Mushroom Emergence Detected by Combining Spore Trapping with Molecular Techniques

Carles Castaño,^{a,b} Jonàs Oliva,^{b,c} Juan Martínez de Aragón,^{a,d} Josu G. Alday,^b Javier Parladé,^e Joan Pera,^e José Antonio Bonet^{b,d}

Forest Bioengineering Solutions S.A., Solsona, Spain^a; Departament de Producció Vegetal i Ciència Forestal, Universitat de Lleida-AGROTECNIO, Lleida, Spain^b; Department of Forest Mycology and Plant Pathology, Swedish University of Agricultural Sciences, Uppsala, Sweden^c; Centre Tecnològic Forestal de Catalunya, CTFC-CEMFOR, Solsona, Spain^d; Protecció Vegetal Sostenible, IRTA, Centre de Cabriels, Barcelona, Spain^e

ABSTRACT Obtaining reliable and representative mushroom production data requires time-consuming sampling schemes. In this paper, we assessed a simple methodology to detect mushroom emergence by trapping the fungal spores of the fruiting body community in plots where mushroom production was determined weekly. We compared the performance of filter paper traps with that of funnel traps and combined these spore trapping methods with species-specific quantitative real-time PCR and Illumina MiSeq to determine the spore abundance. Significantly more MiSeq proportional reads were generated for both ectomycorrhizal and saprotrophic fungal species using filter traps than were obtained using funnel traps. The spores of 37 fungal species that produced fruiting bodies in the study plots were identified. Spore community composition changed considerably over time due to the emergence of ephemeral fruiting bodies and rapid spore deposition (lasting from 1 to 2 weeks), which occurred in the absence of rainfall events. For many species, the emergence of epigeous fruiting bodies was followed by a peak in the relative abundance of their airborne spores. There were significant positive relationships between fruiting body yields and spore abundance in time for five of seven fungal species. There was no relationship between fruiting body yields and their spore abundance at plot level, indicating that some of the spores captured in each plot were arriving from the surrounding areas. Differences in fungal detection capacity by spore trapping may indicate different dispersal ability between fungal species. Further research can help to identify the spore rain patterns for most common fungal species.

IMPORTANCE Mushroom monitoring represents a serious challenge in economic and logistical terms because sampling approaches demand extensive field work at both the spatial and temporal scales. In addition, the identification of fungal taxa depends on the expertise of experienced fungal taxonomists. Similarly, the study of fungal dispersal has been constrained by technological limitations, especially because the morphological identification of spores is a challenging and time-consuming task. Here, we demonstrate that spores from ectomycorrhizal and saprotrophic fungal species can be identified using simple spore traps together with either MiSeq fungus-specific amplicon sequencing or species-specific quantitative real-time PCR. In addition, the proposed methodology can be used to characterize the airborne fungal community and to detect mushroom emergence in forest ecosystems.

KEYWORDS propagules, ectomycorrhizal, saprotroph, DNA barcoding, molecular identification, dispersion, fungi, *Lactarius*

Received 16 March 2017 Accepted 12 April 2017

Accepted manuscript posted online 21 April 2017

Citation Castaño C, Oliva J, Martínez de Aragón J, Alday JG, Parladé J, Pera J, Bonet JA. 2017. Mushroom emergence detected by combining spore trapping with molecular techniques. *Appl Environ Microbiol* 83:e00600-17. <https://doi.org/10.1128/AEM.00600-17>.

Editor Emma R. Master, University of Toronto

Copyright © 2017 American Society for Microbiology. All Rights Reserved.

Address correspondence to Carles Castaño, carles.castanyo@fbs.cat.

Wild edible fungi are highly important nonwood forest products and are increasingly in demand at food markets worldwide (1). Up to 268 fungal taxa have been authorized to be commercialized in Europe (2), of which the most important marketed mushrooms are *Boletus edulis*, *Cantharellus cibarius*, *Lactarius deliciosus*, *Morchella esculenta*, and *Agaricus campestris* (2, 3). Spain, the Netherlands, France, and Poland are the largest mushroom producers in Europe (2). Despite the increasing interest and importance of mushrooms as nonwood forest products, mushroom production has only recently been included as a target in forest management and planning alongside the traditional goal of wood production (4). The inclusion of mushroom production into forest management planning requires the ability to evaluate potential mushroom yields in quantifiable terms. However, the variability in mushroom biomass across years, the ephemeral cycle of several fruiting bodies that are only observable for a few days, and the time needed to sample representative mushroom populations (5) represent serious challenges for monitoring purposes (6). In this context, new techniques that do not require the collection of mushrooms can improve the estimation of mushroom yields. Except for a few cases (7), no correlations have been found between the belowground mycelial biomass of specific species and their mushroom production (8–10), even though positive correlations have been found between root mycorrhizal colonization and soil mycelia (11). In this regard, new monitoring approaches using fungal spores, such as spore trapping, may represent a new way of addressing this challenge. Spore detection could be used to evaluate the fruiting bodies present without the need for regular or repeated samplings over time.

Traditionally, spore trapping has been used to detect and monitor airborne spores, particularly those of fungal pathogens (12). However, recently, spore trap methods have been combined with molecular techniques such as quantitative real-time PCR (qPCR) (13, 14). Spore traps need to be simple, robust devices for effective use in forest stands; they also need to be easy to handle and have low maintenance costs (14, 15). Active traps (e.g., volumetric or cyclonic traps) trap fungal spores more effectively than passive traps (12). However, they have some disadvantages when used under forest conditions; for example, they require electric power. In contrast, passive traps, such as filter or funnel traps, can be placed anywhere (13). Previous studies have shown that passive traps can capture ectomycorrhizal fungal propagules. These passive traps consisted of simple funnels attached to jars (16, 17). However, even simpler spore traps have been used to capture pathogenic fungi, such as filter traps (13). Although these traps collect spores passively, funnel-based traps collect spores in a jar, whereas filter traps retain spores within the filter, which may mean that the spore collection performance of these two traps is different. Despite the potential of these traps to capture fungal spores, to date, no quantitative relationship between fruiting bodies and airborne spores has been established.

Besides the type of trap, the dispersal capacity of the spores also affects spore detection. Unlike highly sporulating fungi, such as puffballs or several mold or yeast species that dominate the airborne community (18), ectomycorrhizal basidiomycetes seem to disperse spores less abundantly (19–21). Problems detecting rare species can be circumvented by using molecular techniques such as qPCR (22), which can detect even small amounts of fungal DNA, although specific primers need to be designed for the target species (13). High-throughput DNA sequencing technologies enable the relative proportions of each of the identified operational taxonomic units (OTUs) to be determined in a given sample (23). Besides monitoring mushroom emergence, this approach can also be used to understand fungal dispersal. To sum up, spore traps combined with qPCR can be used to detect airborne fungal spores of specific species, while spore traps in combination with high-throughput sequencing approaches can be used as a generic tool to detect and identify airborne spores of different taxa.

We hypothesized that (i) funnel and filter traps in combination with qPCR or MiSeq would differ in their capacity to detect spores, and (ii) there is a relationship between fruiting body yields and spore abundance over time (temporal relationship) and over plots (spatial relationship). To test these hypotheses, we identified the relative abun-

TABLE 1 Selected most abundant species^a

Potential species	Ecology ^b	Species hypothesis concept	Species hypothesis	Frequency ^c
<i>Inocybe glabripes</i> Ricken	Ectomycorrhizal	<i>Inocybe glabripes</i>	https://unite.ut.ee/sh/SH174191.07FU	1,620
<i>Lactarius deliciosus</i> (L.) S. F. Gray	Ectomycorrhizal	<i>Lactarius deliciosus</i>	https://unite.ut.ee/sh/SH220107.07FU	227
<i>Lactarius vinosus</i> (Quél.)	Ectomycorrhizal	<i>Lactarius semisanguifluus</i>	https://unite.ut.ee/sh/SH220111.07FU	104
<i>Russula chloroides</i> (Kromb.) Bres.	Ectomycorrhizal	<i>Russula chloroides</i>	https://unite.ut.ee/sh/SH220523.07FU	260
<i>Tricholoma terreum</i> (Sch.) Kumm.	Ectomycorrhizal	<i>Tricholoma gausapatum</i>	https://unite.ut.ee/sh/SH219345.07FU	168
<i>Russula torulosa</i> Bres.	Ectomycorrhizal	<i>Russula torulosa</i>	https://unite.ut.ee/sh/SH186211.07FU	877
<i>Clitocybe phaeophthalma</i> (Pers.) Kuyper	Saprotrophic	<i>Singerocybe umbilicata</i>	https://unite.ut.ee/sh/SH191019.07FU	1,709
<i>Hypholoma fasciculare</i> (Huds.) P. Kumm.	Saprotrophic	<i>Hypholoma fasciculare</i>	https://unite.ut.ee/sh/SH201439.07FU	32
<i>Leucopaxillus gentianeus</i> (Quél.) Kotl.	Saprotrophic	<i>Leucopaxillus gentianeus</i>	https://unite.ut.ee/sh/SH030478.07FU	50
<i>Marasmius androsaceus</i> (L.) Fr.	Saprotrophic	<i>Gymnopus androsaceus</i>	https://unite.ut.ee/sh/SH183805.07FU	1,680
<i>Mycena pura</i> (Pers.) P. Kumm.	Saprotrophic	<i>Mycena diosma</i>	https://unite.ut.ee/sh/SH186350.07FU	79
<i>Lycoperdon perlatum</i> Pers.	Saprotrophic	<i>Lycoperdon perlatum</i>	https://unite.ut.ee/sh/SH175885.07FU	51

^aThese include the species found at least twice in two different plots. Identification was based on comparison with ITS2 reference sequences deposited in UNITE.

^bEcology refers to the functional guild of the species (ectomycorrhizal or saprotrophic).

^cFrequency refers to the order of frequency in terms of the total number of MiSeq reads among the whole propagule community.

dance of the spores by trapping spores during an 8-week period in the fall of 2014 in a *Pinus pinaster* forest in northeastern Spain. In parallel, we also determined the taxonomic identity and the yields of the fruiting body community.

First, we studied *Lactarius vinosus* as a model species to compare the two molecular techniques (qPCR versus MiSeq). We chose this species because of its commercial interest. It belongs to the *Lactarius* group *deliciosus*, which has well-established markets across Europe, Asia, and North Africa (1). Furthermore, this species is highly appreciated both by mushroom pickers and by consumers in Spain (24, 25). The commercialization of *Lactarius* group *deliciosus* has led to the sale of almost 500,000 kg of *L. vinosus* per year at the three most important Spanish markets; the estimated market value is €5.3 million year⁻¹ (25).

Second, we studied the relationship between the spore abundance of a species and the yield of fruiting bodies from temporal (week) and spatial (plot) perspectives. For this, we compared the abundances of the fruiting bodies of the 12 most abundant fungal species (6 ectomycorrhizal and 6 saprotrophic fungal species) found in the fruiting body community.

RESULTS

Fungal species producing fruiting bodies in the studied plots. Among the sampled fruiting bodies, we found 71 fungal species belonging to the ectomycorrhizal or saprotrophic fungal guilds. Among these ectomycorrhizal and saprotrophic species, we identified 37 species that were also present in the spore community. Of those species, 18 species produced fruiting bodies in at least two plots for a 2-week period, and the 12 most abundant species (6 ectomycorrhizal and 6 saprotrophic fungal species) were selected for analysis (Table 1). Thirteen species producing fruiting bodies did not have an internal transcribed spacer (ITS) reference in the UNITE (unified system for the DNA-based fungal species linked to classification) database or in the International Nucleotide Sequence Database (INSD), and, therefore, these species were not searched for within the spore community.

Spore abundance estimated by trapping techniques and molecular methods. MiSeq data revealed that the relative abundance of *L. vinosus* was significantly higher in filter traps than in funnel traps ($F_{[1,15]} = 4.98$; $P < 0.001$) (Table 2), representing on average 5.55 and 0.48 per thousand of the total number of reads, respectively (i.e., nearly 10 times more abundant in filter traps than in funnel traps) (Table 2). However, when using qPCR, the numbers of *L. vinosus* spores trapped by the filter and funnel traps did not seem to be significantly different ($F_{[1,15]} = 0.06$; $P = 0.951$) (Table 2). When using funnel trap data, the spore abundance of *L. vinosus* estimated by qPCR correlated with that estimated by MiSeq ($F_{[1,59]} = 27.53$; $P < 0.001$; $R^2 = 0.3$) (Fig. 1a). However, there was no relationship between the spore abundance of *L. vinosus* detected in the

TABLE 2 Differences in the relative proportions of ectomycorrhizal and saprotrophic species detected by filter traps versus funnel traps^a

Ecology	Species	Mean proportions		Effects				
		Filter trap	Funnel trap	Value	SE	df	t value	P value
Ectomycorrhizal	<i>Russula torulosa</i>	0.354	0.003	−0.262	0.126	15	−2.082	0.055
Ectomycorrhizal	<i>Inocybe glabripes</i>	0.079	0.015	−0.089	0.07	15	−1.267	0.225
Ectomycorrhizal	<i>Lactarius deliciosus</i>	2.408	0.075	−1.09	0.228	15	−4.793	<0.001
Ectomycorrhizal	<i>Lactarius vinosus</i> (MiSeq)	5.551	0.450	−1.531	0.307	15	−4.982	<0.001
	<i>Lactarius vinosus</i> (qPCR)	2.260	2.676	−0.016	0.268	15	−0.062	0.951
Ectomycorrhizal	<i>Russula chloroides</i>	2.031	0.012	−0.898	0.254	15	−3.543	0.003
Ectomycorrhizal	<i>Tricholoma terreum</i>	2.207	0.033	−0.649	0.299	15	−2.17	0.046
Saprotrophic	<i>Lycoperdon perlatum</i>	0.715	0.695	0.068	0.212	15	0.32	0.753
Saprotrophic	<i>Leucopaxillus gentianeus</i>	13.853	2.411	−1.509	0.411	15	−3.67	0.002
Saprotrophic	<i>Clitocybe phaeophthalma</i>	0.022	0.000	−0.057	0.034	15	−1.689	0.112
Saprotrophic	<i>Hypholoma fasciculare</i>	10.706	2.018	−1.06	0.468	15	−2.28	0.038
Saprotrophic	<i>Marasmius androsaceus</i>	0.029	0.015	−0.03	0.049	15	−0.604	0.555
Saprotrophic	<i>Mycena pura</i>	6.853	1.864	−1.107	0.23	15	−4.809	<0.001
Yeast	<i>Cryptococcus</i> sp.	9.830	80.908	4.111	1.088	15	3.78	0.002
Yeast	<i>Rhodotorula baccarum</i>	6.063	32.247	2.241	0.746	15	3.00	0.009
Yeast	<i>Cryptococcus wieringae</i>	3.586	29.628	2.571	0.784	15	3.28	0.005
Yeast	<i>Rhodotorula colostri</i>	0.389	1.348	0.502	0.146	15	3.435	0.004
Mold	<i>Mortierella elongata</i>	0.201	4.509	0.825	0.499	15	1.651	0.119
Mold	<i>Trichoderma</i> sp.	0.498	0.507	0.09	0.154	15	0.587	0.565

^aA few of the most abundant yeast and mold species are also included in the analysis to determine whether the relative proportion of ectomycorrhizal and saprotrophic species detected by funnel traps was smaller than that detected by filter traps owing to the trapping of a larger proportion of yeast or mold species by funnel traps.

filter traps and the abundance detected in the funnel traps ($F_{[1,14]} = 0.92$; $P = 0.352$) (Fig. 1b).

The higher relative abundance of *L. vinosus* spores detected by MiSeq when using filter traps rather than funnel traps was also observed for most of the saprotrophic and ectomycorrhizal fungal species studied ($F_{[1,15]} = 5.71$; $P < 0.001$) (Table 2). When comparing guilds, we found that selected yeast species were better represented in funnel traps than in filter traps ($F_{[1,15]} = 4.70$; $P < 0.001$) (Table 2), but no differences between spore trap types were observed for mold species.

Weekly relationship between fungal spores and the production of fruiting bodies.

The spore community composition varied significantly across weeks ($F_{[7,59]} = 3.72$; $R^2 = 0.27$; $P < 0.001$) and plots ($F_{[7,59]} = 3.45$; $R^2 = 0.25$; $P < 0.001$). The temporal effect (across weeks) was as high as the spatial effect (across plots) and together accounted for more than 50% of the total variation. The abundance of spores of different species found in the traps fluctuated over the sampling period, especially those of ectomycorrhizal fungal species (Fig. 2a and b).

Three saprotrophic (*Marasmius androsaceus*, *Clitocybe phaeophthalma*, and *Hypholoma fasciculare*) and two ectomycorrhizal fungal species (*Russula torulosa* and *Russula chloroides*) showed signs of having highly ephemeral fruiting bodies. Spore production by most of these species peaked 1 or 2 weeks after fruiting body production but then ended abruptly (Fig. 3a and b). Species with at least three spore production peaks during the study period showed a significant relationship between fruiting body yields and their spore abundance, for example, *Mycena pura* ($F_{[1,51]} = 36.97$; $P < 0.001$; $R^2 = 0.96$), *Lactarius deliciosus* ($F_{[1,51]} = 11.56$; $P = 0.001$; $R^2 = 0.68$), *Lactarius vinosus* ($F_{[1,51]} = 57.39$; $P < 0.001$; $R^2 = 0.77$), *Leucopaxillus gentianeus* ($F_{[1,51]} = 12.58$; $P < 0.001$; $R^2 = 0.59$), and *Tricholoma terreum* ($F_{[1,51]} = 20.89$; $P < 0.001$; $R^2 = 0.58$). This relationship was also confirmed for *L. vinosus* using qPCR data ($F_{[1,48]} = 29.24$; $P < 0.001$; $R^2 = 0.79$). *L. vinosus* spore abundance patterns detected using either Illumina MiSeq or qPCR data were very similar, and both techniques showed that the production of *L. vinosus* spores peaked between 20 October and 29 October. This peak was estimated to represent an average of 1.5×10^6 *L. vinosus* spores · trap^{−1} (Fig. 4).

In contrast to the species already mentioned, *Lycoperdon perlatum* ($F_{[1,51]} = 0.01$; $P = 0.917$) and *Inocybe glabripes* ($F_{[1,51]} = 0.89$; $P = 0.349$) did not display a significant

FIG 1 Relationship between the *L. vinosus* abundance measurements determined by qPCR (y axis) and by MiSeq data (x axis) (a) and relationship between the *L. vinosus* abundance measurements obtained at the funnel traps (y axis) and obtained at the filter traps (x axis) (b).

relationship between fruiting body yield and spore abundance. For example, the spore abundance of *L. perlatum* increased with time and did not correspond to variable fruiting body production (Fig. 3a), and the spore abundance of *I. glabripes* peaked at the start of the season, which was not reflected at the fruiting body level (Fig. 3b).

Relationship between spores and fruiting body production at plot level. At plot level, only spores of saprotrophic fungal species correlated with fruiting body production ($F_{[1,48]} = 4.11$; $P = 0.048$) (Fig. 5a). These significant differences were mainly attributed to the effect of one plot, in which large quantities of both spores and fruiting bodies were recorded. Nevertheless, no relationship was observed between spores of ectomycorrhizal fungi and their fruiting body yields ($F_{[1,48]} = 0.41$; $P = 0.527$) (Fig. 5b). Only five species were found in more than two plots: *Inocybe glabripes*, *Lactarius deliciosus*, *Lactarius vinosus*, *Lycoperdon perlatum*, and *Mycena pura*. None of these species showed a significant relationship at plot level between spores and their fruiting bodies ($P > 0.05$). Thus, in most cases, fruiting bodies were found in only a few plots, but their spores were collected in a greater number of plots (e.g., *Mycena pura*) (Fig. 5c). In other cases, the plots with high levels of fruiting body production also had the highest spore abundance, but no quantitative relationships were found (e.g., *Lactarius vinosus*) (Fig. 5d). Among the saprotrophic fungal species, there was a clear altitudinal pattern ($F_{[1,6]} = 2.48$; $P = 0.047$) (Fig. 6), with both fruiting bodies and spores decreasing toward higher altitudes, whereas no clear altitudinal pattern was observed among ectomycorrhizal fungal spores ($F_{[1,6]} = -0.28$; $P = 0.785$) (Fig. 6).

DISCUSSION

In this study, we showed that spore trapping coupled with qPCR or MiSeq can detect the emergence of epigeous mushrooms. This study demonstrates that qPCR and MiSeq can be used to detect fungal emergence. In general, a 1-week delay between mushroom emergence and airborne propagule detection was found using spore traps. This

FIG 2 Temporal fluctuation of mushroom yields (red line) produced by ectomycorrhizal (EcM) species (a) and saprotrophic species (b), the relative abundance of their spores (black line), and the total precipitation recorded each week (blue line). The sampling day and month are shown along the x axis. During the first 2 weeks, there were no precipitation events, and only low levels of precipitation were seen over the following weeks. However, during the first week of December, an extreme precipitation event was recorded (almost 180 mm).

delay was most likely caused by a combination of (i) the time needed for mushrooms to mature and produce spores, and (ii) the time needed for spore ejection and deposition in the spore traps. However, an alternative explanation for the observed delay may be that our fruiting body sampling scheme favors the sampling of immature fruiting bodies before they initiate sporulation. It is generally accepted that spore deposition is driven by meteorological conditions. In theory, the initial phase of spore dispersal of some basidiomycete mushroom-forming species involves the discharge of basidiospores from the gill by a mechanism promoted by a droplet, also known as a Buller's drop (26), which is normally stimulated by the secretion of mannitol and other sugars (27). Then, convective airflows promoted by the pileus enhance the vertical movement of these spores, which may then reach dispersive winds (28). Finally, these spores may fall by gravity or rainfall events. However, in our study, we observed that spore deposition occurred regardless of rainfall events, indicating that spore deposition is not necessarily driven by meteorological conditions such as precipitation events (29). Future studies should focus on gaining a better understanding of the potential delay in spore detection and the factors driving spore deposition, such as the effect of winds on the movement of these spores.

In this study, we observed an interspecific short temporal variation in the abundance of spores, especially those produced by ectomycorrhizal fungal species. This finding agrees with the literature because spores can remain airborne for between a day and up to a few weeks, depending on their size and aerodynamic properties (30). The temporal variation in spore abundance has been reported previously (16, 20), and

FIG 3 (a) Temporal changes in the number of fruiting bodies produced by saprotrophic species and their spores across the sampled weeks in all of the plots considered in this study. The solid black line represents mushroom production (number of fruiting bodies $\cdot$ plot $^{-1}$), and the broken black line indicates the relative spore abundance (%). (b) Temporal changes in the number of fruiting bodies produced by ectomycorrhizal species and their spores across the sampled weeks in all of the plots considered in this study. The solid black line represents mushroom production (number of fruiting bodies $\cdot$ plot $^{-1}$), and the broken black line indicates the relative spore abundance (%).

FIG 4 Relationship between mushroom biomass (red line) and the relative abundance of *L. vinosus* spores detected by the spore traps, quantified by qPCR (black line) and by determining the relative proportions of *L. vinosus* using MiSeq (blue line). Average values are shown with their standard error. The sampling day and month are shown along the x axis. The peak in mushroom production on 20 October was detected the following week (29 October) using both qPCR and MiSeq. The slight increase in mushroom production on 20 November was only detected by MiSeq.

this variation has been attributed to different abiotic parameters, such as wind direction, rainfall, and species-specific life history traits (29, 31, 32). Based on our results, spore detection was related to mushroom production peaks; thus, it seems likely that seasonal differences in the composition of the airborne fungal community can also be partly driven by mushroom phenology (20, 33).

Despite the observed relationship between mushroom yields and spore abundance across the sampling weeks, there was no relationship at the spatial scale where mushroom production was assessed, i.e., plots of 10 by 10 m. Spores were captured in plots without fruiting bodies of the corresponding species, indicating that these spores were produced outside the sampled plots. The spores of ectomycorrhizal fungal species are usually dispersed approximately 10 to 1,000 m (16, 19, 21), depending on the species (17). The large amount of spores produced per fruiting body, e.g., 1.1×10^8 to 1×10^{10} spores (34, 35), makes it possible for spores of these species to be dispersed long distances, particularly if carried away by turbulences. The fine mapping of spore captures in future studies could be used to indirectly estimate dispersal curves for several species and, therefore, understand better how these fungal species disperse.

MiSeq analysis detected significantly greater relative proportions of our 12 selected fungal species belonging to the saprotrophic or ectomycorrhizal guilds in filter traps than in funnel traps. Funnel traps trapped a greater number of species from other fungal guilds, such as yeasts, than the filter traps, which translated into a relative decrease in the proportion of saprotrophic and ectomycorrhizal MiSeq reads. Differences between samples can arise because of the way in which samples were handled. Spores captured in the funnel traps were first filtered *in situ* and subsequently collected inside a jar with water (17), which may favor the growth of some opportunistic fungi such as yeasts. Both fungus-specific amplicon sequencing and species-specific quantitative real-time PCR successfully detected mushroom production peaks. Both methods revealed an almost identical pattern of spore abundance across the weeks for *L. vinosus*. The use of MiSeq avoids the need to design specific primers for each target species. However, the use of high-throughput sequencing data as a semiquantitative measure has been the subject of discussion, mostly because of the variation in ribosomal gene copy numbers among different fungal species (36), the read length biases caused by current high-throughput sequencing platforms (37), the DNA extraction efficiency (38), or biases due to interspecific primer binding differences. Despite these limitations, read abundance may still be used as a semiquantitative measure of abundance (relative proportions) (23).

FIG 5 Spatial relationships between fruiting body yields (number of fruiting bodies) and the relative abundance of spores evaluated by MiSeq for saprotrophic species (a), ectomycorrhizal species (b), *Mycena pura* (c), and *Lactarius vinosus* (d). Blue or green dots represent each of the 8 plots considered in this study. Reddish to yellowish colors in spore abundance represent a gradient from lower to higher spore abundance, respectively, whereas blueish to orange colors represents a gradient from lower to higher fruiting body abundance, respectively. Coordinates X (easting) and Y (northing) are represented in ED50 data.

We showed that a peak in ephemeral fruiting bodies was followed by a peak in the numbers of spores that were trapped. For most of the species, the spore peak mostly disappeared 2 weeks after mushroom emergence reached its highest level. Nevertheless, a quantitative relationship between fruiting body yields and their fungal spores

FIG 6 Relationship between fruiting body yields (solid line) and their propagules (broken line) for ectomycorrhizal (a) and saprotrophic (b) species along an elevation gradient represented by all of the sampled plots considered in this study.

TABLE 3 Main sampling plot characteristics^a

Plot	Altitude (meters above sea level)	East to West x^b	North to South y^b	Mean <i>L. vinosus</i> mushroom yield (kg · ha ⁻¹)
301c	1,010	4578428	336601	0.67
303c	903	4579373	335531	0.64
305c	744	4580525	335839	140.47
307c	796	4580200	335673	115.57
309c	852	4579709	335445	53.09
312c	633	4580848	333675	30.77
313c	609	4580755	336541	1.72
316c	644	4580113	336211	5.59

^aThis includes altitude, location, and mean *Lactarius vinosus* mushroom biomass for the period of 2008 to 2014.

^bCoordinates X (represented as East to West x) and Y (represented as North to South y) are expressed as European Datum 1950 (ED50) data.

was not observed for *Inocybe glabripes* and *Lycoperdon perlatum*. Although the spore abundance and fruiting body yields observed for *I. glabripes* showed a similar pattern, the lack of relationship between these two measures was most likely caused by a peak in spore abundance that was not reflected at the fruiting body level. Interestingly, the spore abundance pattern for *L. perlatum* suggests a progressive and slow spore deposition over time; the lack of relationship between spore abundance and time may be because these spores are rarely falling unless a rainfall event occurs, such as the event that occurred during the week of 4 December, which most likely washed away all of the spores.

Some species produced fruiting bodies, but their spores were not found among the spore community. Possible explanations for this include the following: (i) these species had a very short range of dispersal, or (ii) their sporulation was very small compared with the rest of the species. Alternatively, these species may have a very long ITS2 sequence and, thus, be underrepresented using MiSeq sequencing. We investigated whether this was a plausible explanation by studying the ITS2 region length of these species using ITS sequences from UNITE, specifically if the region theoretically amplified by the primers was longer and if the DNA sequence where each primers binds was different for the fungal species not detected at the spore traps. However, the lengths of the ITS2 regions of these species were similar to those of other species that were successfully captured by the spore traps. Primer sequence mismatch was also discarded as an alternative explanation because these species did not show any sequence mismatch where the primer binds. Thus, discarding these last possibilities, and given that fruiting bodies were collected <10 m from spore traps, we hypothesize that low sporulation may be the reason for the lack of detection of these species among the spore community.

Filter traps are more suitable for high-throughput sequencing approaches targeting saprotrophic or ectomycorrhizal fungal species. Our method represents a potential improvement on mushroom sampling approaches, as current sampling schemes are highly time-consuming and require expert knowledge to identify the fungal taxa (5). Here, we showed that mushroom production and fungal diversity of fungi with epigeous fruiting body structures can be monitored accurately by spore traps. Thus, spore trapping could represent a less time-consuming alternative for monitoring mushrooms, which is fundamental for effective management planning for these important nonwood forest products.

MATERIALS AND METHODS

Study area. The study was carried out at a long-term experimental setup located in the natural area of Poblet (northeastern Spain, 41°21'6.4728"N latitude and 1°2'25.7496"E longitude), which is characterized by a Mediterranean climate with an average annual temperature of 11.8°C and annual rainfall of 665.5 mm. The long-term experimental site comprises 28 fenced plots, of which we selected 8 plots (10 by 10 m), where fruiting bodies have been continuously monitored every fall since 2008 (39). Even-aged

(60 years old) *Pinus pinaster* and *Quercus ilex* are the codominant species growing in the plots. Additional information about the study area and the plots can be found in Table 3.

Fruiting body sampling. All plots were sampled for mushroom production weekly during the fall (October to December 2014) following previously described methods (5). In short, all of the fruiting bodies within the plot and with a minimum cap size of 1 cm were sampled on Thursdays to minimize errors resulting from recreational weekend collectors picking mushrooms. The plots were chosen according to the productivity gradient for *Lactarius vinosus* (from the highest to the lowest production), which was based on continuous production data that have been recorded since 2008 (39). All of the fruiting bodies from all of the species found within the plots were brought to the laboratory on the same day, identified, cleaned, and counted. Fruiting bodies were dried in an air-vented oven at 35°C to 40°C and weighed to the nearest 0.01 g. Data in this study were expressed as the number of fruiting bodies (n) per plot in kilograms · hectare⁻¹ to show the temporal variation of *L. vinosus* fruiting bodies over time.

Spore trapping. Two types of spore traps were installed: filter traps (13) and funnel traps (16). The two traps were placed 1 m apart in the center of each plot and captured spores 30 cm above ground level.

Funnel traps consisted of 15-cm-diameter funnels attached to 1-liter dark jars, with a 50-μm-pore-size nylon mesh fixed at the bottom of the funnel. These traps were erected 1 week after the first mushroom of *L. vinosus* was observed (14 October 2014) and remained in place until 11 December 2014, when no *L. vinosus* fruiting bodies were observed. Each week, the funnels were rinsed with ultrapure water (Milli-Q) to collect any spores, and then the jars were immediately removed and replaced with sterile jars. Liquids from the traps were filtered using sterile filter papers (90-mm diameter; Whatman no. 1) within 48 h of collection. Sample filtering was conducted in a flow chamber to prevent potential contamination. Filters were stored at -20°C until further analysis.

Filter traps consisted of a filter paper (Whatman no. 1; 90-mm-diameter filter paper) placed over a metal mesh, supported by a metal clamp, and attached to a vertical support 30 cm above ground level. The filter papers in the filter traps were sampled simultaneously to the funnel traps during the two most productive weeks when *L. vinosus* fruiting body production was greatest (from 14 October until 29 October 2014) and stored at -20°C after sampling.

Spore trap sample processing and DNA extraction. Filters containing spores from the filter and the funnel traps were cut in half and placed in separate 50-ml Falcon tubes. A solution of 20 ml of sodium dodecyl sulfate (SDS buffer) was added to each tube before incubating at 65°C for 90 min. The tubes were vortexed three times before removing the filter from each tube. Twenty milliliters of 2-propanol was added to the resulting solution and then left overnight at room temperature. After centrifugation (700 × g for 10 min), the supernatant was carefully removed and 700 μl of SL2 lysis buffer (NucleoSpin NSP soil DNA extraction kit; Macherey-Nagel, Duren, Germany) was added. The resultant solution was vortexed 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 × g for 30 s (twice) following the instructions provided by the manufacturer. The DNA obtained was eluted in a 50-μl elution buffer.

Quantification of *Lactarius vinosus* using real-time PCR. For *Lactarius vinosus* spore quantification, we used a species-specific hydrolysis probe (40). In order to obtain comparable results between different plates, we prepared standard curves using known amounts of *L. vinosus* DNA, which was extracted with the NucleoSpin NSP soil kit (Macherey-Nagel, Duren, Germany). Threshold cycle (C_T) values were converted to the number of *L. vinosus* spores in each reaction using serial DNA dilutions of known amounts of spores, starting with 15.10×10^6 spores and ending with 151.2 spores. Results are expressed as the number of spores · trap sample⁻¹.

Real-time PCRs were prepared using 2× Premix Ex Taq (TaKaRa Bio Europe SAS, France) following the manufacturer's recommendations. The reaction mix was prepared as follows: 5 μl of DNA template, 400 nM each oligonucleotide, 200 nM TaqMan probe, 0.8 μl of ROX (rhodamine X), and a volume of water (high-pressure liquid chromatography [HPLC]) to adjust the final reaction volume to 20 μl. The cycling conditions in the StepOnePlus instrument (Applied Biosystems) were as follows: 30 s at 95°C followed by 40 cycles at 95°C for 5 s and at 60°C for 34 s. There were three replicates of each sample and the standards were included in the analysis, as well as a negative control using HPLC water instead of the template. Only amplification efficiencies ranging from 95% to 105% were accepted and considered for analysis. qPCRs, processing of data, and quantification of *L. vinosus* DNA were performed following the methods described in a previous study (11).

Spore trap sample sequencing using Illumina MiSeq. Each spore trap sample was PCR amplified using the primers fITS7 and ITS4 (41), which amplify the ITS2 region of the ribosomal DNA (rDNA). Both primers were tagged using unique DNA sequences composed of eight bases. A PCR cycle test was performed prior to randomly selected samples in order to perform the minimum number of PCR cycles possible. 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 volumes. The final concentrations in the PCR mixture were as follows: 25 ng of template, 200 μM each nucleotide, 2.75 mM MgCl₂, 200 nM each primer, and 0.025 U μl⁻¹ polymerase (DreamTaq Green; Thermo Scientific, Waltham, MA) in 1× buffer PCR. The cycling conditions for PCR were as follows: 5 min at 95°C, followed by 24 to 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. Each sample was amplified in triplicate, purified using an AMPure kit (Beckman Coulter Inc., Brea, CA), and quantified using a Qubit fluorometer (Life Technologies, Carlsbad, CA). Equal amounts of DNA from each sample were pooled before sequencing. The final equimolar mix was purified using an EZNA Cycle Pure kit (Omega Bio-tek). 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.

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 data with a minimum allowed 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. We used “usearch” 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 a value of 1, a gap open penalty of 0, and a gap extension penalty with a value of 1. Sequences were clustered in OTUs with single linkage clustering, using 1.5% as a threshold distance with the closest neighbor. After quality control and clustering, all tags were identified and tag jumps were removed from the database (42). A tag jump has been defined as the generation of artifactual sequences in which amplicons carry tags different from those originally applied.

Identification of the fungal clusters. From a total of 13,408,476 MiSeq DNA sequences, 1,992,529 passed the quality control. Of these, 95,104 (4.77%) sequences were discarded because they had two different tags. Finally, 1,897,425 reads were obtained, with an average of 19,361 reads per sample.

We searched among the most abundant 2,400 OTUs (representing OTUs with more than 20 read counts) for those potential fungal species representing the fruiting body community. We first identified the OTUs using the *seriateBLAST* option in the UNITE database (43). We included DNA sequences from both UNITE and INSD, but preference was given to the UNITE reference for identification. In case we could not find any given species, we verified that each species had a known reference ITS sequence by checking UNITE and INSD in order to differentiate those species not found among the spore community from those without a known ITS reference sequence. Taxonomic assignment of the OTUs was given using a 98.5% sequence similarity threshold, and all of the species found at or above this threshold were included in each OTU. Read count data were transformed to relative proportions, and data are shown as counts per thousand of the total read numbers per sample.

Statistics. All statistical analyses were implemented in the R software environment (version 2.15.3; R Development Core Team 2013) using the “nlme” package for linear mixed models (LME [44]), the *vegan* package (45) for the multivariate analysis, and the “gstat” (46) and “maps” (47) packages for spatial kriging.

Comparison of spore traps and of the two molecular methods used to quantify the fungal spores. LME models were used to test differences between spore traps (filter spore traps and funnel traps) using both qPCR and MiSeq data for *L. vinosus* and only MiSeq data for the other species. In these models, the temporal dependency was considered by defining plots nested with “week” as a random factor, whereas the square root spore abundance of 12 species was defined as a fixed factor. In these analyses, we included the 12 species considered in this study (6 ectomycorrhizal and 6 saprotrophic fungal species) in separate models and randomly selected yeast (i.e., *Cryptococcus* spp. and *Rhodotorula* spp.) and mold species (*Mortierella elongata* and *Trichoderma* sp.) to test whether the hypothetical effect of the spore trap type was due to the increase of some of these fungal guilds.

Temporal relationship between fungal spores and mushroom yields. Temporal (week) and spatial (plot) effects on community data were tested using permutational multivariate analysis of variance (PERMANOVA) based on distance matrices (using the function *adonis* and Bray-Curtis distances). Plot and week were included as factors, and community data were previously Hellinger transformed. For this analysis, the fruiting body community data represented species that produced fruiting bodies in at least two plots for at least 2 weeks.

We analyzed the temporal (week) linear relations between spore abundance and the mushroom yields using LME models, using square root transformed qPCR and MiSeq data. To test for lagged relationships, different LME models were tested, with and without accounting for 1-week temporal autocorrelation among observations (*corAR1*[*form*~week]). The best model fit was selected using the Akaike information criterion (AIC) value. We confirmed this lagged relationship with LME models using data in which spores were 1 week manually lagged. Only species that produced fruiting bodies for at least 2 weeks were considered for analysis. As mushroom fresh weight, dry weight, and the number of mushrooms ha⁻¹ were highly correlated ($P < 0.001$; $R^2 = 0.9$), we only used fruiting body counts for analysis. Data were modeled with plots defined as random to deal with the intraplot variation and either qPCR data (*L. vinosus*) or MiSeq data (for each species) were used as explanatory variables.

Relationship between fungal spores and mushroom yields at plot level. The distribution of fungal spores and mushroom counts at the spatial scale was obtained by ordinary kriging interpolating each value using the inverse distance weighting (IDW) function. Interpolated values were graphically represented using Universal Transverse Mercator (UTM) coordinates (European Datum 1950 [ED50]). The relationship between spores and fruiting bodies over the plots was tested with LME models, using only species found in more than two plots. In this case, data were modeled with week defined as random to deal with the temporal variation.

Accession number(s). Raw sequence reads have been deposited in the NCBI Sequence Read Archive (SRA) under accession number [PRJNA352156](https://www.ncbi.nlm.nih.gov/sra/PRJNA352156).

ACKNOWLEDGMENTS

We are very grateful to the PNIN of Poblet for supporting the process of installing and maintaining the experimental plots.

We also thank Katarina Ihrmark and Johanna Boberg for providing the protocol and for advice on how to process and analyze the spore trap samples. We thank Josep Miró and Francesc Bolaño for their assistance with collecting the spore trap and fruiting body samples. We thank Caroline Woods for linguistic revision and for helping to clarify the manuscript.

This work was supported by an STSM grant from COST Action FP1203 and by the Spanish Ministry of Economy and Competitiveness (MINECO) through the projects MICOGEST AGL2012-40035-C03 and MYCOSYSTEMS AGL2015-66001-C3. Carles Castaño received the support of the Secretaria d'Universitats i Recerca del Departament d'Economia i Coneixement de la Generalitat de Catalunya through the program of Doctorats Industrials. José Antonio Bonet is supported by the Serra-Hunter fellowship, and Josu G. Alday is supported by the Juan de la Cierva fellowship (IJC-2014-21393).

The funders had no role in the study design, data collection, and interpretation, or in the decision to submit the work for publication.

REFERENCES

- Boa E. 2004. Wild edible fungi: a global overview of their use and importance to people. Food and Agriculture Organization of the United Nations, Rome, Italy.
- Peintner U, Schwarz S, Mešić A, Moreau PA, Moreno G, Saviuc P. 2013. Mycophilic or mycophobic? Legislation and guidelines on wild mushroom commerce reveal different consumption behaviour in European countries. *PLoS One* 8(5):e63926.
- De Roman M, Boa E. 2004. Collection, marketing and cultivation of edible fungi in Spain. *Micol Apl Int* 16:25–33.
- Palahí M, Pukkala T, Bonet JA, Colinas C, Fischer CR, Martínez de Aragón J. 2009. Effect of inclusion of mushroom values on the optimal management of even-aged pine stands of Catalonia. *Forest Sci* 55:503–511.
- Martínez de Aragón J, Bonet JA, Fischer CR, Colinas C. 2007. Productivity of ectomycorrhizal and selected edible saprotrophic fungi in pine forests of the pre-Pyrenees mountains, Spain: predictive equations for forest management of mycological resources. *For Ecol Manage* 252:239–256. <https://doi.org/10.1016/j.foreco.2007.06.040>.
- Vogt KA, Blomfield J, Ammirati JF, Ammirati SR. 1992. Sporocarp production by basidiomycetes with emphasis on forest ecosystems, p 563–581. In Dighton J, White JF, Oudemans P, Carroll GC, Wicklow DT, (ed), *The fungal community, its organization and role in the ecosystem*, 2nd ed. Taylor & Francis, Boca Raton, FL.
- Liu B, Bonet JA, Fischer CR, Martínez de Aragón J, Bassie L, Colinas C. 2016. *Lactarius deliciosus* Fr. soil extraradical mycelium correlates with stand fruitbody productivity and is increased by forest thinning. *For Ecol Manage* 380:196–201. <https://doi.org/10.1016/j.foreco.2016.08.053>.
- van der Linde S, Alexander IJ, Anderson IC. 2009. Spatial distribution of sporocarps of stipitate hydroid fungi and their belowground mycelium. *FEMS Microbiol Ecol* 69:344–352. <https://doi.org/10.1111/j.1574-6941.2009.00716.x>.
- De la Varga H, Agueda B, Martínez-Peña F, Parladé J, Pera J. 2012. Quantification of extraradical soil mycelium and ectomycorrhizas of *Boletus edulis* in a Scots pine forest with variable sporocarp productivity. *Mycorrhiza* 22:59–68. <https://doi.org/10.1007/s00572-011-0382-2>.
- De la Varga H, Águeda B, Ágreda T, Martínez-Peña F, Parladé J, Pera J. 2013. Seasonal dynamics of *Boletus edulis* and *Lactarius deliciosus* extraradical mycelium in pine forests of central Spain. *Mycorrhiza* 23: 391–402. <https://doi.org/10.1007/s00572-013-0481-3>.
- Parladé J, Hortal S, Pera J, Galipienso L. 2007. Quantitative detection of *Lactarius deliciosus* extraradical soil mycelium by real-time PCR and its application in the study of fungal persistence and interspecific competition. *J Biotechnol* 128:14–23. <https://doi.org/10.1016/j.jbiotec.2006.09.010>.
- Jackson SL, Bayliss KL. 2011. Spore traps need improvement to fulfil plant biosecurity requirements. *Plant Pathol* 60:801–810. <https://doi.org/10.1111/j.1365-3059.2011.02445.x>.
- Schweigkofler W, O'Donnell K, Garbelotto M. 2004. Detection and quantification of airborne conidia of *Fusarium circinatum*, the causal agent of pine pitch canker, from two California sites by using a real-time PCR approach combined with a simple spore trapping method. *Appl Environ Microbiol* 70:3512–3520. <https://doi.org/10.1128/AEM.70.6.3512-3520.2004>.
- Chandelier A, Helson M, Dvorak M, Gischer F. 2014. Detection and quantification of airborne inoculum of *Hymenoscyphus pseudoalbidus* using real-time PCR assays. *Plant Pathol* 63:1296–1305. <https://doi.org/10.1111/ppa.12218>.
- Savage D, Barbetti MJ, MacLeod WJ, Salam MU, Renton M. 2012. Mobile traps are better than stationary traps for surveillance of airborne fungal spores. *Crop Prot* 36:23–30. <https://doi.org/10.1016/j.cropro.2012.01.015>.
- Peay KG, Bruns TD. 2014. Spore dispersal of basidiomycete fungi at the landscape scale is driven by stochastic and deterministic processes and generates variability in plant-fungal interactions. *New Phytol* 204: 180–191. <https://doi.org/10.1111/nph.12906>.
- Peay KG, Schubert MG, Nguyen NH, Bruns TD. 2012. Measuring ectomycorrhizal fungal dispersal: macroecological patterns driven by microscopic propagules. *Mol Ecol* 21:4122–4136. <https://doi.org/10.1111/j.1365-294X.2012.05666.x>.
- Adams RI, Miletto M, Taylor JW, Bruns TD. 2013. Dispersal in microbes: fungi in indoor air are dominated by outdoor air and show dispersal limitation at short distances. *ISME J* 7:1262–1273. <https://doi.org/10.1038/ismej.2013.28>.
- Galante TE, Horton TE, Swaney DP. 2011. 95% of basidiospores fall within 1 m of the cap: a field- and modeling-based study. *Mycologia* 103: 1175–1183. <https://doi.org/10.3852/10-388>.
- Kivlin SN, Winston GC, Goulden ML, Treseder KK. 2014. Environmental filtering affects soil fungal community composition more than dispersal limitation at regional scales. *Fungal Ecol* 12:14–25. <https://doi.org/10.1016/j.funeco.2014.04.004>.
- Li D-W. 2005. Release and dispersal of basidiospores from *Amanita muscaria* var. *alba* and their infiltration into a residence. *Mycol Res* 109:1235–1242. <https://doi.org/10.1017/S0953756205003953>.
- West JS, Atkins SD, Emberlin J, Fitt BDL. 2008. PCR to predict risk of airborne disease. *Trends Microbiol* 16:380–387. <https://doi.org/10.1016/j.tim.2008.05.004>.
- Amend AS, Seifert KA, Bruns TD. 2010. Quantifying microbial communities with 454 pyrosequencing: does read abundance count? *Mol Ecol* 19:5555–5565. <https://doi.org/10.1111/j.1365-294X.2010.04898.x>.
- Górriz-Mifsud E, Domínguez-Torres G, Prokofieva I. 2015. Understanding forest owners' preferences for policy interventions addressing mushroom picking in Catalonia (north-east Spain). *Eur J For Res* 134:585–598. <https://doi.org/10.1007/s10342-015-0874-2>.
- Voces R, Diaz-Balteiro L, Alfranca Ó. 2012. Demand for wild edible mushrooms. The case of *Lactarius deliciosus* in Barcelona (Spain). *J For Econ* 18:47–60.
- Stolze-Rybczynski JL, Cui Y, Stevens MHH, Davis DJ, Fischer MWF, Money NP. 2009. Adaptation of the spore discharge mechanism in the Basidiomycota. *PLoS One* 4(1):e4163. <https://doi.org/10.1371/journal.pone.0004163>.
- Webster J, Davey RA, Smirnoff N, Fricke W, Hinde P, Tomos D, Turner JCR.

1995. Mannitol and hexoses are components of Buller's drop. *Mycol Res* 99:833–838. [https://doi.org/10.1016/S0953-7562\(09\)80737-5](https://doi.org/10.1016/S0953-7562(09)80737-5).
28. Dressaire E, Yamada L, Song B, Roper M. 2016. Mushrooms use convectively created airflows to disperse their spores. *Proc Natl Acad Sci U S A* 113:2833–2838. <https://doi.org/10.1073/pnas.1509612113>.
29. Oliveira M, Ribeiro H, Delgado JL, Abreu I. 2009. The effects of meteorological factors on airborne fungal spore concentration in two areas differing in urbanisation level. *Int J Biometeorol* 53:61–73. <https://doi.org/10.1007/s00484-008-0191-2>.
30. Després VR, Huffman JA, Burrows SM, Hoose C, Safatov AS, Buryak G, Fröhlich-Nowoisky J, Elbert W, Andreae MO, Pöschl U, Jaenicke R. 2012. Primary biological aerosol particles in the atmosphere: a review. *Tellus B* 64:15598. <https://doi.org/10.3402/tellusb.v64i0.15598>.
31. Burch M, Levetin E. 2002. Effects of meteorological conditions on spore plumes. *Int J Biometeorol* 46:107–117. <https://doi.org/10.1007/s00484-002-0127-1>.
32. di Giorgio C, Krempff A, Guiraud H, Binder P, Tiret C, Dumenil G. 1996. Atmospheric pollution by airborne microorganisms in the city of Marseilles. *Atmos Environ* 30:155–160. [https://doi.org/10.1016/1352-2310\(95\)00143-M](https://doi.org/10.1016/1352-2310(95)00143-M).
33. Levetin E. 1990. Studies on airborne basidiospores. *Aerobiologia* 6:177–180. <https://doi.org/10.1007/BF02539111>.
34. Dahlberg A, Stenlid J. 1994. Size, distribution and biomass of genets in populations of *Suillus bovinus* (L.: Fr.) Roussel revealed by somatic incompatibility. *New Phytol* 128:225–234. <https://doi.org/10.1111/j.1469-8137.1994.tb04006.x>.
35. Kadowaki K, Leschen RA, Beggs JR. 2010. Periodicity of spore release from individual *Ganoderma* fruiting bodies in a natural forest. *Australas Mycol* 29:17–23.
36. Baldrian P, Větrovský T, Cajthaml T, Dobiášová P, Petránková M, Šnajdr J, Eichlerová I. 2013. Estimation of fungal biomass in forest litter and soil. *Fungal Ecol* 6:1–11. <https://doi.org/10.1016/j.funeco.2012.10.002>.
37. Wommack KE, Bhavsar J, Ravel J. 2008. Metagenomics: read length matters. *Appl Environ Microbiol* 74:1453–1463. <https://doi.org/10.1128/AEM.02181-07>.
38. Feinstein LM, Sul WJ, Blackwood CB. 2009. Assessment of bias associated with incomplete extraction of microbial DNA from soil. *Appl Environ Microbiol* 75:5428–5433. <https://doi.org/10.1128/AEM.00120-09>.
39. Bonet JA, De Miguel S, Martínez de Aragón J, Pukkala T, Palahí M. 2012. Immediate effect of thinning on the yield of *Lactarius* group *deliciosus* in *Pinus pinaster* forests in Northeastern Spain. *For Ecol Manage* 265: 211–217. <https://doi.org/10.1016/j.foreco.2011.10.039>.
40. Castaño C, Parladé J, Pera J, Martínez de Aragón J, Alday JG, Bonet JA. 2016. Soil drying procedure affects the DNA quantification of *Lactarius vinosus* but does not change the fungal community composition. *Mycorrhiza* 26:799–808. <https://doi.org/10.1007/s00572-016-0714-3>.
41. Ihrmark K, Bödeker ITM, Cruz-Martinez K, Friberg H, Kubartova A, Schenck J, Strid Y, Stenlid J, Brandström-Durling M, Clemmensen KE, Lindahl BD. 2012. New primers to amplify the fungal ITS2 region—evaluation by 454-sequencing of artificial and natural communities. *FEMS Microbiol Ecol* 82:666–677. <https://doi.org/10.1111/j.1574-6941.2012.01437.x>.
42. Schnell IB, Bohmann K, Gilbert MTP. 2015. Tag jumps illuminated—reducing sequence-to-sample misidentifications in metabarcoding studies. *Mol Ecol Resour* 15:1289–1303. <https://doi.org/10.1111/1755-0998.12402>.
43. Kõljalg U, Larsson K-H, Abarenkov K, Nilsson RH, Alexander IJ, Eberhardt U, Erland S, Høiland K, Kjoller R, Larsson E, Pennanen T, Sen R, Taylor AFS, Tedersoo L, Vrålstad T, Ursing BM. 2005. UNITE: a database providing web-based methods for the molecular identification of ectomycorrhizal fungi. *New Phytol* 166:1063–1068. <https://doi.org/10.1111/j.1469-8137.2005.01376.x>.
44. Pinheiro J, Bates D, DebRoy S, Sarkar D, R Core Team. 2016. nlme: linear and nonlinear mixed effects models. R package version 3.1-128. The R Foundation, Vienna, Austria.
45. Oksanen J, Blanchet FG, Kindt R, Legendre P, Minchin PR, O'Hara RB, Simpson GL, Solymos P, Stevens MHM, Wagner H. 2015. VEGAN v2.1-10: community ecology package v 2.2-1. The R Foundation, Vienna, Austria.
46. Pebesma EJ. 2004. Multivariable geostatistics in S: the gstat package. *Comput Geosci* 30:683–691. <https://doi.org/10.1016/j.cageo.2004.03.012>.
47. Becker R, Wilks A, Brownrigg R. 2016. Package 'maps'. The R Foundation, Vienna, Austria.