences were also screened for primers (using 0.9 as a minimum proportional primer match for both primers) and sample tags. The 'usearch' was used as a search engine, considering a minimum match length of 85%. Homopolymers were collapsed to 3 bp before cluster analysis. Pairwise alignments were conducted using a mismatch penalty assigned of 1, gap open penalty of 0, and a gap extension penalty of 1. Sequences were clustered in Operational Taxonomic Units (OTUs) using single linkage clustering, defining 1.5% as a threshold distance with the closest neighbor. Finally, all sample tags were identified, and tag jumps were removed from the database. The raw sequence reads have been deposited in the NCBI Sequence Read Archive (SRA) under accession number **PRJNA352156**.

2.7 Identification of the fungal clusters

We taxonomically identified the most abundant 2,000 OTUs using the massBLASTer option in the PlutoF database (https://plutof.ut.ee/) implemented in plutoF (Abarenkov et al., 2010). The most abundant sequence from each cluster was selected for taxonomic identification. Taxonomic identities were assigned based on >98.5% similarity with database references. Functional identification of identified taxons at species or genera level was done by using FUNGuild (Nguyen et al., 2016). All filtered DNA sequences, environmental data and fungal identifications are available at Mendeley Data (DOI: **10.17632/42hhdp53zb.1**).

2.8 Climate data

We obtained daily weather variables of precipitation from 2014 for each of 8 plots, following DAYMET methodology (Thornton et al., 2000), as implemented in the R package 'meteoland' (De Cáceres et al., 2017). In this package, daily precipitation and temperature was estimated for each plot by averaging the values of several local meteorological stations recorded during the sampling period, weighting factors that depended on the geographic proximity to the target plot and correcting for differences in elevation between the station and the target plots. Low weekly precipitation values (<20 mm.) were recorded during the mushroom productive season, with the exception of the week between from 28th November to 4th December, that registered an average of 162 mm in less than 48 h. This extreme precipitation episode was caused by a cut-off low, associated to S-SE winds carrying also significant amounts of Saharian dust (A sequence of the geopotential maps from 28th November – 1st December can be seen in Fig. S2A, B, C, D). As a result of this rainfall event, the spore traps were overflown and some spores were probably lost.

2.9 Data analysis

Statistical analyses were implemented in the R software environment (version 2.15.3; R Development Core Team, 2013) using the "vegan" package for multivariate analysis (Oksanen, 2015) and "nlme" package for linear mixed models (Linear Mixed Effects models (LME): Pinheiro et al., 2016). "iNEXT" package (Hsieh et al., 2016) was used for diversity analysis and interpolation of fungal diversity data. Ordination of community data (Detrended Correspondence Analysis: DCA) was also carried out using CANOCO version 5.0 (Biometris Plant Research International, Wageningen, The Netherlands).

2.9.1 Deposited spore compositional changes across weeks

We used variation partitioning analyses to identify whether temporal (weeks identity) or spatial (plots identity) effects were significantly influencing deposited spore composition. Here, we used the "varpart" function on compositional data, down weighting the effect of rare species with Hellinger transformation (Legendre and Gallagher, 2001). We also studied the effect of geographical distance of plots (spatial autocorrelation) on the spore composition. As a geographical distance index, we first calculated the principal coordinates of neighbor matrices (PCNM) spatial eigenvectors, based on UTM coordinates of the sampled plots, using Euclidean distances. We used forward selection of explanatory variables to select for significant eigenvectors, using Bonferroni correction of *P*-values. The scores of the significant spatial eigenvectors (named PCOs) for each plot were used as explanatory variables in the variation partitioning analyses, together with the plot and weeks identity. First, a general variation partitioning analyses was performed, but afterwards separated analyses were done over the most abundant taxonomic fungal groups (i.e. ectomycorrhizal, wood saprotrophs, plant pathogens, other saprotrophs, lichenized and soil saprotrophs). Geographical distance (PCOs), weeks and plots identity were defined as explanatory variables and significance was obtained by redundancy analysis (RDA) with 999 permutations, and stratifying by Week (Strata= Week). The graphical representation of the deposited spore similarity between weeks was obtained using a Detrended Correspondence Analysis (DCA) with 'week' fitted over the ordination. Here, one DCA for each functional group was done to facilitate the outcomes interpretation. Also, spore temporal patterns of most abundant species commonly found at the fruiting body community were assessed using General Additive Models (GAM) and visualized as response curves (16 species only). In these analyses, we included the 12 most abundant functional guilds.

2.9.2 Fruiting body and spore diversity calculations

Hill's series of diversity indices were used to identify diversity patterns of spores across weeks (Hill 1973). Hill's diversity consists of three numbers: N0 is species richness; N1 is the antilogarithm of Shannon's diversity index; and N2 is the inverse of Simpson's diversity index. N0, N1 and N2 were calculated from the asymptotic estimates implemented in "iNEXT" and values were derived in all samples considering the minimum number of reads observed across all data set (Number of reads=6,625). For the fruiting body community, only richness was included at the analyses.

Finally, since rarefaction may result in a potential loss of information or incorrect interpretation of results (McMurdie and Holmes, 2014), we also performed spore diversity analyses on non-rarefied samples. As an alternative to take in account the uneven read distribution, we used the square root transformation of read counts as an explaining variable when testing for the Hill's numbers (Bálint et al., 2015), and using the same previous models. Similar results were obtained with or without rarefaction.

2.9.3 Rainfall and fruiting body effects on spore composition, abundance and diversity

Rainfall effects on weekly compositional changes of deposited spores were studied by redundancy analysis (RDA) with 200 permutations. Here, Hellinger transformed community data was considered as a response data, whereas log-transformed precipitation data was considered as an explanatory variable. To study whether precipitation washed-out airborne spores, we calculated the spores compositional heterogeneity across weeks, which was determined by calculating the area of the standard deviational ellipse resulting from weekly composition dispersion, and adjusted with the weekly precipitation using linear models. Rainfall and mushroom emergence effects on deposited spore diversity were tested with a temporal perspective using LME. LME models considered the interaction "fruiting body diversity × rainfall" as a fixed variable and the plots and week identities as a random factors.

LME models were also used to study relationships in a temporal scale between the relative abundance of each functional guild and the weekly precipitation, by defining precipitation values as fixed factor and plots and week identities as random factors. Relationships between rainfall and i) spore abundance as measured by qPCR, ii) relative abundance of guilds or specific fungal groups (e.g. puffballs) were also studied by LME following the previous scheme.

Since increase of fungal spore diversity may be related to increasing number of spores washed-out by rainfall, relative proportions of each fungal species were normalized by the total number of spores as derived by qPCR, and rarefaction curves were again obtained by interpolating the diversity values with the number of spores using "iNEXT". Here, three precipitation classes were considered to be able to construct rarefaction curves (Low= P < 5 mm. (n=3), Moderate= P > 10 mm. (n=4), Severe= P > 100 mm. (n=1)). However, significance of precipitation effects on richness was tested, using precipitation as a continuous variable in LME models following the previously described schemes. Finally, to study the number of specific OTUs associated to each week, we used indicator species analyses (De Caceres and Legendre, 2009), using weeks identity as explanatory factor (n=8). Here, values $P<0.05$ were considered significant.

3. Results

3.1 Fungal spore composition and structure

We identified 521 OTUs belonging to 12 functional guilds. OTUs belonging to undefined saprotrophs were the most abundant (16%), followed by plant pathogens (15%), soil saprotrophs (5%) and mycorrhizal species (4%). The most abundant guilds were; undefined

saprotrophs (209 OTUs), mycorrhizal (109 OTUs), plant pathogens (75 OTUs) and wood saprotrophs (41 OTUs; Table 1).

Table 1. Functional guilds detected at the spore traps, as well as, the number of OTUs, % of reads that represent each functional guild. The most abundant species and the most diverse genera within the guild are also shown. Guilds were identified by FUNGuild and 45.88% of the reads could not be ecologically identified and they were unassigned.

Guild	Richness	% reads	Most abundant species	Most diverse genera
Undefined saprotroph	209	16.27	*Phaeococcomyces catenatus*	*Agaricus, Mortierella*
Ectomycorrhizal	109	3.97	*Suillus bellinii*	*Cortinarius, Tomentella, Russula*
Plant pathogen	75	14.92	*Mycosphaerella tassiana*	*Melampsora, Puccinia*
Wood saprotroph	41	1.87	*Crepidotus cesatii*	*Postia, Trechispora*
Soil saprotroph	21	5.14	*Bovista promontorii*	*Lepiota*
Lichenized	21	0.72	*Physcia stellaris*	*Hyperphyscia, Lecanora, Ramalina*
Fungal parasite	19	3.07	*Sporobolomyces oryzicola*	*Sporobolomyces*
Leaf saprotroph	13	7.98	*Phallus impudicus*	*Mycena*
Ericoid mycorrhizal	4	0.07	*Oidiodendron sp.*	*Oidiodendron*
Endophyte	4	0.05	*Cadophora epimyces*	*Cadophora*
Animal pathogen	3	0.02	*Beauviera bassiana*	*Beauviera*
Epiphyte	2	0.03	*Bullera sp.*	*Bullera*
TOTAL	521	54.120		

3.2 Spore compositional changes across weeks and plots (spore succession)

The highest compositional changes in deposited spores occurred across weeks (weeks within one autumn season, Table 2). While this pattern was consistently strong across all functional guilds with the exception of lichenized fungi, only mycorrhizal, wood saprotrophs and other saprotrophs showed also significant changes across plots. Nevertheless, temporal variation

was twice as big as spatial variation (Table 2). Similarly, spore composition showed a high spatial autocorrelation, since shared variance of geographical distance and plot identity was about 8.6% (Fig. S3). Mycorrhizal fungi showed the highest spatial autocorrelation in comparison with pathogens, wood saprotrophs and other saprotrophs (group mostly represented by dung saprotrophs), since shared variance of plot identity and geographical proximity was about 3.2% of the 4.4% total variation explained by plot identiy (Fig. S3). Soil saprotrophs, wood saprotrophs and other saprotrophs were the guilds with greater relative abundance fluctuations across weeks, while wood saprotrophs displayed the large fluctuations across space (Table 1, Table S1).

Table 2. Variation partitioning analyses considering the temporal (a) vs spatial (b) variation in total deposited spore composition and splitted by functional guilds.

	Temporal variation (a)	Spatial variation (b)	
	Adj R^2	Adj R^2	Residuals
Whole composition	**0.23(*P*<0.001)**	**0.14(*P*<0.001)**	0.63
Functional guilds			
Ectomycorrhizal	**0.16(*P*<0.001)**	**0.04(*P*=0.045)**	0.80
Wood saprotrophs	**0.23(*P*<0.001)**	**0.15(*P*<0.001)**	0.62
Pathogens	**0.25(*P*<0.001)**	0.09(*P*=0.053)	0.66
Other saprotrophs	**0.26(*P*<0.001)**	**0.10(*P*<0.001)**	0.66
Lichenized	**0.06(*P*<0.009)**	0.04(*P*=0.063)	0.90
Soil saprotrophs	**0.49(*P*<0.001)**	0.07(*P*=0.487)	0.51

There was a succession in spore deposition from early autumn to early December (light blue circles vs. dark blue circles in Fig. 1, S4). The successional trend was especially evident for mycorrhizal fungi, saprotrophs and soil saprotrophs, and almost inexistent for lichenized species (Fig. 1). The successional changes of fruiting body community were stronger (P<0.001, R^2= 72%, Fig. S5) than those of spores (P<0.001, R^2=18%; Fig. 1).

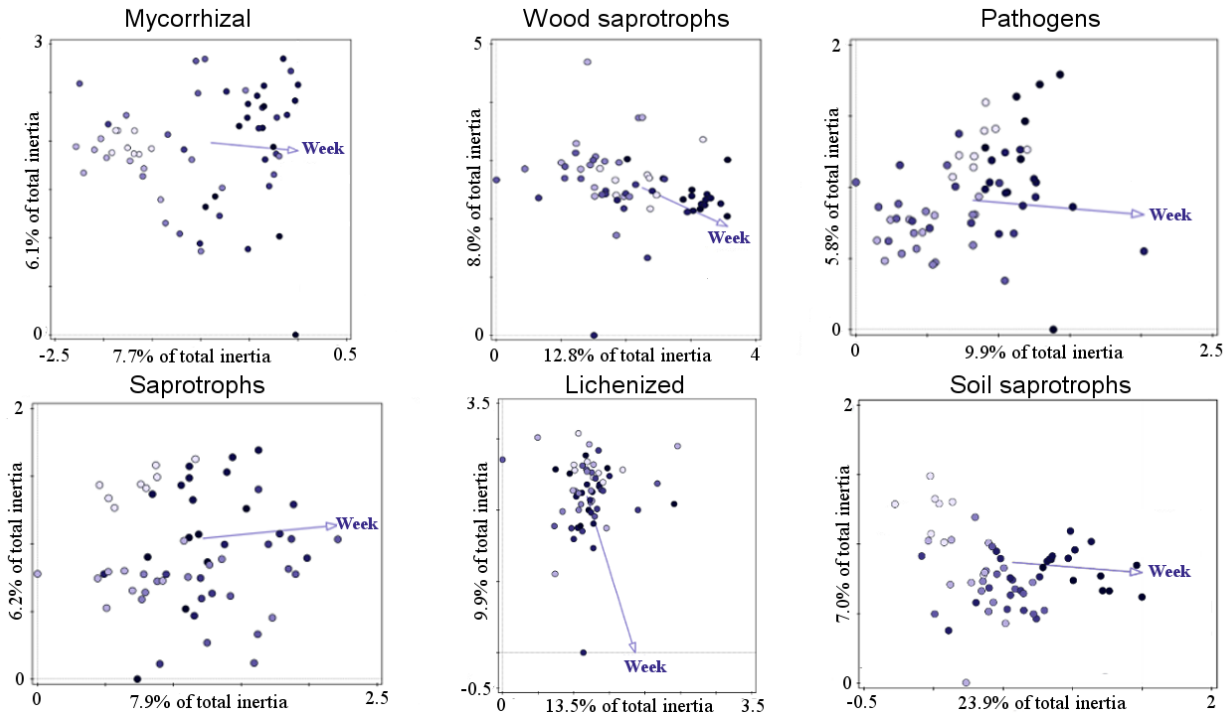

Fig. 1. DCA analysis of the deposited fungal spore composition in a Mediterranean pine forest, as analysed by sequencing of ITS2 amplicons. The figures illustrate variation in deposited spore composition across weeks. Here, 'week' was defined as supplementary variable. Shift from light blue to dark blue in colour represents a gradient from the beginning of sampling season to final of sampling season.

Trends in relative abundance of spores from early to late autumn were different across guilds (Fig. S6). Mycorrhizal and soil saprotrophs followed a U-shape pattern, whereas wood saprotrophs increased linearly and fungal parasites decreased with time (Fig. S6). In addition, there were also different species-specific fluctuations across weeks within the same guilds.

For example, many saprotroph and ectomycorrhizal species followed a unimodal trend and were more abundant during the beginning of the sampling period (e.g. *Macrolepiota procera*, *Lactarius vinosus*, most of the *Suillus* sp.; Fig. 2) or at the end of the sampling period (e.g. *Tricholoma terreum, Lycoperdon perlatum*, *Inocybe* spp.; Fig. 2).

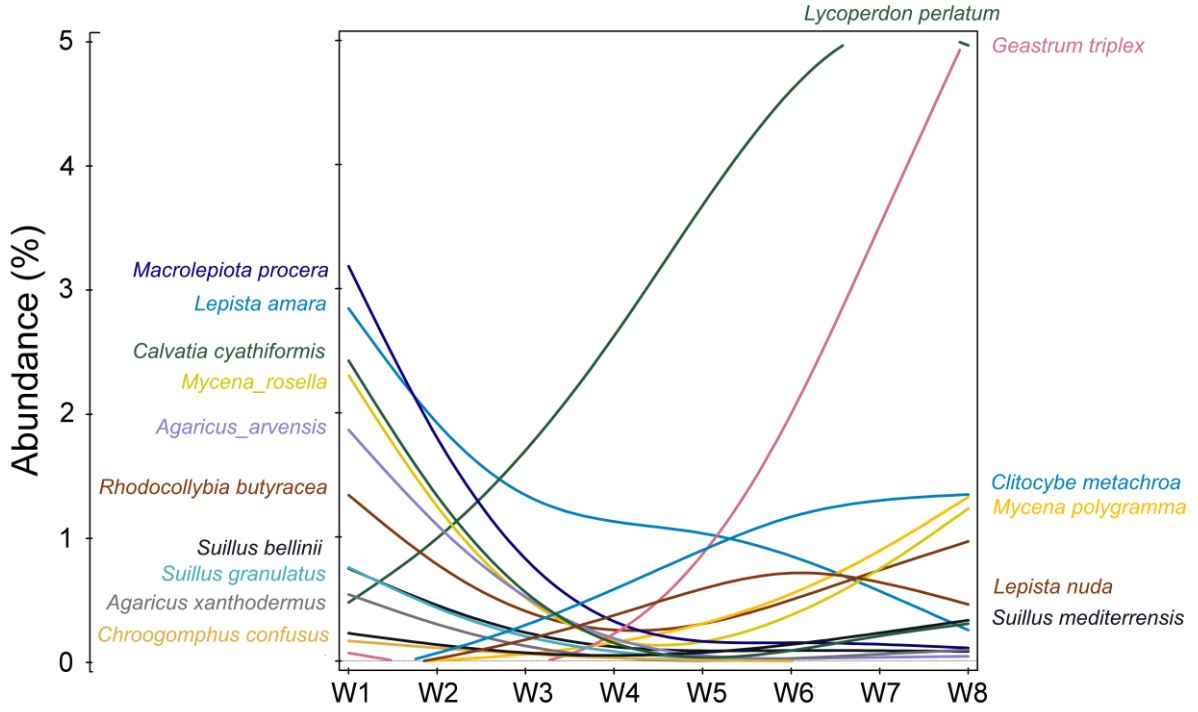

Fig. 2. Species-response curve using GAM models of the most abundant mycorrhizal and saprotrophic species. Weeks are shown in X-axis, starting from the 1st sampling week (W1=20/10) to the last week (W8=11/12).

3.3 Rainfall and mushroom emergence effects on spore composition, diversity and abundance

Rainfall significantly affected the deposited spore composition (F=3.11, *P*=0.006, R^2= 4.7%). However, a detailed inspection of rainfall effects over deposited spore composition showed a significant negative correlation between rainfalls and weekly compositional multivariate variance (i.e. beta diversity decreasing with precipitation (r= -44%): Fig. S7). Rainfall

reduced the spatial heterogeneity of the deposited spores, making them more homogeneous during the rainy weeks (7 ± 4% of multivariate variance) than in no rainy weeks (low or no precipitation, < 5 mm, = 46 ± 7% and moderate precipitation, > 5 mm. = 26 ± 7%). Remarkably, the reduction of compositional multivariate variance in rainy weeks was identical in all sampled plots. In the same way, deposited spore diversity was also positively influenced by rainfall (analyzed as continuous variable) and mushroom emergence (Table 3; Fig. 3). This positive effect was still evident after correcting the relative proportions of each OTU by their total number of spores recorded in each trap (Fig. 3). Diversity was greatest during the 1st, 6th and 7th sampling week, coinciding with the three peaks in precipitation recorded. During the 7th sampling week, and overlapping with the extreme rainfall event, spore diversity was especially higher than the other weeks (Fig. 3, S8). Species indicator analysis showed a greater number of OTUs associated with heavy rainy weeks (e.g. Week 1: 157 OTUs, Week 7: 252 OTUs) than weeks with lower or no rainfall (e.g. Week 5: 11 OTUs, Week 8: 30 OTUs).

Table 3. Effect of fruiting body emergence (Richness), rainfall and its interaction on Hill's numbers derived from the spore community, as evaluated by LME, and considering subsampled composition to the sample with the lowest number of reads.

		Richness (N0)		Shannon (N1)		Simpson (N2)	
Effects	**dF**	**F-value**	***P*-value**	**F-value**	***P*-value**	**F-value**	***P*-value**
Intercept	1	675.20	**<0.001**	97.4	**<0.001**	72.1	**<0.001**
Fruiting bodies (FB)	1	9.56	**0.003**	8.95	**0.043**	4.50	**0.039**
Rainfall	1	10.68	**0.001**	4.94	**0.031**	1.25	0.268
FB × Rainfall	1	11.49	0.309	0.23	0.635	0.10	0.752

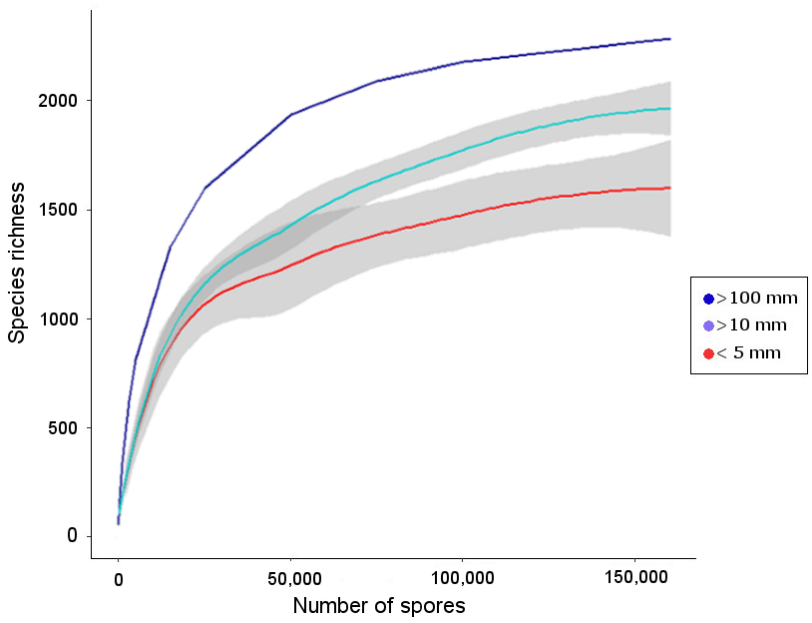

Fig. 3. Rarefaction curves based on the ITS relative proportions of each species as evaluated by Illumina MiSeq, and corrected by the total number of spores as evaluated by qPCR. Here, we considered three precipitation classes: (Low= P < 5 mm., Moderate= P > 10 mm., Severe= P > 100 mm.). Here three precipitation classes were considered, but statistical significance of precipitation effects on richness was tested over precipitation values taken as a continuous variable.

Abundance of deposited spores quantified by qPCR was also positively associated with rainfall ($F_{[1,48]}$=8.10, P=0.006; Fig. 4a). This effect was again especially evident during the 7th week, when there was a strong peak in spore abundance followed by a large rainfall event (>150 mm/24 h). At functional level, rainfall associated positively with the relative abundance of soil saprotrophs ($F_{[1,48]}$=40.4, P<0.001) and undefined saprotrophs ($F_{[1,48]}$=5.2, P=0.028), whereas rainfall was negatively associated with fungal parasites ($F_{[1,48]}$=12.4, P<0.001; Fig. 5a). Soil saprotroph group was mainly composed by OTUs forming puffballs. When studying the correlation of these and other species forming puffballs from other guilds, a great majority

of them showed a high and significant positive correlation with rainfall (average across species of R^2=0.75, Fig. 4b, Fig. 5b).

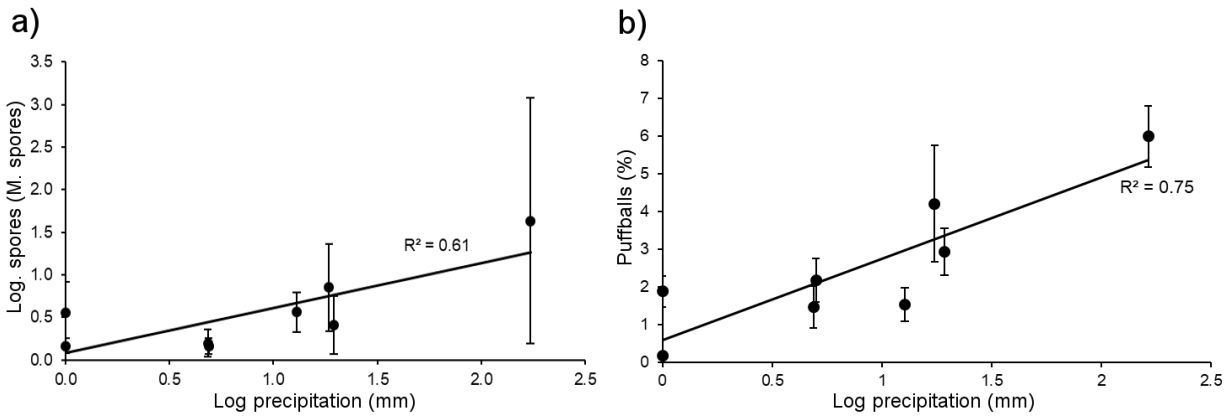

Fig. 4. Correlation between weekly averaged precipitation and (a) the total number of spores recorded at the spore traps, and (b) the relative abundance of puffballs. Error bars indicate standard error.

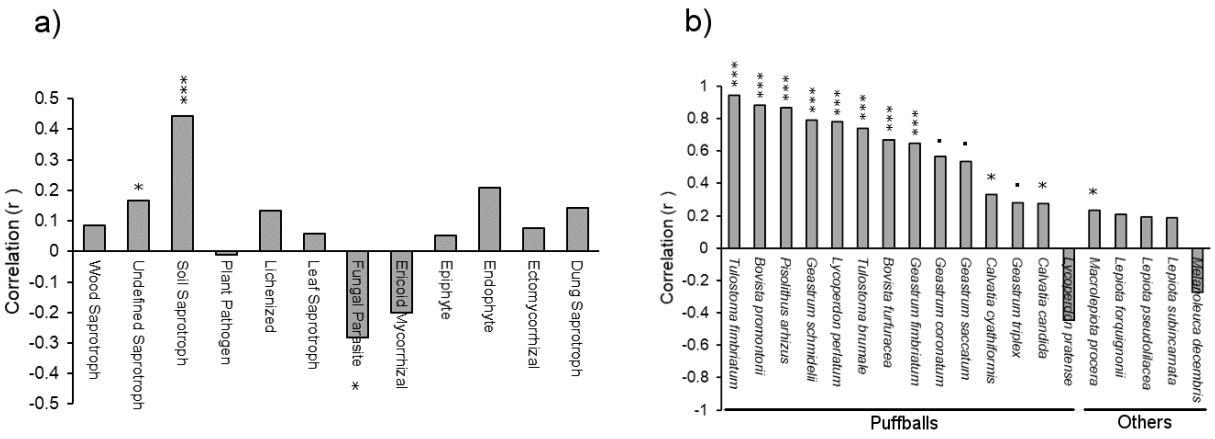

Fig. 5. (a) Pearson correlation between weekly precipitation and relative abundance of each guild and, (b) Correlation between weekly precipitation and relative abundance of each species belonging to soil saprotrophs, including puffball species and non-puffball species (others). In (b), puffballs belonging to other functional guilds, such as *Pisolithus arhizus*, which forms puffballs but belongs to the ectomycorrhizal guild, or the species belonging to *Lycoperdon* genera, classified as undefined saprotrophs are also included. Significance values: "*" P<0.05, "**" P<0.01, "***" P<0.001.

4. Discussion

382 Our results using spore traps in a local-scale set up showed that deposited fungal spores were
383 succesionally changing during autumn, following the fungal fruiting body season. Rainfall
384 influenced positively fungal spore deposition (whole spores), especially from soil saprotrophs
385 and other saprotrophs. This positive correlation was especially high for puffballs. Spatial
386 homogenization of deposited spore composition was related with rainfall, with higher spatial
387 homogenization during wet weeks. Our findings point out the importance of both fruiting
388 body emergence and rainfall events in determining temporal spore deposition in
389 Mediterranean forests, where both mushroom emergence and rainfall are irregular and often
390 scarce events. It seems that predicted irregularity of both rainfall events and fruiting body
391 emergence in Mediterranean regions (Alday et al., 2017) may cause potential alterations in
392 fungal dispersal and deposition, potentially affecting fungal community renewal and
393 colonization of Mediterranean forest ecosystems.

394 We observed variation in spore deposition amongst time and space, although temporal
395 changes were more important than spatial changes at local scale. Changes in airbone spore
396 composition at temporal scales are well-known (Abrego et al., 2018; Kivlin et al., 2014;
397 Nicolaisen et al., 2017), and recent studies showed low spatial changes of airborne spores at
398 local scale (Abrego et al., 2018). In this study, we showed that variation across time and space
399 is highly affected by rainfall, which should be considered in future aerospore diversity
400 studies.

401 Significant positive rainfall relationships with specific fungal spores were previously reported
402 (Oliveira et al., 2009; Peay and Bruns, 2014). In contrast, negative correlation between
403 rainfall and airborne spores has been reported (Pakpour et al., 2015), suggesting that rainfall
404 washed-out airborne spores, increasing deposited spores. There was a lack of knowledge
405 about the effects of rainfall events on deposited spore diversity or the differential effects

406 across functional guilds. Our local-scale study supports a positive effect of rainfall on spore
407 deposition, with an increase in both diversity and number of spores trapped. In addition, the
408 negative association between rainfall and beta diversity across weeks suggest that rainfall may
409 be a driver of community homogenization in time and space. Rainfall often occurs irregularly
410 amongst spatial units considered and in a stochastic way in Mediterranean areas, thus perhaps
411 homogenization is not as strong as in other environments where rainfall is more frequent and
412 affects larger areas. Thus, the observed positive effect of rainfall on deposited spores could be
413 especially relevant in Mediterranean forests, with potential ecological implications with
414 respect to spore establishment.

415 Lack of strong dispersive winds may prevent the fungal spores to reach the turbulent
416 boundary layer (Lacey and West, 2006). However, rainfall events are often accompanied by
417 strong winds that may promote both spore mixing and deposition, thus, favouring the
418 homogenization of airborne spores. In our study, we found a higher number of species during
419 high rainfall events than during low rainfall events in autumn. We could speculate that in the
420 heaviest rain events not only spores were lifted from the ground but also spores coming from
421 distant sources or present in higher atmospheric layers, such as the turbulent layer of the
422 atmosphere (Lacey and West, 2006; Nicolaisen et al., 2017). However, at community level,
423 successional changes in spore composition were much greater (R^2=28%) than compositional
424 changes caused by rainfall (R^2= 4.7%), suggesting a small contribution of spores from the
425 turbulent layer. Thus, our results suggest that most of spores gathered by rain originate from
426 nearby sources, and just a small fraction from distant sources.

427 New OTUs were exclusively found during specific rainfall events. This seems to be more
428 evident during the 7th week of sampling, as was showed with the high number of indicator
429 species. The rain event of that week, was not only strong but also seemed to carry some

Saharian dust, with estimated peaks of wet deposition around 50-400 mg of dust m^{-2} in only 3 h during the precipitation event (Barcelona Dust Forecast Center), which may potentially be the source of the new species. Similar observations were made by Reche et al., (2018), who showed that deposition rates of bacteria were significantly higher during rain events and Saharian dust intrusions, with bacterial deposition rates ranging from 0.3×10^7 to $>8\times10^7$ m^{-2} per day. Thus, as already demonstrated with bacteria, it is possible that some spores coming from far distances were deposited in our plots, but in our study it is not possible to know which spores neither their abundance.

A high positive correlation between rainfall and deposited spores was found for soil saprotrophs and undefined saprotrophs. However, the highest correlation was found for puffball species (e.g. *Lycoperdon* spp. and *Bovista* spp.), included within the soil saprotrophs, which are assumed to be very efficiently transported by wind (Hitchcock et al., 2011). Interestingly, no association between fruiting body production and spores were found in previous studies for these species (Castaño et al., 2017). These results suggest that, for this group of species, rainfall may be a driver for both spore ejection and deposition, since no spores were found outside rain events even though fruit bodies were present. Thus, despite this mechanism is not tested here, we hypothesize that raindrops during rain may hit the puffball and favor ejection.

This study provides new knowledge to understand how spatio-temporal changes in deposited spores and how rainfall affect fungal spore deposition. For example, successional changes in the fungal fruiting body community paralleled partially with the successional changes observed in the deposited spores (Fig. 6A), which means that most of the spore deposition rely on mushroom emergence and gravity at short-time scales. However, previous studies have shown that a little fraction of ejected spores potentially remains airborne or reach

dispersive winds (Dressaire et al., 2016), forming a spore bank in the atmospheric layer (seed-bank model theory; Reche et al., (2018)). Among environmental factors, rainfall may contribute to increase both; i) the amount and diversity of deposited spores and ii) the spore composition homogeneity amongst plots compared with drier weeks (Fig. 6B). Thus, it seems that in our local-scale study the rainfall events may have potentially efficiently washed-out the circulating atmospheric spores. In addition, some rainfall events may be a source of new spores coming from geographical distant sources (Fig. 6C). For some species, such as puffballs, rainfall may also promote spore ejection (Fig. 6D). These results provide a step further in predicting fungal assemblages in forest ecosystems.

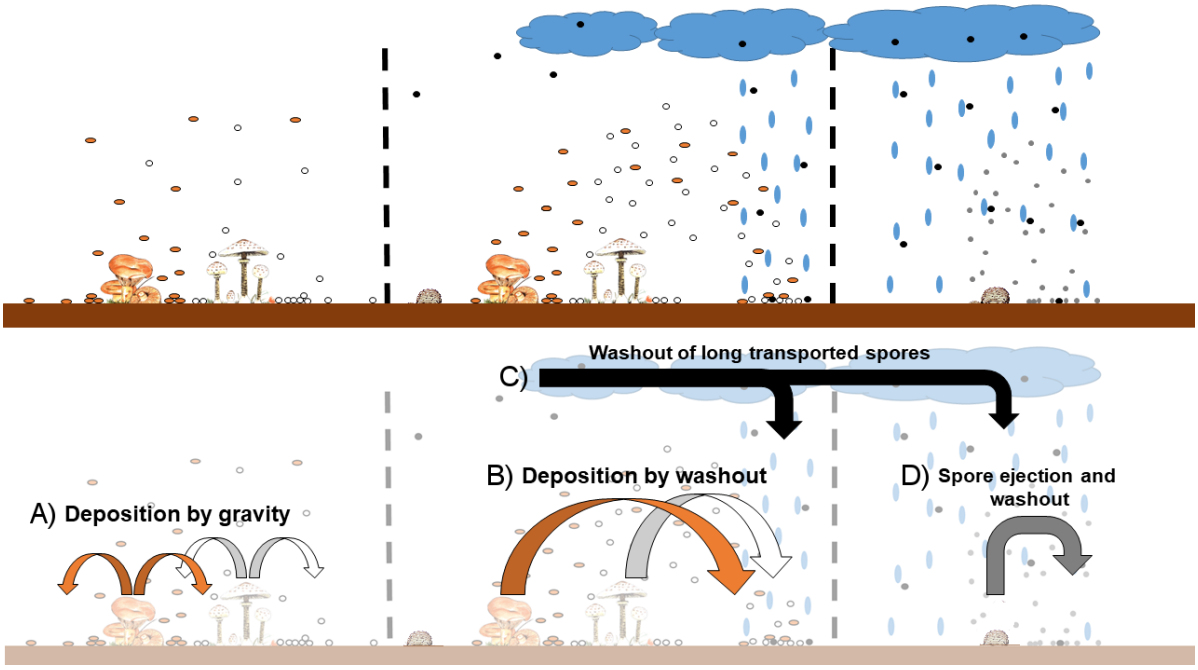

Fig. 6. Summary of the drivers and processes influencing spore deposition observed in this study. Billions of spores are produced by many species and a very important fraction of them fall close to the spore source, despite a little fraction of them may still reach dispersive winds (A). Here, we suggest that rainfall washed-out the atmospheric spores, concurrent with the greater spore diversity and abundance during rainy days (B). Winds and clouds may transport

particles from sites located further (e.g. the week from 27th November to 4th December) that may fall during the rainfall event (C). Finally, rainfall may not only promote spore deposition but also cause spore ejection (e.g. puffballs; D).

Conflict of interests

The authors declare no conflict of interests associated with this publication.

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6. References

Abarenkov, K., Nilsson H. R., Larsson, K. H, Alexander, I.J., Eberhardt, U., Erland, S., …. Pennanen, T., 2010. The UNITE database for molecular identification of fungi – recent

491 updates and future perspectives. New Phytol. 186, 281–285.

492 Abrego, N., Norros, V., Halme, P., Somervuo, P., Ali-Kovero, H., Ovaskainen, O., 2018.
493 Give me a sample of air and I will tell which species are found from your region:
494 Molecular identification of fungi from airborne spore samples. Mol. Ecol. Resour. 18(3),
495 511–524.

496 Aguayo, J., Fourrier-Jeandel, C., Husson, C., Ioos, R., 2018. Assessment of Passive Traps
497 Combined with High-Throughput Sequencing To Study Airborne Fungal Communities.
498 Appl. Environ. Microbiol. 84 (11), e02637-17.

499 Alday, J.G., Bonet, J.A., Oria-de-Rueda, J.A., Martínez de Aragón, J., Aldea, J., Martín-Pinto,
500 P., De-Miguel, S., Hernández-Rodríguez, M., Martínez-Peña, F., 2017. Record breaking
501 mushroom yields in Spain. Fungal Ecol., 26, 144-146.

502 Bahram, M., Kohout, P., Anslan, S., Harend, H., Abarenkov, K., Tedersoo, L., 2016.
503 Stochastic distribution of small soil eukaryotes resulting from high dispersal and drift in
504 a local environment. ISME Journal, 10(4), 885–896.

505 Bálint, M., Bartha, L., O'Hara, R.B., Olson, M.S., Otte, J., Pfenninger, M., Robertson, A.,
506 Tiffin, P., Schmitt, I., 2015 Relocation, high-latitude warming and host genetic identity
507 shape the foliar fungal microbiome of poplars. Mol Ecol, 24, 235–248.

508 Burch, M., Levetin. E., 2002. Effects of meteorological conditions on spore plumes. Int J
509 Biometeorol 46:107–117.

510 Castaño, C., Oliva, J., Martínez de Aragón, J. M., Alday, J. G., Parladé, J., Pera, J., Bonet, J.
511 A., 2017. Mushroom emergence detected by combining spore trapping with molecular
512 techniques. Appl. Environ. Microbiol. 83(13), e00600-17.

513 Castaño, C., Lindahl, B.D., Alday, J.G., Hagenbo, A., Martínez de Aragón, J., Parladé, J.
514 Pera, J., Bonet, J.A., 2018. Soil microclimate changes affect soil fungal communities in a
515 Mediterranean pine forest. New Phytol. http://doi.org/10.1111/nph.15205 (in press).

516 Clemmensen, K.E., Bahr, A., Ovaskainen, O., Dahlberg, A., Ekblad, A., Wallander, H.,
517 Stenlid, J., Finlay, R.D., Wardle, D.A., Lindahl, B.D., 2013. Roots and associated fungi
518 drive long-term carbon sequestration in boreal forest. Science, 339, 1615‑1618.

519 Bonet, J.A., de-Miguel, S., Martinez de Aragón, J., Pukkala, T., Palahí, M. 2012. Immediate
520 effect of thinning on the yield of *Lactarius* group *deliciosus* in *Pinus pinaster* forests in
521 Northeastern Spain. For. Ecol. and Manage. 265, 211–217.

522 Dahlberg, A., Stenlid, J., 1994. Size, distribution and biomass of genets in populations of
523 *Suillus bovinus* (L.: Fr.) Roussel revealed by somatic incompatibility. New Phytol. 128,
524 225–234.

525 Dam, N., 2013. Spores do travel. Mycologia, 105(6), 1618–1622.

526 De Caceres, M., Legendre, P., 2009. Associations between species and groups of sites: indices
527 and statistical inference. Ecology, 90, 3566–3574.

528 De Cáceres, M., Martin, C., Cabon, A., 2017. Package ‘meteoland’. R package version
529 0.5.9. https://cran.rproject.org/web/packages/meteoland/index.html.

530 Després, V. R., Alex Huffman, J., Burrows, S. M., Hoose, C., Safatov, A. S., Buryak, G., …
531 Jaenicke, R., 2012. Primary biological aerosol particles in the atmosphere: A review.
532 Tellus, Series B: Chem. Phys. Meteorol. 64(1), 15598.

533 Dressaire, E., Yamada, L., Song, B., Roper, M., 2016. Mushrooms use convectively created

534 airflows to disperse their spores. Proc. Natl. Acad. Sci. USA, 113(11), 2833–2838.

535 Galante, T.E., Horton, T.E., Swaney, D.P., 2011. 95% of basidiospores fall within 1 m of the
536 cap: a field- and modeling-based study. Mycologia, 103, 1175–1183.

537 Gardes, M., Bruns, T., 1993. ITS primers with enhanced specificity for basidiomycetes—
538 application to the identification of mycorrhizae and rusts. Mol. Ecol., 2, 113–118.

539 Hartmann, M., Brunner, I., Hagedorn, F., Bardgett, R. D., Stierli, B., Herzog, C., … Frey, B.,
540 2017. A decade of irrigation transforms the soil microbiome of a semi-arid pine forest.
541 Mol Ecol, 26(4), 1190–1206.

542 Hill, M.O., 1973. Diversity and evenness: a unifying notation and its consequences. Ecology,
543 *54*, 427‑432

544 Hitchcock, C. J., Chambers, S. M., Cairney, J. W. G., 2011. Genetic population structure of
545 the ectomycorrhizal fungus *Pisolithus microcarpus* suggests high gene flow in south-
546 eastern Australia. Mycorrhiza, 21(2), 131–137.

547 Hsieh, T.C., Ma, K.H., Chao, A., 2016. iNEXT: iNterpolation and EXTrapolation for Species
548 Diversity. R package version 2.0.8. https://cran.r-project.org/web/
549 packages/iNEXT/index.html.

550 Ihrmark, K., Bödeker, I. T. M., Cruz-Martinez, K., Friberg, H., Kubartova, A., Schenck, J., …
551 Lindahl, B. D., 2012. New primers to amplify the fungal ITS2 region-evaluation by 454-
552 sequencing of artificial and natural communities. FEMS Microbiol. Ecol. 82(3), 666–77.

553 Kadowaki, K., Leschen, R. A., Beggs, J. R., 2010. Periodicity of spore release from individual
554 Ganoderma fruiting bodies in a natural forest. Australasian Mycologist, 29, 17–23.

555 Kennedy, P. G., Peay, K. G., Bruns, T. D., 2009. Root tip competition among ectomycorrhizal
556 fungi: Are priority effects a rule or an exception? Ecology, 90(8), 2098–2107.

557 Kivlin, S. N., Winston, G. C., Goulden, M. L., Treseder, K. K., 2014. Environmental filtering
558 affects soil fungal community composition more than dispersal limitation at regional
559 scales. Fungal Ecol. 12, 14–25.

560 Kohout, P., Charvátová, M., Štursová, M., Mašínová, T., Tomšovský, M., Baldrian, P., 2018.
561 Clearcutting alters decomposition processes and initiates complex restructuring of fungal
562 communities in soil and tree roots. ISME Journal, 12(3), 692–703.

563 Lacey, M.E., West, J.S., 2006. The Air Spora. Dordrecht, The Netherlands: Springer 156 pp.

564 Legendre, P., Gallagher, E., 2001. Ecologically meaningful transformations for ordination of
565 species data. Oecologia, 129, 271–280.

566 Martínez de Aragón, J., Bonet, J.A., Fischer, C.R., Colinas C., 2007. Productivity of
567 ectomycorrhizal and selected edible saprotrophic fungi in pine forests of the pre-
568 Pyrenees mountains, Spain: predictive equations for forest management of mycological
569 resources. For. Ecol. Manage. 252, 239–256.

570 McMurdie, P.J., Holmes, S., 2014. Waste not, want not: why rarefying microbiome data is
571 inadmissible. PLoS Comput. Biol. 10, e1003531.

572 Nguyen, N. H., Song, Z., Bates, S. T., Branco, S., Tedersoo, L., Menke, J., … Kennedy, P. G.,
573 2016. FUNGuild: An open annotation tool for parsing fungal community datasets by
574 ecological guild. Fungal Ecol. 20, 241–248.

575 Nicolaisen, M., West, J. S., Sapkota, R., Canning, G. G. M., Schoen, C., Justesen, A. F., 2017.

576 Fungal communities including plant pathogens in near surface air are similar across
577 northwestern Europe. Front Microbiol. 8, 1–11.

578 Oksanen, J., Blanchet, F. G., Kindt, R., Legendre, P., Minchin, P. R., O'Hara, B., . . . Wagner,
579 H., 2015. VEGAN: Community ecology package. R package version 2.2-1.

580 Oliva, J., Messal, M., Wendt, L., Elfstrand, M., 2017. Quantitative interactions between the
581 biocontrol fungus *Phlebiopsis gigantea*, the forest pathogen *Heterobasidion annosum*
582 and the fungal community inhabiting Norway spruce stumps. For. Ecol. Manage. 402,
583 253–264.

584 Oliveira, M., Ribeiro, H., Delgado, J. L., Abreu, I., 2009. The effects of meteorological
585 factors on airborne fungal spore concentration in two areas differing in urbanisation
586 level. Int. J. Biometeorol. 53(1), 61–73.

587 Pakpour, S., Li, D.W., Klironomos, J. 2015. Relationships of fungal spore concentrations in
588 the air and meteorological factors. Fungal Ecol. 13, 130-134.

589 Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D. R Core Team. 2016. nlme: Linear and
590 Nonlinear Mixed Effects Models. R package version 3.1-128, http://CRAN.R-
591 project.org/package=nlme

592 Peay, K. G., Bruns, T. D., 2014. Spore dispersal of basidiomycete fungi at the landscape scale
593 is driven by stochastic and deterministic processes and generates variability in plant-
594 fungal interactions. New Phytol. 204(1), 180-191.

595 Peay, K. G., Schubert, M. G., Nguyen, N. H., Bruns, T. D., 2012. Measuring ectomycorrhizal
596 fungal dispersal: macroecological patterns driven by microscopic propagules. Mol Ecol.
597 21(16), 4122–4136.

598 Reche, I., D'Orta, G., Mladenov, N., Winget, D. M., Suttle, C. A., 2018. Deposition rates of
599 viruses and bacteria above the atmospheric boundary layer. The ISME Journal, 12,
600 1154–1162.

601 Rincón, A., Santamaría-pérez, B., Rabasa, S. G., Coince, A., Marçais, B., Buée, M., 2015.
602 Compartmentalized and contrasted response of ectomycorrhizal and soil fungal
603 communities of Scots pine forests along elevation gradients in France and Spain.
604 Environ. Microbiol. 17, 3009–3024.

605 Smith, G. R., Steidinger, B. S., Bruns, T. D., Peay, K. G., 2018. Competition–colonization
606 tradeoffs structure fungal diversity. ISME Journal, doi:10.1038/s41396-018-0086-0. In
607 press.

608 Thornton, P.E., Hasenauer, H., White, M.A., 2000. Simultaneous estimation of daily solar
609 radiation and humidity from observed temperature and precipitation: an application over
610 complex terrain in Austria. Agr. For. Meteor., 104, 255‑271.

611 Vilgalys, R., Hester, M., 1990. Rapid genetic identification and mapping of enzymatically
612 amplified ribosomal DNA from several Cryptococcus species. J. Bacteriol. 172, 4238-
613 4246.

614 # Supplementary material

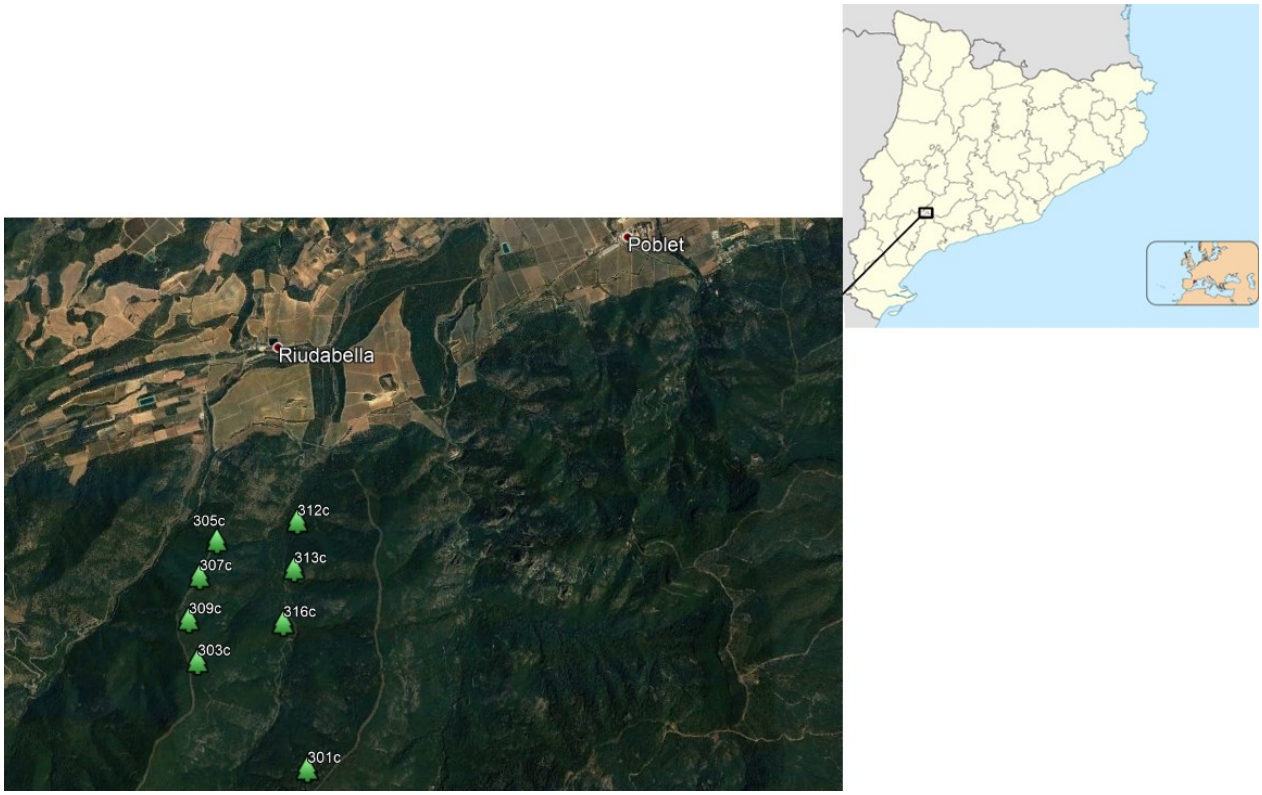

Fig. S1. Geographical localization of the study plots.

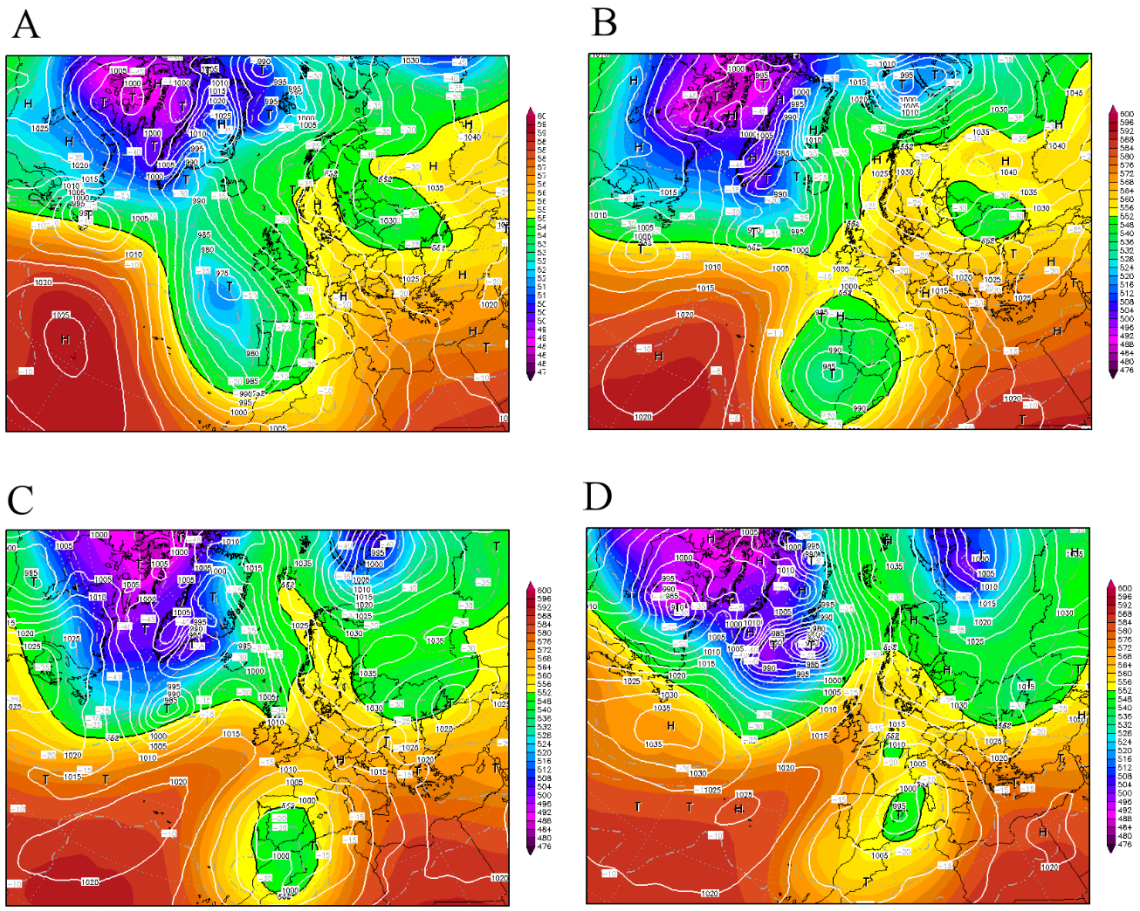

Fig. S2. Synoptic geopotential maps at 500 hPa during the days 28 November (A), 29th November (B), 30th November (C) and 1st December (D) at European scale. Maps show a strong cut-off low in the Iberian Peninsula, as a result of the isolation of cold air temperatures at high altitudes (lower than -25ºC at 5,000 m.) from the *polar jet stream* and subsequent isolation by an undulated belt with high pressures in central Europe. The influence of this cut-off low, together with humid winds with sea influence (note the SE winds in B and C) resulted in rainfall events >160 mm in less than 48 h in our study plots. Sequential geopotential maps were obtained using the "reanalysis" option from the Global Forecast System (GFS) at www.wetterzentrale.de. Colors indicate a gradient in geopotential values. "H" indicate high air pressures and "T" low air pressures. In "H", air direction at the isolines follow clock-wise direction whereas in "T" air direction follows counter clock-wise directions. Here, especially in B and C, maps indicate strong S-SE air directions that carried significant amounts of dust coming from North Africa.

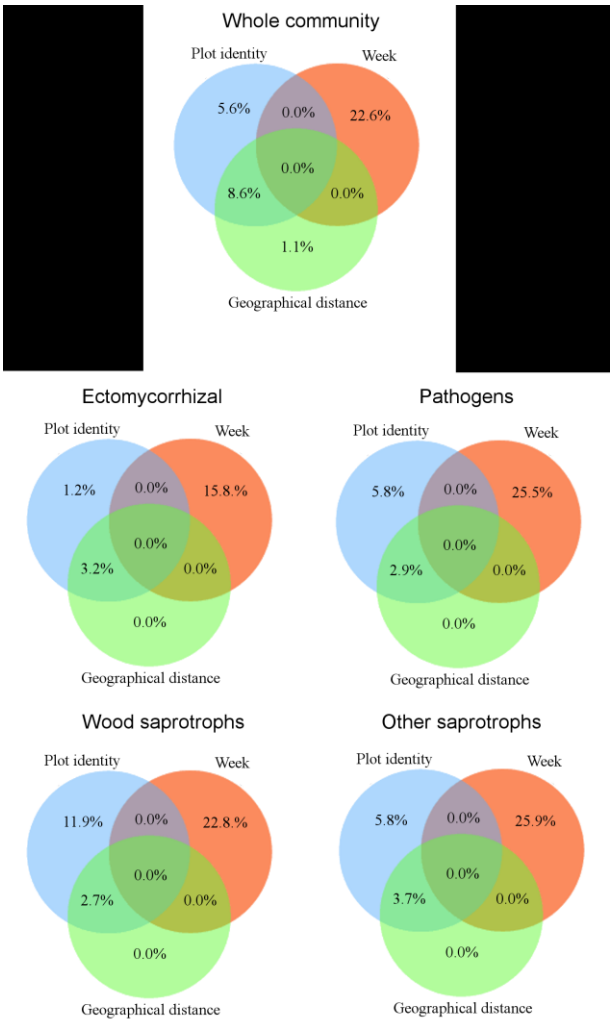

Fig. S3. Variation partitioning analysis considering the spores from the whole community, ectomycorrhizal species, pathogens, wood saprotrophs and other saprotrophs. In these analyses, plot identity, week and geographical distance factors are tested.

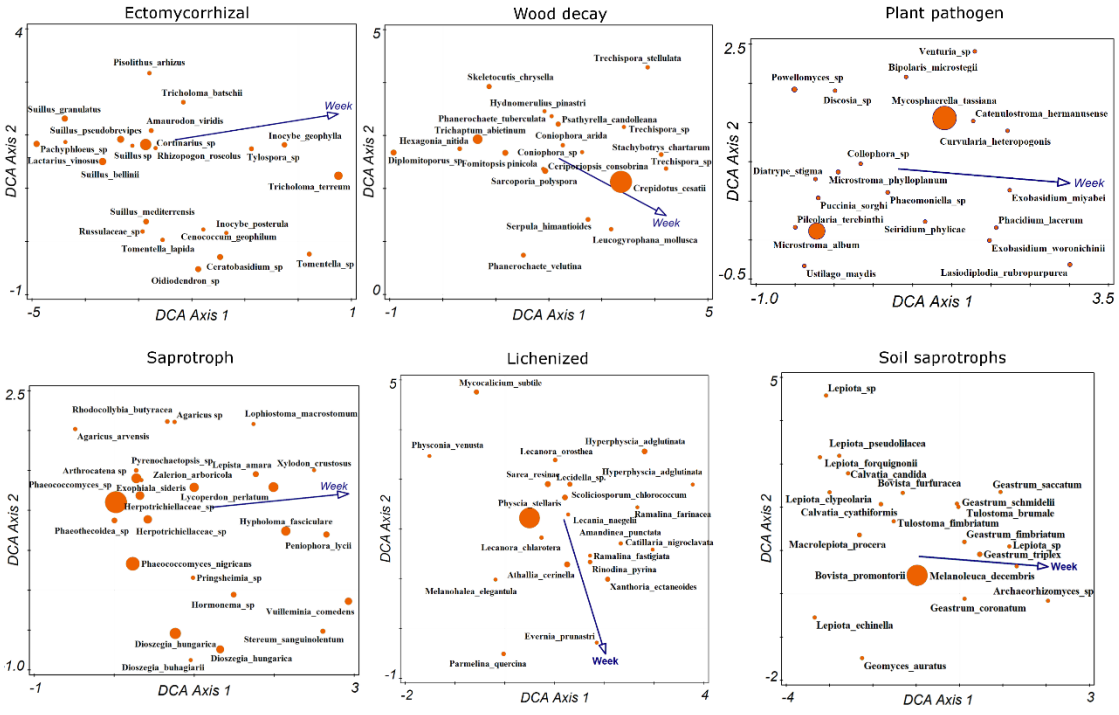

Fig. S4. Species biplots DCA analyses of the deposited fungal spore composition in a Mediterranean pine forest, as analysed by sequencing of ITS2 amplicons. The figures illustrate variation in taxa occurrence of spores across weeks. Here, 'week' was defined as supplementary variable. Symbol size is proportional to the relative abundance of the given taxa.

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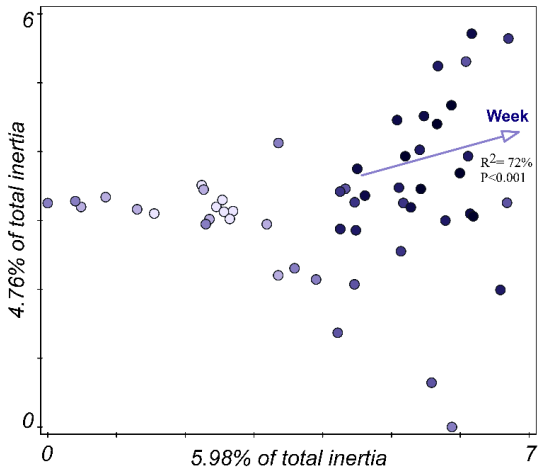

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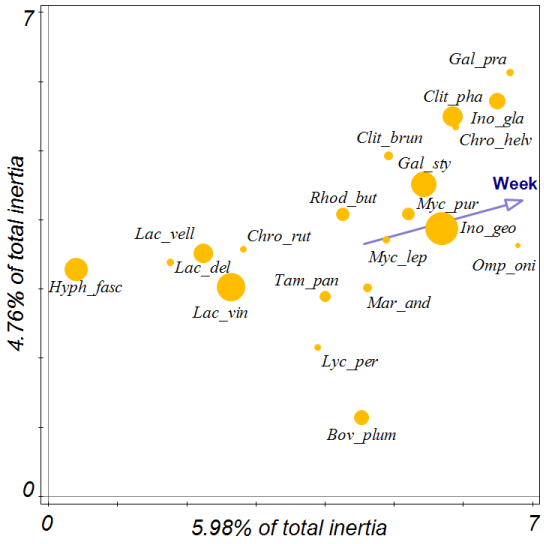

670 **Fig S5.** DCA analysis of the fruiting body community composition in a Mediterranean pine forest,
671 with (a) the sample plot and (b) the species plot showing the 20 most abundant species. The figures
672 illustrate changes in fruiting body community composition across weeks. Here, 'week' was defined as
673 supplementary variable. Shift from light blue to dark blue in colour represents a gradient from the
674 beginning of sampling season to final of sampling season. Symbol size in the species plot is
675 proportional to the relative abundance of the given taxa.

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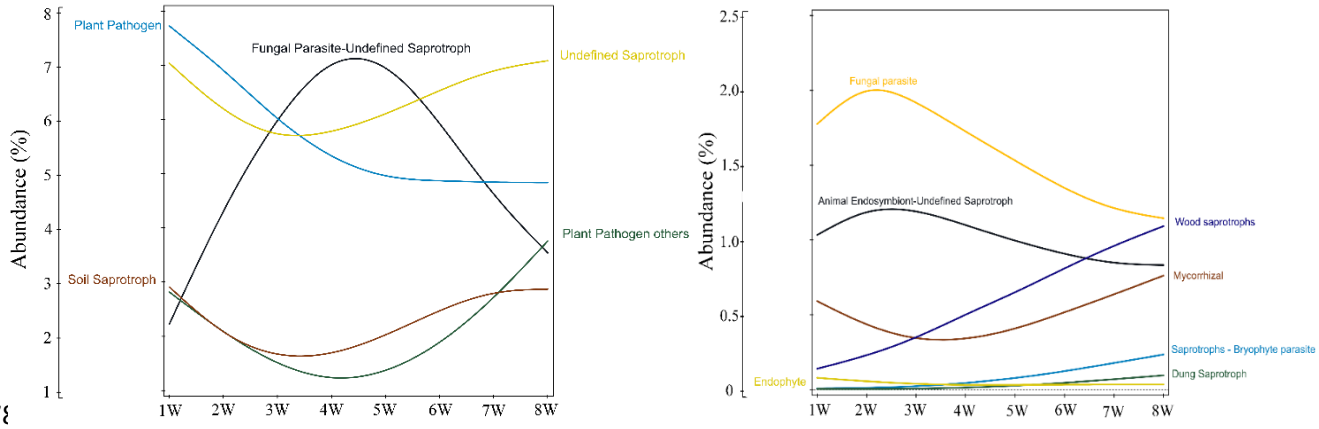

Fig. S6. Temporal fluctuation of the functional guilds that showed significant changes across weeks and visualized by GAM models.

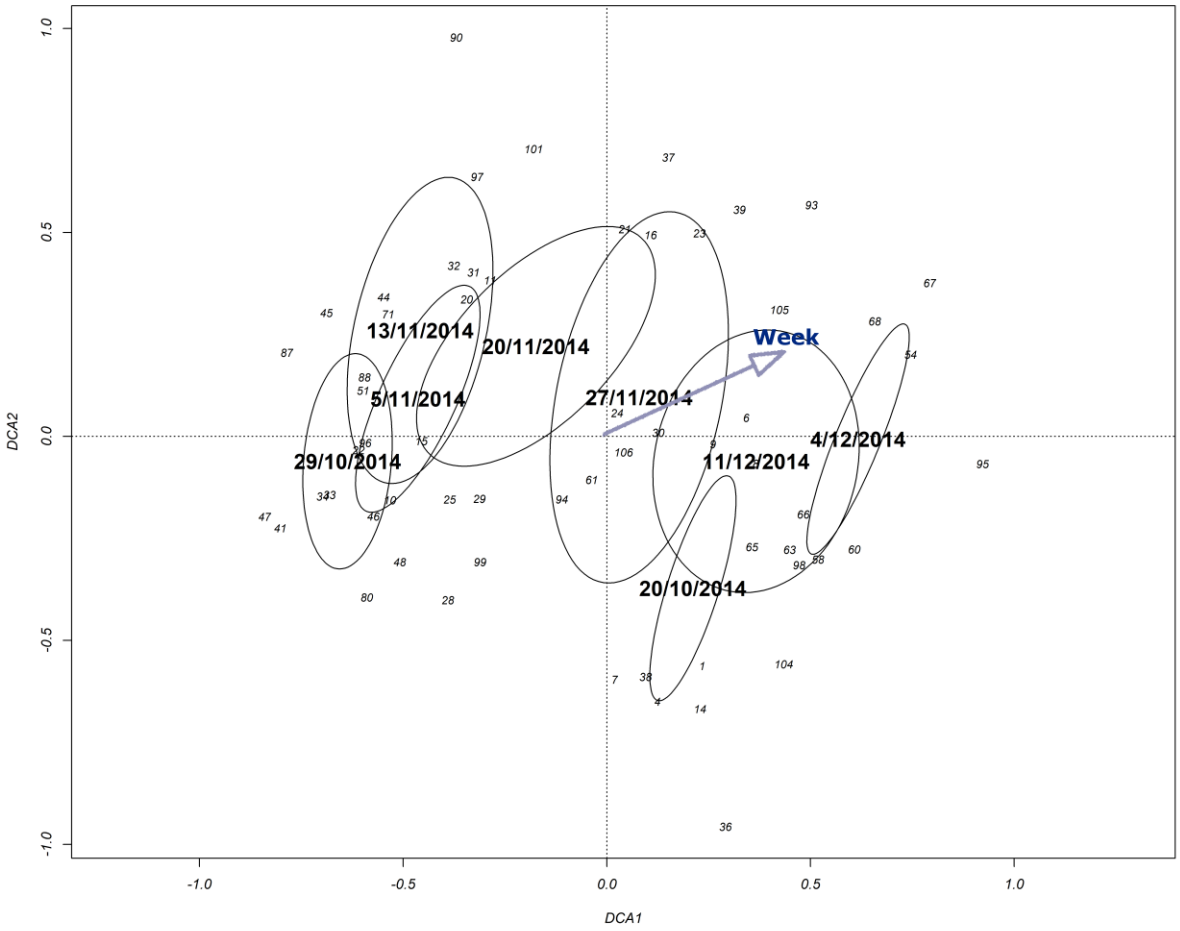

Fig. S7. DCA analysis of the deposited fungal spore composition in a Mediterranean pine forest, as analysed by sequencing of ITS2 amplicons, considering all the fungal guilds. Ellipses show data heterogeneity and data dispersion across weeks. Narrow ellipses indicate low heterogeneity, meaning that compositional changes across plots within each week are low.

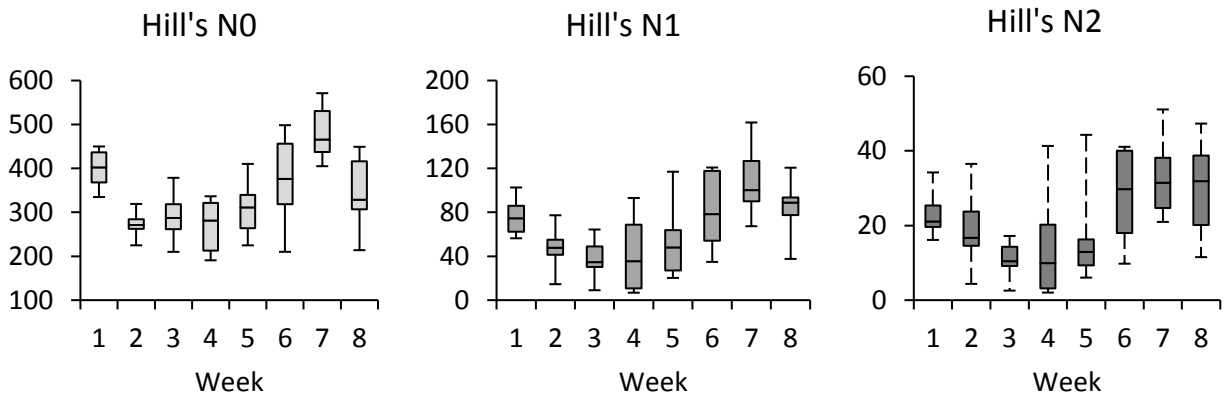

Fig. S8. Hill's diversity numbers of the whole fungal spore composition across the 8 sampling weeks.

Table S1. Temporal changes in relative abundance of each functional guild, analysed by GAMM models. Here, relative abundance of each functional guild was considered the sum of each OTUs belonging to the same guild.

	Temporal changes		
Guilds	**F**	***P*-value**	**Adj. R^2**
Animal pathogen	1.98	**0.011**	**8.6**
Moulds	0.05	0.946	1.7
Dung saprotrophs	19.57	**<0.001**	**43.1**
Mycorrhizal	6.25	**0.001**	**21.8**
Endophyte	7.36	**<0.001**	**23.3**
Epiphyte	9.91	**0.020**	**10.4**
Fungal parasite	0.17	0.678	1.4
Lichenized	0.61	0.436	0.1
Plant pathogen	8.28	**0.006**	**8.1**
Soil saprotrophs	12.67	**<0.001**	**46.7**
Undefined saprotrophs	6.2	**<0.001**	**21.6**
Wood saprotrophs	36.06	**<0.001**	**34.4**