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Summary

- Trees, as foundation species, play a pivotal role in the species interaction networks that constitute forest ecosystems. From the seed stage, they interact with microbial communities that affect their growth, health and fitness. Despite their eco-evolutionary importance, the processes shaping seed microbial communities in natural forests have received little attention.

- To unravel these processes, we analyzed the microbial communities of seeds collected in populations of sessile oak (*Quercus petraea*) growing along elevation gradients. We focused on the fungal communities as this group includes seed pathogens. Ecological processes shaping the communities were quantified using joint species distribution models.

- Fungi were present in all seed tissues, including the embryo. Fungal communities differed significantly among oak populations along the elevation gradients, and among mother trees within the same population. These maternal effects remained significant after seed fall, despite colonization by fungal species on the ground. Associations between tree pathogens and their antagonists were detected in the seeds.

- Our results demonstrate that both maternal effects and environmental filtering shape seed microbial communities of sessile oak. They provide a starting point for future research aimed at identifying the seed extended phenotypic traits that influence seed dispersal and germination, and seedling survival and growth across environments.

Keywords

Microbial ecology; disease ecology; community genetics; ecological networks; joint species distribution models; endophyte; host-parasite interaction; vertical transmission

Introduction

Seeds are colonized by a wide range of microorganisms that positively and negatively influence plant growth, health and fitness. They associate with many endophytic and epiphytic microorganisms that foster the growth of seedlings and protect them against natural enemies (Baker & Smith, 1966; Nelson, 2004; Links, *et al.* 2014). For example, the seed-borne endophytic fungus *Epichloë festucae* limits pest attacks and pathogen development by producing alkaloids (Clay & Schardl, 2002; Faeth, 2002). Seeds are also colonized by pathogens that reduce germination rate (Nelson, 2017) and seedling survival (Kremer, 1987; Gilbert, 2002). For example, the fungal pathogen *Ciboria batschiana*, the causal agent of acorn black rot, can damage up to 80% of acorns when conditions are wet (Prochazkova *et al.*, 2005). By affecting the early stages of the plant life cycle, seed pathogens have significant impacts on natural ecosystems, including forests in which they shape tree species diversity and spatial structure (Janzen, 1970; Gilbert, 2002). Understanding how pathogens and other microorganisms are acquired and transmitted to the seeds of foundation tree species (i.e. tree species that structure and stabilize forest communities and ecosystem processes; Whitham *et al.,* 2006), and how these microorganisms associate to regulate seed germination and seedling development, is thus crucial to our ability to predict and manage the regeneration of forest ecosystems.

Up to now, processes of seed microbiota assembly have received more attention in crop plants than in natural ecosystems. In crops, microorganisms are transmitted by the mother plant to its seeds at the floral and during early seed development stages, through vascular tissues or contact between vegetative and reproductive organs (Maude, 1996). They can also be transmitted from the pollen of the father plant, insect vectors, or bioaerosols (Escobar Rodríguez *et al.,* 2018; Frank *et al.*, 2017). Once seeds fall on the ground, epiphytic microbial communities coalesce with microbial communities of litter and upper soil (Rillig *et al.*, 2015). Germinating seeds then release molecules that attract soil microbes, surrounding themselves with a microbiologically active soil area called the spermosphere (Nelson, 2004; Schiltz *et al.*, 2015). The emergence of the plant radicle creates cracks in the seed tegument, enabling microbes to colonize internal tissues (Nelson *et al.*, 2018). These events lead to intense biotic interactions among microorganisms (Nelson, 2004) and drastic changes in seed microbiota composition and function (Ofek *et al.*, 2011; Yang *et al.*, 2017; Torres-Cortés *et al.*, 2018). Recent studies suggest that seed colonization by soil microorganisms

represents the most influential microbial acquisition for seedling growth and health (Nelson *et al.*, 2018).

The seed microbiota is therefore a dynamic entity that is shaped, like all ecological communities, by four fundamental processes: selection by the abiotic environment (environmental filtering) and biotic interactions (biotic filtering), dispersal, ecological drift and evolutionary diversification (Vellend, 2010; Nemergut *et al.*, 2013; Ovaskainen *et al.*, 2017; Zhou & Ning, 2017). Unraveling these assembly processes is particularly important because they govern seed and seedling extended phenotypes (defined as the diversity and composition of associated communities; Whitham *et al.,* 2006), as well as the performance and eventually fitness of the plant host (Compant *et al.*, 2010; Truyens *et al.*, 2015; Brader *et al.*, 2017). Some processes are deterministic and depend on taxon-specific functional traits (Minard *et al.,* 2019), such as the response to selection. Other processes are partly or purely stochastic (Zhou & Ning, 2017; Rezki *et al.*, 2018) and generate divergences among communities occupying identical environments (Chase & Myers, 2011). The dispersal process is particular in the case of seed microbial communities because seeds are mobile. Microorganisms are recruited from a sequence of species pools: first from the microbiota of the mother tree's aboveground organs, and later from surrounding microbial sources such as bioaerosols, litter and soil (Barret *et al.,* 2016). The microorganisms that are directly transferred from the vascular system of mother plant to the seedlings through seeds are termed vertically-transmitted (Truyens *et al.,* 2015), while the others are called horizontally-acquired. Vertically-transmitted microorganisms can trigger maternal effects (defined as the causal influence of the maternal genotype or phenotype on the offspring phenotype; Wolf & Wade, 2009) in seed and seedling extended phenotypes (Vivas *et al.*, 2015).

To gain insight into processes of seed microbiome assembly in natural ecosystems, we analyzed the microbial turnover among acorns of sessile oak (*Quercus petraea*) populations growing along elevation gradients. We collected acorns in the canopy and on the ground beneath individual trees, and used Hierarchical Models of Species Communities (HMSC; Ovaskainen *et al.,* 2017) to quantify the ecological processes shaping seed microbial communities at each spatial level (oak population, mother tree and seed) and to generate hypotheses of interactions among microorganisms. We focused on fungal communities as they include pathogens that are detrimental to seed survival (such as *Ciboria batschiana*; Prochazkova *et al.*, 2005). After having described and visualized the fungal communities associated to acorn tissues, we tested the following

hypotheses: (**H1**) acorn fungal communities are shaped by environmental filtering and maternal effects, (**H2**) the maternal effects tend to disappear after acorn fall because of horizontal acquisition of fungi from the ground, (**H3**) the response of fungal species to variations in the acorn environment depend on their lifestyle, and (**H4**) biotic interactions among fungal species play a role in the protection of acorns against pathogens.

Materials and methods

Sampling design

Acorns were collected on October 21st and 22nd 2015 in four populations of sessile oak located in the Pyrenees Mountains (France). Two populations (Ade and Bager) were at an elevation of ~400 m a.s.l. and the other two (Gedre-Bas and Gabas) at ~1200 m a.s.l. The sampling date was chosen as close as possible to the fruiting peak at both elevations (see Caignard *et al.,* 2017 for a complete description of the sampling sites and fruiting phenology). In each site, acorns were collected from three trees randomly selected among the dominant adults. For each tree, we collected four acorns from the canopy using a slingshot and four acorns from the ground beneath the crown (within a distance of 2 m from the trunk). We also collected the biotic microenvironment of canopy acorns, defined as all tree tissues present in a cylinder of 4 cm diameter and 6 cm length around the acorn (including the acorn cupule, the cupules of other acorns, the twig to which the acorn was attached, the leaf petioles and the base of leaves) and the biotic microenvironment of ground acorns, defined as all substrates beneath the acorn within a cylinder of 4 cm diameter and 1 cm depth (including dead oak leaves, dead leaves of other plant species, acorn caps, twigs, pieces of bark, granules of soil, mosses, lichens or herbs). Each sample was collected aseptically, using new plastic gloves and scissors cleaned with 96% ethanol to minimize contamination. Samples were stored in individual plastic vials in a cooler with ice until they could be stored at -80°C.

Ten additional acorns were harvested from five mother trees (two in Gedre-Bas, two in Gabas and one in Ade). For each tree, one acorn was harvested in the canopy and one acorn was harvested on the ground. These acorns were surface-sterilized and dissected to characterize the endophytic fungal communities associated with acorn internal tissues. Surface-sterilization was completed using a three-step process: immersion for 3 min in a 70% ethanol solution, immersion for 2 min in

a 3% calcium hypochlorite solution and rinsing with DNAway and sterilized water. After drying the acorns on sterilized filter papers, the fruit walls and the embryos were collected using a sterilized nutcracker and pliers and stored at -80°C.

DNA extraction, amplification and sequencing of fungal communities

Samples were ground into a homogeneous powder using liquid nitrogen. Mortars and pestles were cleaned using DNAway and autoclaved for 20 min at 121°C between each sample. Approximately 45 mg of powder from each sample was transferred to a microplate under a laminar flow hood. Microplates were stored at -80°C until DNA extraction. Total DNA was extracted using DNeasy Plant Mini Kits (Qiagen, USA) according to the manufacturer's protocol except that DNA extracts were eluted twice with 50µL of elution buffer (10mM Tris-Cl, 0.5mM EDTA; pH 9.0).

The ITS1 region of the nuclear ribosomal internal transcribed spacer, considered the universal barcode marker for fungi (Schoch *et al.*, 2012), was amplified using the ITS1F (5′-CTTGGTCATTTAGAGGAAGTAA-3′; Gardes & Bruns, 1993) and ITS2 (5′-GCTGCGTTCTTCATCGATGC-3′; White, Bruns, Lee, & Taylor, 1990) primers. The reaction mixture (12.5 µL of final volume) consisted of 1.25 µL of template DNA, 2.5µL of 1µM of each of the forward and reverse primers and 6.25µL of 2X KAPA HiFi HotStart ReadyMix (Kapa Biosystems) containing 0.6 mM of each dNTP, 5 mM $MgCl_2$, and 2 units of Kapa Taq DNA polymerase. PCR cycling reactions were conducted on a Veriti 96-well Thermal Cycler (Applied Biosystems) using the following conditions: initial denaturation at 95 °C for 3 min followed by 20 cycles at 98°C for 20 s, 55°C for 45 s, 72°C for 15 s with final extension of 72 °C for 1 min. ITS1 amplification was confirmed by electrophoresis on a 2% agarose gel. Templates that were not successfully amplified using this protocol were amplified again after DNA dilution to 20ng/µL or 10ng/µL. Two marine fungal species (*Yamadazyma barbieri* and *Candida oceani*) were used as positive controls as they were unlikely to be found in our samples. One positive control included DNA of a strain of *C. oceani*, and the other included an equimolar mixture of the DNA of both species. A first negative control was represented by 1 mL of water washes of 4 empty plastic vials opened during the sampling campaign and washed with sterile water. The PCR mix was used as a second negative control.

PCR products were diluted five times in PCR-grade water and used as a DNA template for a second PCR performed using the tailed primers ITS1F_PlaGe (5'-CTTTCCCTACACGACGCTCTTCCGATCTCTTGGTCATTTAGAGGAAGTAA-3') and ITS2_PlaGe (5'-GGAGTTCAGACGTGTGCTCTTCCGATCTGCTGCGTTCTTCATCGATGC-3') designed by the Get-PlaGe sequencing facility (Toulouse, France). The second PCR was performed twice (once in 12.5 μL and once in 25μL of final volume) using the same reaction mixture as the first PCR. The PCR conditions were as follows: initial denaturation at 95 °C for 3 min followed by 10 cycles at 98°C for 20 s, 60°C for 15 s, 72°C for 15 s and a final extension step of 72°C for 1 min. PCR products were purified (CleanPCR, MokaScience), multiplex identifiers and sequencing adapters were added, and library sequencing on an Illumina MiSeq platform (v3 chemistry, 2x250 bp) and sequence demultiplexing (with exact index search) were performed at the Get-PlaGe sequencing facility (Toulouse, France).

Bioinformatic analyses

Paired-end sequences were joined using PEAR v0.9.10 (Zhang *et al.*, 2014). Only pairs with a minimum overlap of 50 bp and without any uncalled bases were kept. Assembled sequences were filtered using DADA2 v1.4.0 (Callahan *et al.*, 2016). Only sequences with less than one expected error and longer than 100 bp were retained in the dataset. Amplicon sequence variants (ASVs) were inferred using DADA2 and chimeric sequences were removed using the consensus method of the *removeBimeras* function. Taxonomic assignments were performed using the RDP classifier (Wang *et al.*, 2007) implemented in DADA2 and trained with the UNITE database 7.2 (UNITE Community 2017), with an 80% confidence threshold. The ASV table was then imported in R using the phyloseq package v1.26.0 (McMurdie & Holmes, 2013) and filtered. Only ASVs assigned to a fungal phylum were kept. Positive and negative controls were used to remove contaminants (Galan *et al.,* 2016). The cross-contamination threshold (T_{cc}) was defined as the maximal number of sequences of each ASV found in negative or positive control samples. The false-assignment threshold (T_{FA}) was defined as the highest sequence count of a positive control strain in a non-control sample, divided by the total number of sequences of the strain in the whole run and multiplied by the total number of sequences of each ASV. ASVs were removed from all samples where they harbored fewer sequences than either threshold (T_{FA} or T_{cc}).

SNP genotyping and maternity exclusion analyses

Acorns collected on the ground were genotyped to confirm that they belonged to the mother tree above them. Genotyping was performed using 39 polymorphic single nucleotide polymorphism (SNP) markers (Gerzabek *et al.,* 2017). DNA was diluted to a final concentration of 15 to 20 ng/µL and sequenced using the iPLEX Gold Genotyping kit (Agena, San Diego, CA, USA) at the Genome Transcriptome Facility of Bordeaux (PGTB, Bordeaux, France) according to the manufacturer's instructions (see Chancerel *et al.*, 2013 for more details). Two samples of aboveground tissues of each tree were genotyped and compared to estimate the typing error rate of false calls during genotyping. Loci with poor performance during the clustering procedure (call rates <60%) were excluded, resulting in a final set of 28 loci. Acorn genotypes were compared to the genotype of their putative mother tree. Considering the low error rate of these SNPs (Gerzabek *et al.,* 2017), we took a deliberately conservative approach and assumed that if tree and acorn shared no alleles for at least one locus, the mother-offspring relationship was not confirmed and the acorn sample was removed from the dataset.

Confocal microscopy

Sixteen additional acorns were collected in autumn 2017 from the ground of the oak forest of Bellebat (44°43'36.4"N 0°13'22.5"W, Southwest of France) to visualize fungal colonization outside and inside acorns. Acorns were cut in half with secators and fixed overnight at 4°C in a paraformaldehyde solution (4% w/v in PBS, pH 7.2). Samples were then rinsed three times with PBS, immersed in 15 mL PBS containing 50 µg ml^{-1} of wheat germ agglutinin (WGA)-AlexaFluor488 conjugate (Life Technologies, USA), incubated 2 hours at 37°C, and rinsed again three times with PBS. The samples were observed under a confocal microscope (Olympus Fluoview FV1000 with multiline laser FV5-LAMAR-2 and HeNe(G) laser FV10-LAHEG230-2). Pictures were taken with X, Y, Z coordinates at 405, 488, 594 nm and with 10X, 20X, 40X or 60X objectives. Images were merged (RGB) using Image J software (Schneider *et al.* 2012). Pictures were created using Z Project Stacks (Campisano *et al.* 2014), then cropped, and the light/contrast balance was improved (Glassner *et al.* 2015). Images presented in this publication correspond to the average colonization level observed.

Statistical analyses

Comparison of acorn fungal communities among mother trees and environments

To test hypotheses H1 and H2, we analyzed the effects of mother tree, local environment (oak population), and microenvironment (acorn position in the canopy *versus* on the ground) on acorn fungal community richness and composition. Fungal richness was defined as the total number of ASVs per acorn sample and was modelled using generalized linear mixed models (GLMMs) with a negative binomial distribution and a log-link function. The first model had oak population, acorn position and their interaction as fixed effects and the mother tree as a random effect. We then tested the effect of the mother tree on fungal richness for acorns in the canopy and acorns on the ground separately. The two models had the mother tree as fixed effect and the population as random effect. The natural logarithm of the total number of sequences per sample (sequencing depth) was introduced as an offset in all models.

We then analyzed the effects of the three same factors on fungal community composition by using permutational multivariate analyses of variance (PERMANOVAs) with 9999 permutations. Compositional dissimilarities among acorn samples were estimated using quantitative and binary versions of the Jaccard index (Jaccard, 1901) and visualized with principal coordinate analyses (PCoA). We first tested the effects of oak population, acorn position and their interaction on compositional dissimilarities among samples, by constraining or not constraining the permutations by elevation. We then assessed the effect of mother tree on community composition for acorns in the canopy and on the ground separately. The two models had the mother tree as a fixed effect and permutations were constrained by population. The natural logarithm of the total number of sequences per sample (sequencing depth) was introduced as the first effect in all models.

In addition, we investigated whether changes in fungal community composition after acorn fall were due to either the substitution of canopy-associated fungal species by ground-associated fungal species or gain of ground-associated fungal species without loss of canopy-associated fungal species, by partitioning Jaccard binary dissimilarities among acorns of the same mother tree using the betapart package v1.5.1 (Baselga *et al.,* 2018). The proportion of fungal species of acorns on the ground also found in acorns in the canopy was calculated for each mother tree.

Quantification of maternal effects, environmental filtering and biotic interactions

Hierarchical Models of Species Communities (HMSC; Ovaskainen *et al.,* 2017) were then used to quantify processes of community assembly. The models assumed that variation in fungal community composition among acorns (i.e. the **Y** matrix in the HMSC framework) was accounted for by four ecological predictors (*Mother mycobiota*, *Microenv mycobiota*, *Acorn position* and *Site elevation*) introduced as fixed effects in the **X** matrix. The **Y** matrix represented ASV sequence counts in all acorn samples, out of which we included only the ASVs that were present in five or more acorns. *Mother mycobiota* and *Microenv mycobiota* represented fungal communities in the canopy of mothers trees and in the microenvironment of acorns, respectively. They were ASV-specific predictors and thus the **X** matrix was different for every ASV of the **Y** matrix. *Mother mycobiota* was calculated for all acorn samples as the average relative abundance of the focal ASV in the twigs and leaves of mother trees, and was included at the tree level to model vertical transmission of fungi from the mother tree to its acorns. *Microenv mycobiota* was calculated as the residuals of the regression of the relative abundance of the focal ASV in the microenvironment of each acorn over its relative abundance in the canopy of the mother tree, and was included at the sample level to model horizontal acquisition of fungi from the materials surrounding each acorn. *Site elevation* and *Acorn position* represented filtering of fungal communities by climate and microclimate, respectively. *Site elevation* was included at the site level to model selection exerted by site-level abiotic factors, such as average air temperature, on acorn fungal communities. *Acorn position* (canopy vs. ground) was included at the sample level to model selection exerted by microclimate, such as higher humidity on the ground, on acorn fungal communities. *Mother mycobiota* and *Microenv mycobiota* were included in interaction with *Acorn position* to test the hypothesis that their contribution to fungal communities differ between acorns in the canopy and acorns on the ground (H2). We also introduced the log-transformed sequencing depth of each sample (*Sequencing depth*) as a fixed effect in the **X** matrix, to take into account methodological biases influencing ASV sequence counts. Random effects at each hierarchical level (oak population, mother tree and acorn sample) were also introduced to model variations in ASV sequence counts that can neither be attributed to the four ecological predictors nor sequencing depth. In addition, we tested the hypothesis that fungal lifestyle modulates fungal ASV responses to environmental variations (H3), by including the trophic mode (saprotroph, plant pathogen or other) and the degree of specialization toward acorns of each ASV in the **T** matrix. The putative trophic mode of each ASV was determined using the FUNGuild database (Nguyen *et al.*, 2016).

Their degree of specialization toward acorns was defined as the log-ratio of ASV relative abundance in acorns *versus* other sample types (i.e. branches, leaves, litter and upper soil), calculated using DESeq2 (Love *et al.*, 2014). To account for the zero-inflated nature of the data, we applied a hurdle modelling approach. We first fitted a probit model on ASV presence-absence data and then fitted a linear model on sequence count data conditional on presence, in which counts were log-transformed and scaled to zero mean and unit variance for each ASV and absences masked as non-available data. We fitted both models with default prior distributions (Ovaskainen *et al.,* 2017). For each of the four MCMC chains, we sampled the posterior for 1,500,000 iterations, out of which we excluded the first 500,000 as burn-in and thinned the remaining iterations by 1000, thus producing a total of 4,000 posterior samples. We examined MCMC convergence through the distributions of potential scale reduction factors (PSRF) of the model parameters. To examine model fit, we applied a two-fold cross validation across the samples and evaluated predictive performance by AUC for the presence-absence model and R^2 for the linear model. Finally, residual correlations among fungal ASV sequence counts at the acorn level were interpreted as hypothetical biotic interactions among fungal strains (see Ovaskainen *et al.,* 2017). We examined associations among ASVs assigned at the species level to test hypothesis H4.

Results

All acorn tissues, including the fruit wall, seed coat, and embryo were colonized by fungi, with a dense colonization under the endocarp (Fig. 1A-E). Ascomycota represented 91.1% and 89.4% of the sequences of canopy and ground acorns, respectively. *Dothideomycetes*, *Leotiomycetes* and *Sordariomycetes* were the three main classes of ascomycetes present (Fig. S1). Among the ten most abundant species associated to whole acorns, five are known as plant pathogens (*Gnomoniopsis paraclavulata, Taphrina carpini*, *Epicoccum nigrum*, *Mycosphaerella tassiana* and *Polyscytalum algarvense*) and two are known as antagonists of other microorganisms (*Cladosporium delicatulum, Cylindrium elongatum*) (Table 1; Tables S2 and S3 for all ASVs and subset of ASVs used in HMSC models, respectively). The ubiquitous fungi *Curvibasidium cygneicolum* and *Epicoccum nigrum* were dominant in the internal tissues of acorns, including the embryo (Table S1).
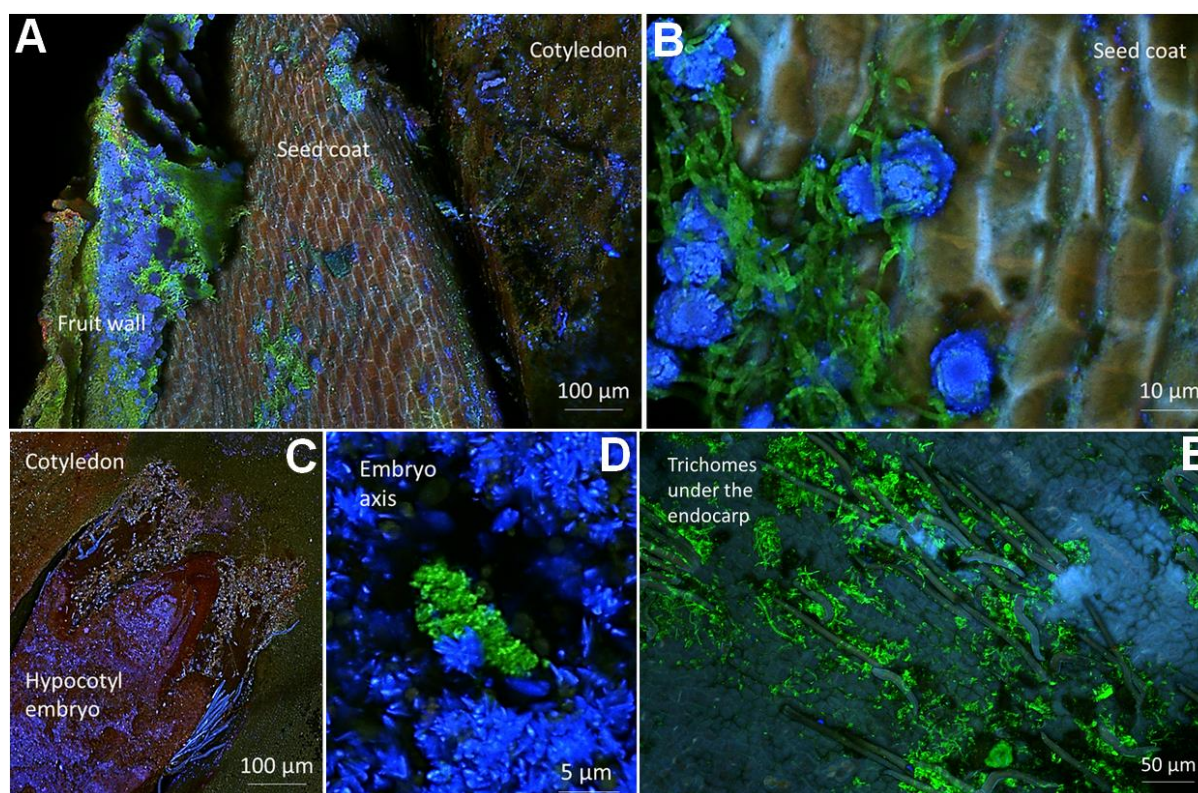
Figure 1. Fungi in cross sections of seeds of sessile oak collected on the ground. Fungi (green fluorescent) were revealed by confocal microscopy and WGA-ALEXA fluor488 staining. (A) Fruit wall and seed coat. (B) Zoom-in view of panel A. (C) Embryo and cotyledon. (D) Zoom-in view of panel C. (E) Internal surface of the endocarp.

Table 1. Most abundant fungal species associated with seeds of sessile oak and their microenvironment. Seeds were either collected in the tree canopy or on the ground. Materials from the seed microenvironment (twigs and leaves, or litter and upper soil) were also collected. The fungal community of all 4 sample types was analyzed using a metabarcoding approach. Only Amplicon sequence variants (ASV) assigned to Ascomycota (A) or Basidiomycota (B) with the UNITE database were kept. Average relative abundances of all ASVs were computed for each sample type, after merging ASVs assigned to the same fungal species. Only ASVs identified at the species level are shown in the table.

Fungal species	Average relative abundance per sample type (%)					Lifestyle	Reference(s)
	All seeds	Seeds in the canopy	Canopy samples (twigs, leaves)	Seeds on the ground	Ground samples (litter, upper soil)		
Gnomoniopsis paraclavulata (A)	13.4	5.3	1.5	21.9	0	Pathogen isolated in leaves, buds, cupules and shoots of *Castenea sativa*. Commonly isolated from overwintered leaves of *Quercus* sp.	Tosi *et al.* (2014) ; Sogonov *et al.* (2008)
Stromatoseptoria castaneicola (A)	2.7	5.2	0.9	0	0.2	Causes leaf spots on *Castenea sativa*.	Quaedvlieg *et al.* (2013)
Taphrina carpini (A)	2.4	3.4	0.7	1.4	0.3	Common pathogen encountered on *Fagaceae* leaves.	Bacigálová (1991); Inácio *et al.* (2004); Cordier *et al.* (2012)
Epicoccum nigrum (A)	2.3	0.4	0.1	4.2	0.2	Ubiquitous fungus found in soil, leaves and seeds described as primary saprotroph and plant pathogen.	Ahumada-Rudolph *et al.* (2014)
Mycosphaerella tassiana (A)	1.7	1.5	0.1	1.9	0.3	Common pathogen found in the phyllosphere including that of oak.	Schubert *et al.* (2007); Jakuschkin *et al.* (2016)
Curvibasidium cygneicollum (B)	1.5	1.2	0.2	1.9	0.1	Endophyte of fruits, leaves, trunk and soil behaving as a phytopathogen or a mycoparasite. The species is insensitive to the mycocins produce by *Filobasidium* and *Cystofilobasidium*.	Sampaio *et al.* (2004); Mašínová *et al.* (2017) ; Sampaio *et al.* (2004)
Cylindrium elongatum (A)	1.5	1.4	0.1	1.5	4.3	Bacterial and fungal antagonist found on oak leaves.	Reyes-Estebanez (2011) ; Duarte *et al.* (2015)
Polyscytalum algarvense (A)	1.5	1.9	0.1	1	5.3	Necrotroph fungi found on *Eucalyptus* leaves.	Cheewangkoon et al. (2009); Crous (2018)
Fusarium pseudensiforme (A)	1.3	1.4	0	1.2	0	Found on bark of trees.	Nalim *et al.* (2011)
Cladosporium delicatulum (A)	1.3	1.3	0.2	1.3	0.3	Found in cereal seeds, mycoparasite of *Taphrina spp* and *Magnaporthe oryzae*	Amanelah Baharvandi & Zafari (2015) ; Chaibub *et al.* (2016)

Acorn fungal communities are shaped by environmental filtering and maternal effects

Acorn fungal community richness (Table 2) and composition (Tables 3 and S2) differed significantly among oak populations along elevation gradients, and among mother trees within the same population, confirming the hypothesis that both environmental filtering and maternal effects shape acorn fungal communities (H1). PCoAs suggested that fungal communities were more similar between populations at the same elevation (Fig. 2). However, the population effect remained significant when PERMANOVAs were constrained by elevation (Pseudo-F = 1.24, P < 0.01), implying that selection exerted by abiotic conditions along the gradients was not the only process triggering variation in fungal community composition among oak populations.

Table 2. Generalized linear mixed-effects models (GLMM) of fungal community richness of seeds of sessile oak. Richness was defined as the number of amplicon sequence variants (ASV) per sample. The total number of sequences per sample (sequencing depth, SD) was introduced as an offset in all models. The effects of tree population (T), seed position (canopy *versus* ground, P) and their interaction were tested on the whole seed dataset while the effect of mother tree was tested separately on canopy seeds and ground seeds.

|	d.f.	chi-square	*P*-value
All seeds			
Tree population (T)	3	12.5	**<.01**
Seed position (P)	1	12.6	**<.001**
T × P	3	5.74	0.12
Seeds in the canopy			
Mother Tree	10	30.6	**<.01**
Seeds on the ground			
Mother Tree	11	28.3	**<.01**

Table 3. Permutational multivariate analyses of variance (PERMANOVA) of compositional dissimilarities among fungal communities of seeds of sessile oak. Dissimilarities among seeds were estimated using the binary Jaccard distance. The total number of sequences per sample (sequencing depth, SD) was log-transformed and introduced as the first explanatory variable in all models. The effects of tree population (T), seed position (canopy *versus* ground, P) and their interaction were tested on the whole seed dataset while the effect of mother tree was tested separately on canopy seeds and ground seeds.

	d.f.	F-value	*P*-value	R^2
All seeds				
Log(SD)	1	1.3	**<.01**	0.01585
Tree population (T)	3	2.4	**<.001**	0.08711
Seed position (P)	1	1.8	**<.001**	0.02226
S x P	3	1.2	**<.001**	0.04494
Seeds in the canopy				
Log(SD)	1	1.3	0.191	0.03052
Mother tree	10	1.4	**0.010**	0.32742
Seeds on the ground				
Log(SD)	1	1.1	0.319	0.02834
Mother tree	11	1.2	**0.025**	0.34149

PCoAs and HMSC models gave different estimates of the relative contribution of environmental filtering and maternals effects. PCoAs suggested that mother tree identity had a lower influence on fungal community composition than elevation (Fig. 2), whereas HMSC models indicated the opposite trend (Table 4). The model of ASV presence-absence explained (in units of AUC, averaged over the ASVs) 80% of the variation for the model fitted to all data and 69% of the variation based on the two-fold cross-validation approach. According to this model, elevation was a minor direct driver of fungal community composition (only 2% of the explained variance). In contrast, the average relative abundance of a fungal ASV in the tissues of a mother tree (*Mother mycobiota*) was the second most important predictor of the occurrence of this ASV in an acorn from this tree, in interaction with the acorn position (39% of the explained variance). *Mother mycobiota* was the unique predictor of occurrence for several ASVs belonging to the orders *Helotiales*, *Venturiales* and *Xylariales* (Fig. S2). A similar ranking of the predictors was obtained for the model of ASV sequence counts (Table 4), except that sequencing depth and random effects had a much larger influence on ASV sequence counts than on ASV presence-absence (17% *versus*

1% and 21% *versus* 6%, respectively). This model only explained 34% of the variation for the model fitted to all data and 18% of the variation based on the two-fold cross-validation approach.
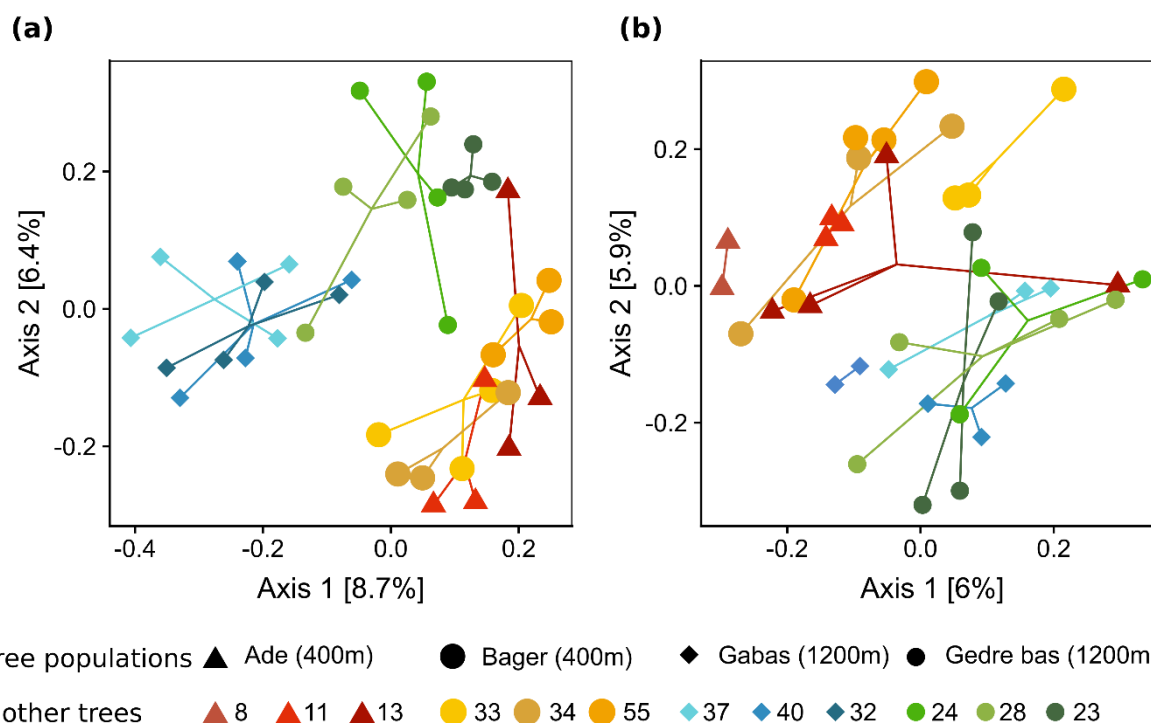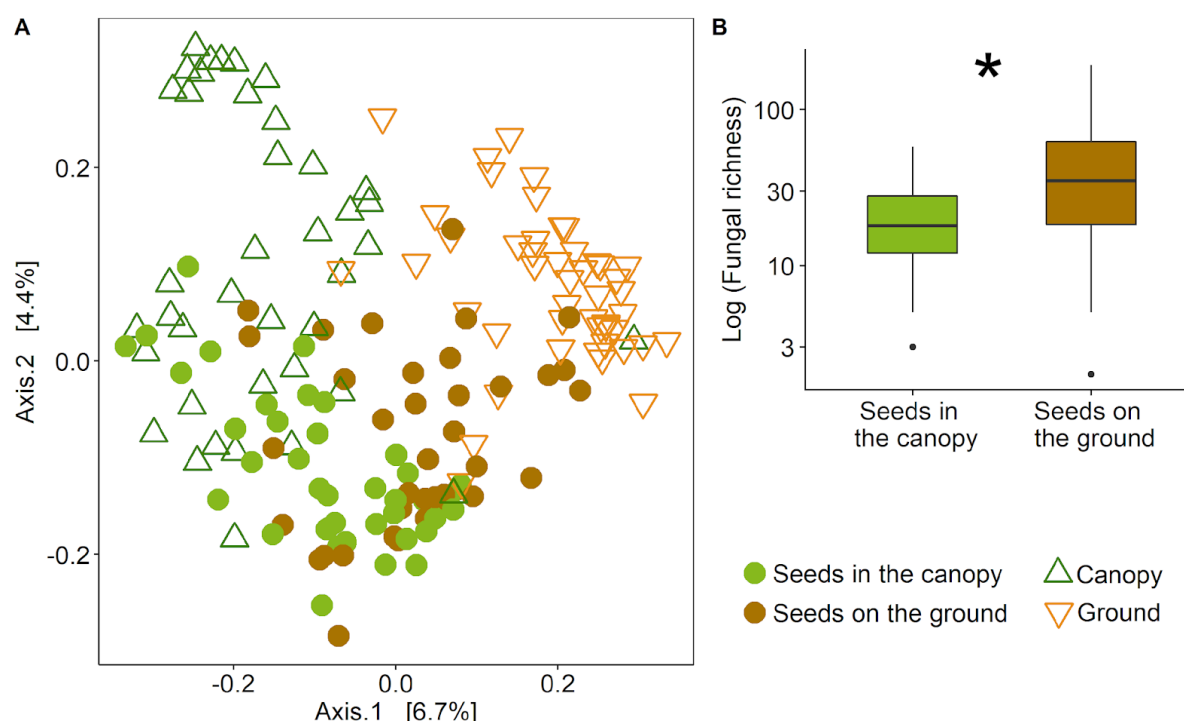

Figure 2. Compositional dissimilarities among fungal communities of seeds collected (A) in the canopy and (B) on the ground in four populations of sessile oak. Dissimilarities among seeds were estimated using binary Jaccard distance and represented with a PCoA plot. Fungal community composition differed significantly among tree populations and among mother trees (Table 3).

Table 4. Partitioning of the variance in fungal community composition of seeds of sessile oak. Four fixed effects were included in the HMSC models to explain variations in the presence-absence (PA) or the sequence count (SC) of a focal fungal ASV among seeds: *Sequencing depth* (total number of sequences per sample), *Seed position* (canopy or ground), *Microenv. mycobiota* (relative abundance of the focal ASV in the seed biotic microenvironment), *Mother mycobiota* (average relative abundance of the focal ASV in the mother tree aboveground tissues), and *Elevation*. Random effects were included at each spatial scale (seed, tree and population). Results of variance partitioning are given as percentages (%) of total explained variance.

	Predictor		Explained variance (%)	
Type	**Level**	**Name**	**PA model**	**SC model**
Fixed	Seed	Sequencing depth	1	17
Fixed	Seed	Seed position (P)	4	8
Fixed	Seed	Microenv. mycobiota * P	47	23
Fixed	Tree	Mother mycobiota * P	39	25
Fixed	Population	Elevation	2	6
Random	Seed	Seed	3	9
Random	Tree	Tree	1	6
Random	Population	Population	2	6

Maternal effects persist after acorn fall despite horizontal acquisition of fungi from the ground

Fungal community richness (Table 2) and composition (Table 3 and S2) differed significantly between acorns in the canopy and acorns on the ground. For instance, *Gnomoniopsis paraclavulata* was four times more abundant in acorns on the ground than in acorns in the canopy, while *Stromatospheria castaneicola* was only present in canopy acorns (Table 1). Fungal richness increased and composition shifted toward that of ground materials after acorn fall (Fig. 3), confirming the horizontal acquisition of fungi from the ground. Partitioning of Jaccard beta-diversity indicated that these temporal changes in community composition were mainly driven by turnover (replacement of fungal species rather than net gains or losses in species number; Table S4). HMSC models confirmed the large influence of horizontal transmission on acorn fungal communities. The relative abundance of a fungal ASV in the microenvironment of an acorn (*Microenv mycobiota*), in interaction with the acorn position, was generally the best predictor of ASV occurrence (47% of the explained variance), especially for the *Capnodiales*, *Dothideales* and *Taphrinales* orders (Fig. S2).

Figure 3. Fungal community composition and richness of seeds of sessile oak collected in the canopy and on the ground. (A) PCoA plot of compositional dissimilarities among fungal communities associated to seeds, canopy (leaves and twigs) and ground materials (litter and upper soil). Dissimilarities among samples were estimated using binary Jaccard distance. Fungal community composition differed significantly among the four sample types (PERMANOVA; F=4.3, *P*<0.001). (B) Richness (log-transformed) of seed fungal communities, defined as the number of ASVs per sample. Richness was significantly higher in seeds on the ground (Table 2).

Despite the turnover in fungal community composition after acorn fall and in contrast with our hypothesis (H2), the maternal effects were significant for acorns on the ground (Table 3), indicating that some maternal species were retained after acorn fall. Overall, acorns on the ground shared 10 to 40% of their fungal community with acorns in the canopy of the same mother tree, and 21 to 50% with mother tree tissues (Table S5). On average, 38% of fungal ASVs of acorns on the ground were present in both acorns in the canopy and mother tree tissues. Fungal species most often retained after acorn fall were *Taphrina* sp., *Cladosporium delicatulum*, a mycoparasite of *Taphrina* sp. (Baharvandi & Zafari, 2015), and the ubiquitous *Epicoccum nigrum*.

The response of fungal species to variations in the acorn environment depend on their lifestyle

HMSC models showed that fungal lifestyle influenced the response of acorn fungal communities to environmental variations, in accordance with our hypothesis (H3). High elevation selected for

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Fort, T., Pauvert, C., Zanne, A. E., Ovaskainen, O., Caignard, T., Barret, M., Compant, S., Hampe, A., Delzon, S., Vacher, C. (2019). Maternal effects and environmental filtering shape seed fungal communities in oak trees. BioRxiv. , DOI : 10.1101/691121

saprotroph species and seed specialists, increasing their proportion in acorn fungal communities (Table S3). Acorn fall favored pathogen species and saprotrophs. Their proportion increased after acorn fall while their abundance (conditional on presence) was not altered (Table S3). These findings suggest that vertically-transmitted pathogens did not increase in abundance after acorn fall, and that acorns were colonized by pathogen and saprotroph species of the ground.

Biotic interactions among fungal species might play a role in the protection of acorns against pathogens

Fungal colonization was very dense on acorn external surfaces (Fig. 1A) but also on internal surface of the fruit wall (Fig. 1E), indicating that fungal colonizers might enter into contact and compete for space and eventually resources. In contrast to this expectation, residual co-occurrence patterns of HMSC models at the acorn level revealed only positive associations between fungal ASVs. Among fungal ASVs associated with each other, 14 could be assigned at the species level (Fig. 5). Six of them are described as plant pathogens and two of them, *Mycosphaerella tassiana* and *Taphrina carpini*, have already been found in association with oak (Table S6). Four of the species are described as mycoparasites, including *Cladosporium delicatulum*. The positive association between *Taphrina carpini* and *Cladosporium delicatulum* (Fig. 5), which are both found in the embryo and the fruit wall (Table S3), might therefore represent a vertically-transmitted host-parasite interaction. This interaction might play a role in oak disease regulation, in accordance with our hypothesis (H4).
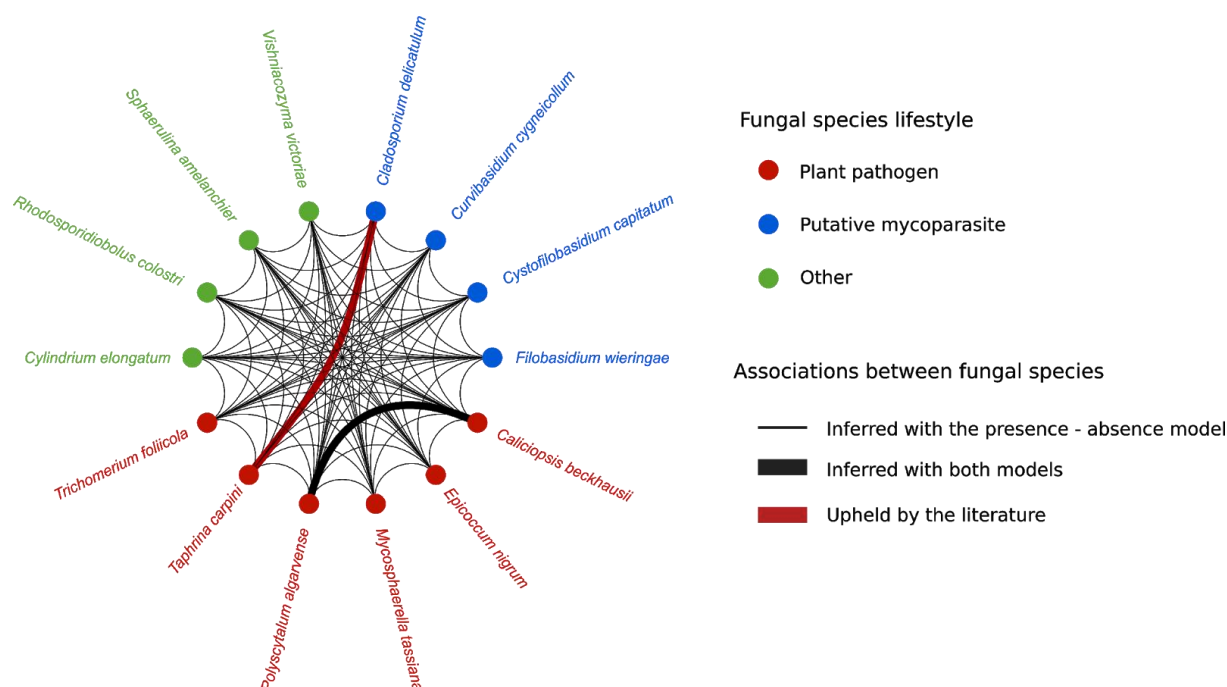
Figure 4. Network of fungal associations estimated by HMSC models. Associations were all positive. Network nodes correspond to fungal ASVs assigned to the species level and their color correspond to putative functions according to FUNGuild and the literature (Table S6). Network links indicate associations with at least 95% posterior probability estimated by the presence-absence model (thin black plain line) or by both the presence-absence model and the sequence count model (thick black plain line). The positive association between *Taphrina carpini* and *Cladosporium delicatulum* is indicated in red because it is described in the literature as a host-parasite interaction (Table S6).

Discussion

Seed microbial communities of trees have received little attention so far despite their potential influence on forest dynamics and evolution. In this study, we compared fungal communities of seeds among four populations of sessile oak (*Quercus petraea*), a dominant tree species of deciduous temperate European forests (McShea *et al.,* 2006). The populations were located along elevation gradients in the Pyrenees Mountains (France). They differed by up to 800 m in elevation. Our analyses revealed that the richness and composition of seed fungal communities differed significantly among oak populations along gradients, suggesting that abiotic filtering was a major driver of community assembly. However, Hierarchical Models of Species Communities (HMSC; Ovaskainen et al. 2017) revealed that the direct influence of elevation on seed fungal communities was small. Elevation accounted for only 2% of the explained variance in fungal species presence-absence, and 6% of the variance in species abundance. The apparently large effects of elevation

suggested by multivariate analyses might therefore be indirect effects. HMSC models estimated that fungal community composition in the microenvironment of each seed (i.e. twigs and leaves surrounding seeds in the canopy, or ground materials surrounding dropped seeds), explained 47% of the variance in species presence-absence, and was by far the strongest driver of seed fungal communities. These findings suggest that environmental filtering act directly on the fungal communities of leaves, litter and soil (as shown by Cordier et al., 2012; Coince et al., 2014; Vacher et al., 2016), to then indirectly shape seed communities.

Seed fungal communities also differed among mother trees within the same population and these differences remained significant after seed fall. Such maternal effects have already been found for the abundance of some fungal genera in seeds (Johnston-Monje & Raizada, 2011; Pinciroli *et al.*, 2013). Here we showed that maternal effects extend to the whole fungal community of seeds through to seed fall. HMSC indicated that the fungal community composition of mother tree tissues (twigs and leaves) had a major, direct influence on that of seeds. It accounted for 39% of the explained variance in fungal species presence-absence, and 25% of the variance in species abundance. Recent findings by Vivas *et al.,* (2017) on *Eucalyptus* trees indicate that these maternal effects can persist in seedlings and influence their growth and resistance to pathogens. Together, these results suggest that maternal effects in seed and seedling extended phenotypes could be a major driver of forest regeneration success.

In addition, our confocal microscopy analyses revealed, for the first time, the presence of fungal aggregates within embryos of acorns of sessile oak, as well as a dense fungal colonization on internal surfaces of fruit walls. These endophytic fungal populations contained foliar pathogens of *Fagaceae* tree species, such as *Mycosphaerella tassiana* and *Taphrina carpini* (Schubert *et al.,* 2007; Bacigálová, 1991), ubiquitous fungal species, such as *Epicoccum nigrum* (Andrews & Harris, 2000), and endophytic yeasts previously described in other fruits, such as *Curvibasidium cygneicollum* (Sampaio *et al.,* 2004; Mašínová *et al.,* 2017). Network inference analyses revealed a positive association between the foliar pathogen *Taphrina carpini* and the mycoparasite *Cladosporium delicatulum,* suggesting that mother trees do not only transmit pathogens but also pathogen antagonists. Our results hence confirm that fungal pathogens use seeds for their own dispersion, and that the fungal pathogen's parasites can follow them using the same dispersion mode (Ewald, 1989; Feldman *et al.*, 2008). Unraveling the genetic architecture of these tripartite

interactions, involving tree seedlings, seed-borne pathogens and their hyperparasites, could improve our understanding of forest ecosystem dynamics and evolution.

Finally, our analyses confirmed that seed fall corresponds to a major transition in seed fungal communities. Our results showed that fungal community richness significantly increased and that composition shifted toward that of ground materials after seed fall, confirming that seeds on the ground are rapidly colonized by the species present in the surrounding microenvironment (Crist, 2009, Qin *et al.,* 2016; Truyens *et al.,* 2015; Klaedtke *et al.,* 2016). For instance, the species *Gnomoniopsis paraclavulata*, that was previously found in association with oak litter (U'Ren *et al.,* 2016), drastically increased in abundance after acorn fall. Our analyses also suggested that seed fall triggers a replacement of canopy-inherited species by ground-derived species, rather than an addition of species associated to ground materials. This replacement was however only partial. Seeds on the ground shared up to 50% of their fungal colonizers with the twigs and leaves of their mother tree. The mechanisms of community filtration during vertical transmission (Vannier *et al.*, 2018) will have to be investigated in future studies.

Conclusions

Our study revealed that acorns of sessile oak harbor diversified fungal communities in their internal tissues, including the embryo, and on their surfaces. These communities were shaped by maternal effects, environmental filtering and biotic interactions. Maternal effects persisted after seed fall, despite seed colonization by soil and litter fungi. Environmental filtering did not shape directly seed fungal communities, but rather influenced communities in the microenvironments surrounding seeds. Biotic interactions included several host-parasite interactions between tree pathogens and their antagonists, one of which was likely to be vertically-transmitted. Future research will have to investigate the maternal and environmental drivers of the rate of vertical transmission of microorganisms (e.g. Cavazos *et al.,* 2018; Sneck *et al.,* 2017; Leff *et al.,* 2017), and assess the role of these microorganisms on seed survival and germination, seedling growth and health (e.g. Vivas *et al.,* 2017; Leroy *et al.,* 2019), and ultimately tree fitness. The influence of vertically-transmitted microorganisms on seed and seedling secondary metabolites (Chen *et al.,* 2018; Shazad *et al.,* 2018), and their cascading effects on tree biotic interactions (e.g. Peris *et al.,* 2018) will also have to be investigated. Previous research on oak trees showed for instance a

significant relationship between a fungus-like pathogen associated with acorns and the abundance of several oak-dependant bird species, including seed dispersers (Monahan & Koenig, 2006). A combination of germination experiments in controlled conditions (e.g. Leroy *et al.,* 2019), seed microbiome analyses in common gardens (Vivas *et al.,* 2015), and seed microorganisms manipulation across environments (Gundel *et al.,* 2017) will be required to integrate seed microbial ecology into predictive models of forest dynamics and evolution.

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Author contributions

TF participated in the sampling campaigns, processed all samples, extracted and amplified DNA, performed the statistical analyses, interpreted the results and wrote the first draft of the manuscript. CP helped process the samples, performed the bioinformatic analyses and provided statistical analysis scripts. AEZ assisted with supervision and writing. OO conducted the HMSC analysis and helped interpreting the results. TC organized the sampling campaigns and provided environmental data. MB provided advice on molecular biology protocols, bioinformatics and statistical analyses. SC took the microscopy pictures and interpreted the results. AH provided advice and analysis tools for SNP genotyping. SD, AEZ and CV had the original idea for the study. CV participated in the sampling campaigns, coordinated and supervised all stages of the work, and made a major contribution to the writing of the manuscript. All authors revised the manuscript and approved the final version.

Data availability

All raw sequences obtained from the sequencing of acorns and their biotic microenvironment are available from the National Center for Biotechnology Information Sequence Read Archive (http://www.ncbi.nlm.nih.gov/sra) under the accession number PRJNA551388. The code and ASV tables are available as an archive at https://doi.org/10.15454/SM6OCR.

Version preprint

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Supplementary Materials

Table S1. Most abundant fungal species found as endophytes in seeds of sessile oak. Seeds were collected in the tree canopy or on the ground and were surface sterilized. The fruit wall and embryo were separated and their fungal community was analyzed using a metabarcoding approach. Only Amplicon sequence variants (ASV) assigned to Ascomycota (A) or Basidiomycota (B) with the UNITE database were kept. Average relative abundances of all ASVs were computed for each sample type, after merging ASVs assigned to the same fungal species. Only ASVs identified at the species level are shown in the table.

Fungal species	Average relative abundance (%)			Lifestyle	Reference(s)
	All internal tissues	Embryos	Fruit walls		
Curvibasidium cygneicollum (B)	8.7	6.8	10.4	Endophyte of fruits, leaves, trunk and soil behaving as a phytopathogen or a mycoparasite. The species is insensitive to the mycocins produce by *Filobasidium* and *Cystofilobasidium*.	Sampaio *et al.* (2004); Mašínová *et al.* (2017)
Epicoccum nigrum (A)	6.1	7.2	5	Ubiquitous fungus found in soil, leaves and seeds described as primary saprotroph and plant pathogen.	Ahumada-Rudolph *et al.* (2014)
Mycosphaerella tassiana (A)	4.7	1	8.2	Common pathogen found in the phyllosphere including that of oak.	Schubert *et al.* (2007); Jakuschkin *et al.* (2016)
Gibberella baccata (A)	4.5	4.5	4.5	Worldwide pathogen found in the phyllosphere and rarely found in seeds or soils.	Douglas Boyette & Lynn Walker (1986); Desjardins, (2003)
Cladosporium delicatulum (A)	3.2	3.5	3	Found in cereal seeds, mycoparasite of *Taphrina spp* and *Magnaporthe oryzae*.	Amanelah Baharvandi & Zafari (2015) ; Chaibub *et al.* (2016)
Caliciopsis beckhausii (A)	1.9	1.7	2	Most species of the genus are saprotrophs or parasites growing on bark of *Quercus* sp.	Garrido-Benavent & Perez-Ortega (2015)
Rhodosporidiobolus colostri (B)	1.5	0.7	2.2	Ubiquitious yeast behaving as a fungal antagonist.	Golubev and Tomashevskaya (2009)
Angustimassarina acerina (A)	1.4	2.4	0.5	Found on twigs of *Acer platanoides* and in bioaerosols.	Thambugala (2015); Banchi *et al.* (2018)
Penicillium paczoskii (A)	1.1	1.1	1.1	Pathogen causing post-harvest fruit rots recovered from harvested seeds.	Palou *et al.* (2010)
Taphrina carpini (A)	1	1	1.1	Common pathogen encountered on *Fagaceae* leaves.	Bacigálová (1991); Inácio *et al.* (2004); Cordier *et al.* (2012)

Table S2. Permutational multivariate analyses of variance (PERMANOVA) of compositional dissimilarities among fungal communities of seeds of sessile oak. Dissimilarities among seeds were estimated using the Jaccard quantitative distance. The total number of sequences per sample (sequencing depth, SD) was log-transformed and introduced as the first explanatory variable in all models. The effects of tree population (T), seed position (canopy *versus* ground, P) and their interaction were tested on the whole acorn dataset while the effect of mother tree was tested separately on canopy seeds and ground seeds.

	d.f.	F-value	P-value	R^2
All seeds				
Log(SD)	1	1.3	0.018	0.01630
Tree population (T)	3	2.1	**<.001**	0.07967
Seed position (P)	1	1.8	**<.001**	0.02240
T x P	3	1.2	**<.01**	0.04619
Seeds in the canopy				
Log(SD)	1	1.0	0.626	0.02588
Mother tree	10	1.2	**0.011**	0.30870
Seeds on the ground				
Log(SD)	1	1.1	0.238	0.02935
Mother tree	11	1.2	0.150	0.34904

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Fort, T., Pauvert, C., Zanne, A. E., Ovaskainen, O., Caignard, T., Barret, M., Compant, S., Hampe, A., Delzon, S., Vacher, C. (2019). Maternal effects and environmental filtering shape seed fungal communities in oak trees. BioRxiv. , DOI : 10.1101/691121

Table S3. Response of fungal ASVs to site elevation and seed position depending on their lifestyle, in the HMSC presence-absence (PA) and sequence count (SC) models. In the PA model, values are the posterior probabilities that the community weighted mean specialization, or the proportion of pathogens or saprotrophs is higher in the reference modality of the ecological factor. In the SC model, values are the posterior probabilities that the community weighted mean specialization, or the abundance of pathogens or saprotrophs (conditional on presence) is higher in the reference modality of the ecological factor. Probabilities higher than 0.9 are in bold.

Ecological factor	Reference modality	PA model			SC model		
		Specialization	Pathogen	Saprotroph	Specialization	Pathogen	Saprotroph
Elevation	High	**0.96875**	0.57875	**0.97825**	0.71475	0.84475	0.75225
Seed position	Ground	0.78575	**0.92525**	**0.9225**	0.38525	0.42925	0.33575

Table S4. Nestedness and turnover components of compositional dissimilarities between fungal communities of seeds in the canopy and seeds on the ground. Compositional dissimilarities among fungal communities were estimated for each mother tree using the binary Jaccard dissimilarity index and partitioned with the beta.temp function of the betapart package (Baselga et al. 2018). A mother tree at the Bager site was excluded from the analysis because it had only two seeds on the ground.

Tree population	Mother tree	Dissimilarity between ground seeds and canopy seeds		
		Turnover	Nestedness	Total
Ade	33	0.75	0.06	0.81
	34	0.78	0.09	0.86
	55	0.85	0.08	0.92
Bager	11	0.66	0.09	0.75
	13	0.54	0.35	0.90
Gabas	32	0.62	0.17	0.79
	37	0.84	0.03	0.87
	40	0.76	0.08	0.84
Gedre bas	23	0.77	0.13	0.90
	24	0.91	0.01	0.92
	28	0.84	0.05	0.89

Table S5. Number and percentage of fungal ASVs of seeds on the ground also found in seeds in the canopy and mother tree aboveground tissues. A mother tree at the Bager site was excluded from the analysis because it had only two acorns on the ground.

| Site	Mother tree	Total number of ASVs in seeds on the ground	Number (%) of ASVs in seeds on the ground shared with	
			Seeds in the canopy	Mother tree aboveground tissues
Ade	33	35	14 (40%)	17 (48%)
	34	142	26 (18%)	30 (21%)
	55	109	11 (10%)	44 (40%)
Bager	11	121	39 (32%)	59 (48%)
	13	247	27 (10%)	58 (23%)
	32	101	26 (25%)	51 (50%)
Gabas	37	67	18 (26%)	30 (44%)
	40	169	37 (21%)	56 (33%)
Gedre bas	23	105	12 (11%)	30 (28%)
	24	42	6 (14%)	11 (26%)
	28	102	16 (15%)	25 (24%)

Table S6. Fungal ASVs positively associated with another other ASV at the seed level according to HMSC models. Putative functions were inferred using FUNGuild and literature search. Only ASVs assigned at the species level are shown. The network of positive associations is represented on Figure 4.

Fungal species	Guild inference		Lifestyle	References
	FUNGuild	Literature		
Caliciopsis beckhausii	Pathotroph	Plant pathogen	Most species of the genus are saprotrophs or parasites growing on bark of *Quercus* sp.	Garrido-Benavent & Perez-Ortega (2015)
Cladosporium delicatulum	Unknown	Seed endophyte Putative mycoparasite	Found in cereal seeds, mycoparasite of *Taphrina spp* and *Magnaporthe oryzae*.	Amanelah Baharvandi & Zafari (2015) ; Chaibub *et al.* (2016).
Curvibasidium cygneicollum	Unknown	Generalist ubiquitous Putative mycoparasite	Endophyte of fruits, leaves, trunk and soil behaving as a phytopathogen or a mycoparasite. The species is insensitive to the mycocins produce by *Filobasidium* and *Cystofilobasidium*.	Sampaio *et al.* (2004); Mašínová *et al.* (2017)
Cylindrium elongatum	Unknown	Fungal antagonist Ubiquitous	Bacterial and fungal antagonist found on oak leaves.	Reyes-Estebanez (2011) ; Duarte *et al.* (2015)
Cystofilobasidium capitatum	Unknown	Seed endophyte Putative mycoparasite	Ubiquitious yeast which colonize the cotyledon after germination of english oak. The genus *Cystofilobasidium* have hyphal structures with haustoria only in the dikaryotic phase suggesting mycoparasites capacities.	Oberwinkler *et al.* (1983); Isaeva *et al.* (2009); Bills, Muller & Foster (2004)
Epicoccum nigrum	Pathotroph	Plant pathogen	Ubiquitous fungus found in soil, leaves and seeds described as primary saprotroph and plant pathogen.	Ahumada-Rudolph *et al.* (2014)
Filobasidium wieringae	Saprotroph	Generalist ubiquitous Putative mycoparasite	Yeast found in the phyllosphere. The genus *Filobasidium* have hyphal structures with haustoria only in the dikaryotic phase suggesting mycoparasites capacities.	Glushakova & Kachalkin (2017); Bills, Muller & Foster (2004)
Mycosphaerella tassiana	Pathotroph	Plant pathogen	Common pathogen found in the phyllosphere including that of oak.	Schubert *et al.* (2007); Jakuschkin *et al.* (2016)
Polyscytalum algarvense	Pathotroph	Plant pathogen	Necrotroph fungi found on *Eucalyptus* leaves.	Cheewangkoon *et al.* (2009); Crous (2018)
Rhodosporidiobolus colostri	Unknown	Fungal antagonist Ubiquitous	Ubiquitious yeast behaving as a fungal antagonist.	(Golubev & Tomashevskaya, 2009)
Sphaerulina amelanchier	Symbiotroph	Leaf and litter endophyte	Found in leaf litter of *Amelanchier*, *Betula*, *Castanea* and *Quercus*.	Quaedvlieg *et al.* (2013)
Taphrina carpini	Pathotroph	Plant pathogen	Common pathogen encountered on *Fagaceae* leaves.	Bacigálová (1991); Inácio *et al.* (2004); Cordier *et al.* (2012)
Trichomerium foliicola	Symbiotroph	Leaf epiphyte	Foliar epiphytes. *Trichomerium* is a genus apparently gaining their nutrients from insect exudates.	Chomnunti *et al.* (2012)
Vishniacozyma victoriae	Unknown	Generalist ubiquitous Fungal antagonist	Microbial antagonist of *Penicillium expansum* and *Botrytis cinerea*. Ubiquitous yeast able to tolerate stressful environments.	Pertot *et al.* (2017); Santiago *et al.* (2016)
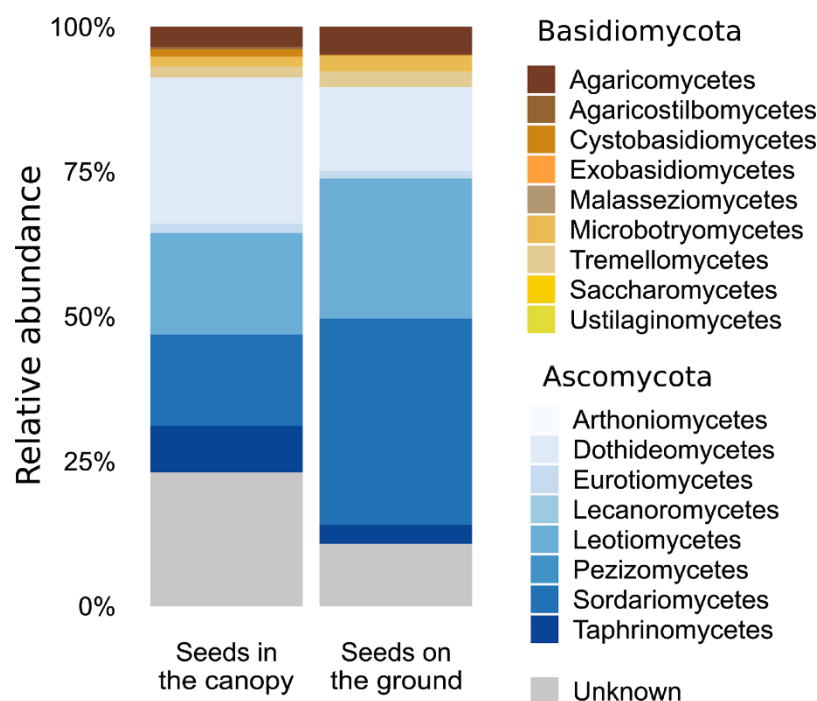
Figure S1. Taxonomic composition of fungal communities of seeds of sessile oak. The barplot indicates the average sequence percentage assigned to each fungal class.
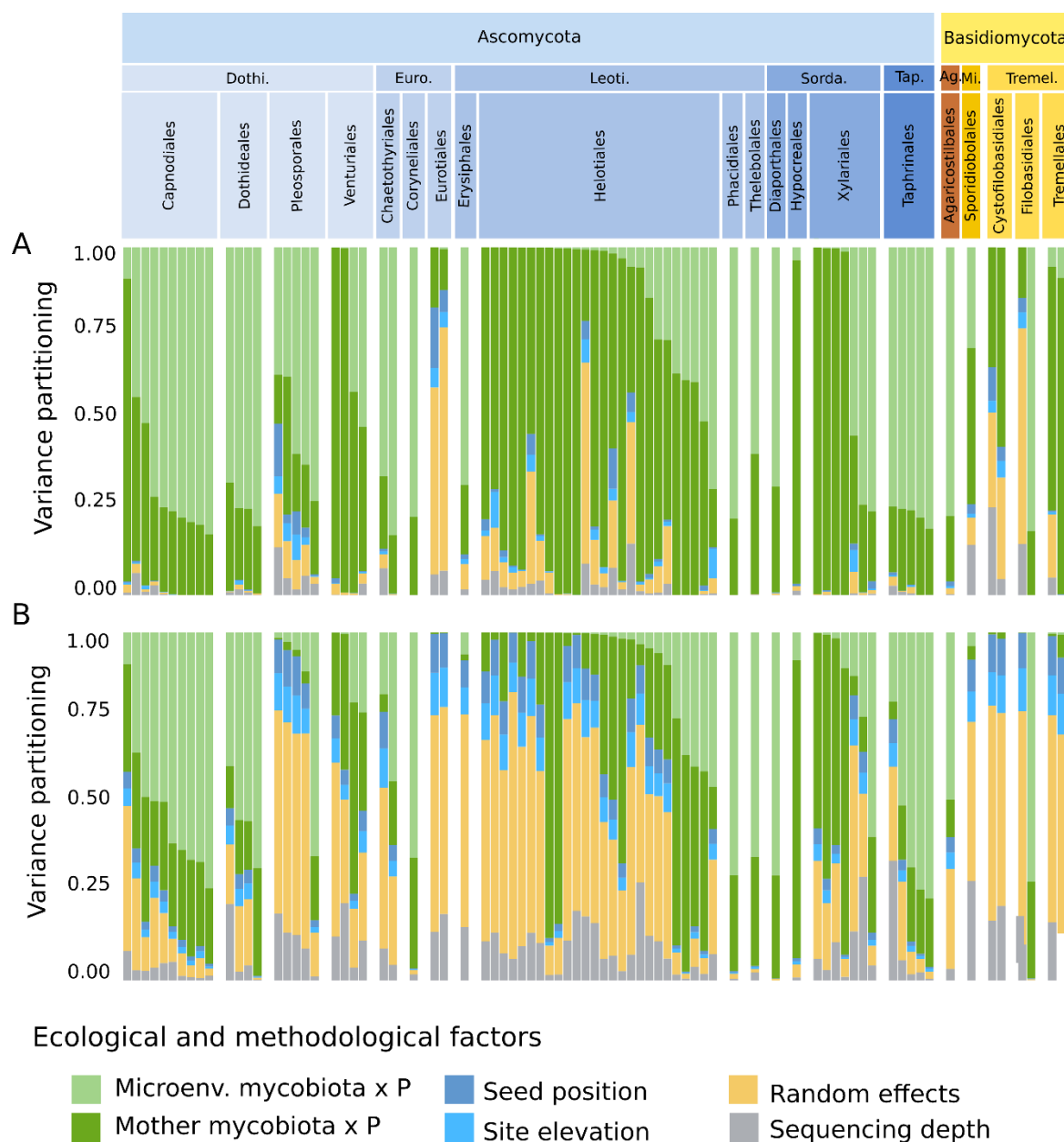
Figure S2. Partitioning of the variance in the composition of seed fungal communities of sessile oak. Four fixed effects were included in the HMSC models to explain variations in (A) the presence-absence or (B) the sequence count of a focal fungal ASV among seed samples: *Sequencing depth* (total number of sequences per sample), *Seed position* (P, canopy or ground), *Microenv. mycobiota* (relative abundance of the focal ASV in the seed microenvironment), *Mother mycobiota* (relative abundance of the focal ASV in the mother tree aboveground tissues), and *Site elevation*. *Mother mycobiota* and *Microenv. mycobiota* were introduced in interaction with P. Random effects were included at each spatial scale (seed, tree and population). Results of variance partitioning are given as percentages (%) of total explained variance. ASV are ranked by fungal phylum, class and order (Dothi: *Dothideomycetes*, Euro: *Eurotiomyecetes*, Leo: *Leotiomycetes*, Sorda: *Sordariomycetes*, Tap: *Taphrinomycetes*, Ag: *Agaricostilbmoyecetes*, Mi: *Microbotryomycetes*, Tremel: *Tremellomycetes*). Only ASVs assigned at the order level are shown.