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ORIGINAL ARTICLE

Combining high-throughput sequencing with fruit body surveys reveals contrasting life-history strategies in fungi

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Before the recent revolution in molecular biology, field studies on fungal communities were mostly confined to fruit bodies, whereas mycelial interactions were studied in the laboratory. Here we combine high-throughput sequencing with a fruit body inventory to study simultaneously mycelial and fruit body occurrences in a community of fungi inhabiting dead wood of Norway spruce. We studied mycelial occurrence by extracting DNA from wood samples followed by 454-sequencing of the ITS1 and ITS2 regions and an automated procedure for species identification. In total, we detected 198 species as mycelia and 137 species as fruit bodies. The correlation between mycelial and fruit body occurrences was high for the majority of the species, suggesting that high-throughput sequencing can successfully characterize the dominating fungal communities, despite possible biases related to sampling, PCR, sequencing and molecular identification. We used the fruit body and molecular data to test hypothesized links between life history and population dynamic parameters. We show that the species that have on average a high mycelial abundance also have a high fruiting rate and produce large fruit bodies, leading to a positive feedback loop in their population dynamics. Earlier studies have shown that species with specialized resource requirements are rarely seen fruiting, for which reason they are often classified as red-listed. We show with the help of high-throughput sequencing that some of these species are more abundant as mycelium in wood than what could be expected from their occurrence as fruit bodies.
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Introduction

Field surveys of fungi are often based solely on fruit bodies because it is difficult to acquire observational data on mycelia, which are usually cryptic and morphologically indistinctive. As a consequence, in fungi inhabiting dead wood, knowledge on ecological characteristics such as resource use is mainly based on data on fruit bodies (Jonsson *et al.*, 2005; Junninen *et al.*, 2006; Ódor *et al.*, 2006; Penttilä *et al.*, 2006; Hottola *et al.*, 2009; Gates *et al.*, 2011). Much less is known about the processes of dispersal (but see Edman *et al.*, 2004a, b; Norros *et al.*, 2012), mycelial growth and formation of fruit bodies (but see Straatsma *et al.*, 2001; Lindner *et al.*, 2011; Olsson *et al.*, 2011; Vetrovsky *et al.*, 2011), as these are still difficult to study under field conditions.

The total number of dead wood-inhabiting polypore and corticioid species is over 600 in Finland (Kotiranta *et al.*, 2009), in addition to which many species of other fungi such as ascomycetes and agarics also utilize dead wood as their resource. As dead wood is subject to decay, the species follow colonization extinction dynamics at the scale of individual pieces of dead wood, making the system highly dynamic. An individual dead tree can host a large number of fungal species as fruit bodies (for example, Stokland and Larsson, 2011) and

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especially as mycelia (for example, Ovaskainen *et al.*, 2010b; Rajala *et al.*, 2011; Kubartova *et al.*, 2012), which interact, typically compete (Boddy, 2000), with each other. Particular species-to-species interactions have been reported in laboratory studies (Holmer and Stenlid, 1997; Holmer *et al.*, 1997) and are likely to influence non-random co-occurrence patterns observed in the field (Ovaskainen *et al.*, 2010a).

In areas of intensive forest management, the diversity of fungi that inhabit dead wood has greatly declined (as evidenced by fruit bodies), and a large fraction of the species community is classified as red-listed in the Nordic countries (Brandrud *et al.*, 2010; Dahlberg *et al.*, 2010; Kotiranta *et al.*, 2010). As judged by fruit body occurrences, many species of fungi that inhabit dead wood have very specialized resource requirements, fruit bodies appearing for example, mainly at a specific decay stage of a specific host tree species (Nordén *et al.*, 2013). Earlier studies based on fruit body inventories have shown that the occurrence of highly specialized species is much higher in well-connected forests than in isolated fragments, while in generalist species such a response is largely lacking (Hottola *et al.*, 2009; Nordén *et al.*, 2013).

Recent advances in high-throughput sequencing have started a new era in fungal research, as it is now possible to study the previously hidden life stages under field conditions (Das *et al.*, 2008; Buée *et al.*, 2009; Gillevet *et al.*, 2009; Jumpponen and Jones, 2009; Jumpponen *et al.*, 2010; Lim *et al.*, 2010; Lumini *et al.*, 2010; Öpik *et al.*, 2010; Suchodolski *et al.*, 2010; Ovaskainen *et al.*, 2010b; Kubartova *et al.*, 2012). High-throughput sequencing has revealed patterns of high fungal diversity in environmental samples acquired from the soil (Buée *et al.*, 2009; Lim *et al.*, 2010) and dead wood (Ovaskainen *et al.*, 2010b; Kubartova *et al.*, 2012), in phyllosphere communities (Jumpponen and Jones, 2009) and in ectomycorrhizal (Jumpponen *et al.*, 2010) and endomycorrhizal fungi (Lumini *et al.*, 2010).

Most biodiversity oriented studies applying high-throughput sequencing have so far been based on the classification of the sequences into operational taxonomical units (Zhang *et al.*, 2008; Gillevet *et al.*, 2009; Jumpponen and Jones, 2009; Jumpponen *et al.*, 2010; Lumini *et al.*, 2010; Kubartova *et al.*, 2012). Although operational taxonomical units provide a practical means of examining patterns of species diversity and community turnover, being able to identify the environmental sequences to the species level would make it possible to relate sequence data to information acquired by other methods, such as fruit body surveys. Molecular identification, however, requires the availability of a reliable and high coverage reference database against which the environmental samples can be compared (Öpik *et al.*, 2010; Ovaskainen *et al.*, 2010b), and an assessment of the reliability of the identifications (Ovaskainen *et al.*, 2010b).

In this paper, we combine (i) 454-pyrosequencing of environmental samples, (ii) an extensive annotated reference database, (iii) an automated method of molecular species identification and (iv) a conventional fruit body inventory to study the relationship between mycelial and fruit body occurrences in a community of fungi inhabiting dead wood. We first examine whether 454-sequencing applied to wood samples carries over a reliable signal of the dominating fungal community inhabiting a log. To do so, we measure a number of population dynamic parameters for each species, namely prevalence and abundance as DNA and as fruit bodies, fruiting rate and time delay in formation of fruit bodies. We use these data to conduct species-specific analyses, which contrast patterns of DNA and fruit body occurrences. We then move to the community level, where we test hypothesized links between different life history parameters and population dynamic parameters. The specific hypotheses are illustrated in Figure 1 and described in detail in Materials and methods section after defining the relevant parameters related to population dynamics and the species traits.

Materials and methods

Sampling scheme

In November 2008, we took wood samples from 100 Norway spruce (*Picea abies*) logs in a protected natural-like spruce-dominated forest (Rörstrand, Sipoo) 30 km north of Helsinki, Finland. The logs were all large (20–42 cm in diameter), and covered the range of decay classes from 1 to 4, that is, from little to strongly decomposed (*cf.* Hottola and Siitonen, 2008). As the stage of decomposition varies along the log, we estimated an average decay class by weighting each class with the volume of wood in that class. The number of logs in decay classes 1 to 4 was 19, 31, 34 and 16, respectively. We also measured a number of physical and chemical parameters of the log (wet weight, dry weight and mass percentages for C and N), but use in this paper decay class as a proxy for the stage of decay. The samples were obtained using a 10 mm electric drill, except for strongly decayed logs, for which a sampling cylinder was used. The logs had variation in the fall type (uprooted or broken) and in their ground contact. Epixylic bryophytes and lichens, tree bark as well as 2–3 mm of surface wood were removed from the drilling points before the sampling. Each log was sampled at 1, 2.5, 4, 5.5 and 7 m from the base (representing the basal part of the log), and at 9, 10.5, 12, 13.5 and 15 m from the base (representing the middle part of the log). The resulting 1000 samples were combined to 200 pooled samples: two for each log, representing the basal and the middle parts of the log.

All logs were inspected for fruit bodies of polyporoid and particular easily recognized

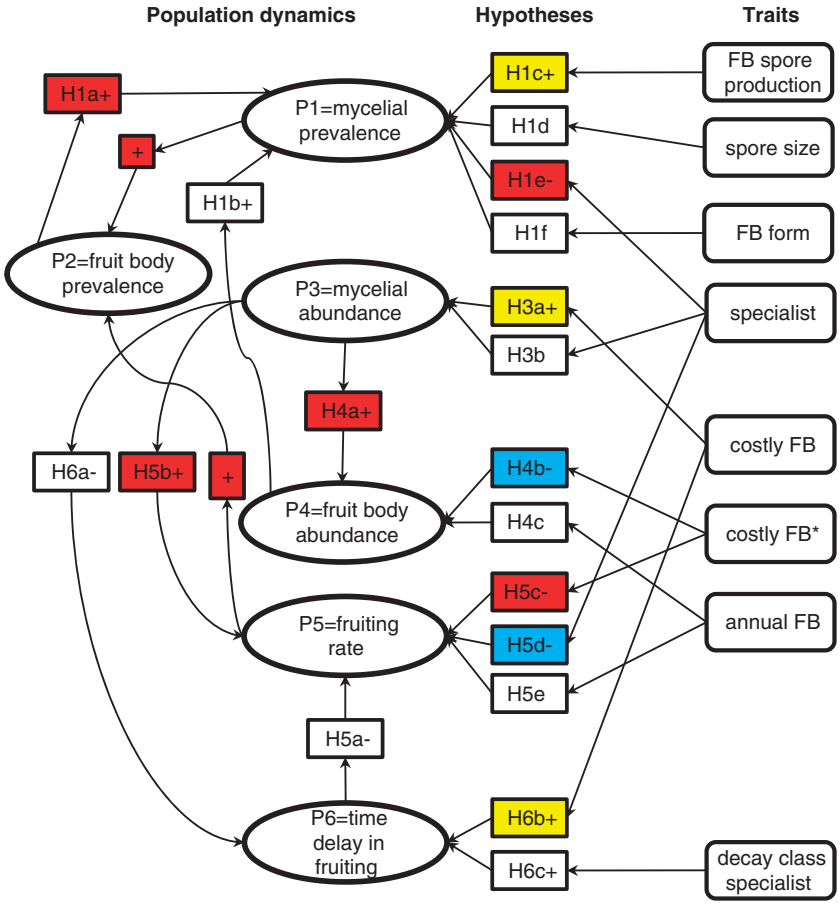

Figure 1 Hypothesized and observed links between species traits and population dynamics in the community of wood-inhabiting fungi. The hypothesized causal links (described in Materials and methods section) are indicated by the boxes with symbols + and − for cases where we expected a positive or negative influence, respectively. The colored hypotheses represent our interpretation of the results (Table 3) so that red, blue and yellow refer to cases where we found evidence for a positive, negative or mixed (depending on the specific parameter used to measure spore production or the cost of fruit body production) effect, respectively. For example, we expected and found (Table 3; column H4, row P3) that fruit body abundance is the highest for species, which reach a high mycelial abundance (hypothesis H4a+). For another example, we expected that fruit body abundance is lower for species for which the production of fruit bodies is costly (H4b−), which expectation is in line with the result that species with resupinate fruit bodies reach a higher fruit body abundance than species with pileate fruit bodies (Table 3; column H4, row E3). Costly fruit body (FB) refers to the following combination of traits: perennial, thick, pileate and dimitic. Costly FB* refers to the same combination except that the duration of the fruit body is not included. Fruit body (FB) spore production (per season and unit area) is assumed to be the highest for perennial and dimitic fruit bodies.

corticioid and hydnoid basidiomycetes twice in 2008 (September and November). Another survey including all polyporoid, corticioid and hydnoid basidiomycetes as well as particular easily recognized ascomycetes was carries out in 2009 (October). The list of surveyed species is given in the Supplementary Information. We used multiple surveys to account for species-specific variation in timing and duration of fruiting (Halme and Kotiaho, 2012). We included both living and dead fruit bodies for species with annual fruit bodies but only living fruit bodies for species with perennial fruit bodies. Fruit bodies that could not be reliably identified in the field were sampled and identified microscopically. In the latter two fruit body surveys, the hymenophore area of each occurrence of all polypores and those corticioids and hydnoid species that could be reliably identified in the field was estimated to give a measure of abundance per log. In the present work, we use fruit body findings from the first 16 m of the log length, that is, the part of the log from which wood samples were taken, up to 1 m after the last drilling hole (in 15 m).

DNA extraction and sequencing

Wood samples (*ca* 10–50 g each) from each drilling point were stored at −20 °C, and the samples were mixed (see above) to two per log before DNA extraction. The mixed samples were grinded using liquid nitrogen. One DNA extraction from 150 to 250 mg (fresh weight) of wood was carried out for each sample using Power Soil DNA isolation kit (MoBio Laboratories, Inc., Carlsbad, CA, USA). Release of DNA was performed by shaking the samples horizontally (Vortex Genie 2, Scientific Industries, Bohemia, NY, USA; Vortex Adapter, MoBio Laboratories, Inc.) for 15 min and incubating

for 45 min at 60 °C. Samples were amplified with 35 cycles using Taq polymerase enzyme (Thermo Fisher Scientific, Waltham, MA, USA) with the primers ITS1 and ITS4 (White *et al.*, 1990) to verify that PCR is functional, that is, the compounds that might inhibit the reaction were successfully removed from the sample. The lack of PCR product, especially in case of some strongly decomposed samples, was considered to be caused by remaining sample impurities, and an additional precipitation with polyethylene glycol (PEG) was performed according to the protocol of Vainio *et al.* (1998).

Real-time quantitative PCR

Real-time quantitative PCR (qPCR) assays were performed on the 18S ribosomal RNA gene with primer pair FF390–FR1 (Vainio and Hantula, 2000), as recommended by Prevost-Boure *et al.* (2011). All samples with at least 0.05 ng μl^{-1} DNA concentration, measured with Qubit fluorometer (Invitrogen by Life technologies, Carlsbad, CA, USA), were included in the qPCR analyses. These 163 samples (out of 200) were then diluted into 0.05 and 0.025 ng μl^{-1} total DNA concentrations for the reactions. The reaction mixtures contained 1 × ABsolute qPCR SYBR Green mix (ABgene, Thermo Fisher Scientific), 0.5 μM of both primers, and 0.05 or 0.025 ng of template DNA, making a final volume of 10 μl^{-1}. The reactions were run with CFX384 Real-Time PCR detection system (Bio-Rad, Hercules, CA, USA) according to the following program: initial step at 95 °C for 15 min, followed by 40 cycles of 15 s at 95 °C, 30 s at 52 °C and 30 s at 72 °C. After the amplification, a melting curve was created from 70 °C to 90 °C with 0.5 °C increments per second to verify the specificity of the PCR product.

Each reaction plate contained three negative controls and three standard dilution series on which the generation of a standard curve was based. The standards were prepared by amplifying fungal DNA (with Taq DNA polymerase, Fermentas, Thermo Fisher Scientific) of three different species, extracted from pure cultures. The PCR product was run on an agarose gel, from which the product was cut off, purified with Illustra GFX purification kit (GE Healthcare, Buckinghamshire, UK) and measured for concentration with Qubit fluorometer (Qubit, Invitrogen by Life Technologies, Carlsbad, CA, USA). A fourfold dilution series was prepared from 0.05 ng μl^{-1} downward to eight standard concentrations. Both the standard and the sample reactions were run in triplicates.

454-Sequencing protocol

We processed the samples following the procedure of Ovaskainen *et al.* (2010b) with some modifications. Altogether *ca* 400 PCR fragments were produced for the primers ITS1 and ITS2. We used Phusion polymerase (Finnzymes, Espoo, Finland) with buffer GC for all of the amplifications in 50 μl reactions. PCR cycles as following were used: 98 °C 30 s, (98 °C 10 s, 55 °C 30 s × 15–25), 72 °C 30 s, 4 °C 5 min. The PCR reactions were not replicated because of low amount of sample DNA.

For those 297 samples that had a DNA concentration >1.43 ng μl^{-1}, the PCR reactions were directly amplified as follows. For ITS1 region, we used the primer pair ITS1F (Gardes and Bruns, 1993) and ITS2 (White *et al.*, 1990). The composite primer for ITS1F contained of the 454-A-adapter sequence followed by a tag and the specific sequence (5′-GCCTCCCTCGCGCCATCAG tag 6-bp CTTGGTCATT TAGAGGAAGTAA-3′), whereas the composite primer for ITS2 contained the 454-B-adapter sequence followed by the specific sequence (B-adapter 5′-GCC TTGCCAGCCCGCTCAGGCTGCGTTCTTCATCGAT GC-3′). For ITS2 region, we used the primer pair ITS4 and ITS3 (White *et al.*, 1990). The composite primer for ITS4 contained the 454-A-adapter sequence followed by a tag and the specific sequence (5′-GCCTCCCTCGCGCCATCAG tag 6-bp TCCTCCGCTTATTGATATGC-3′) and the composite primer for ITS3 contained the 454-B-adapter sequence followed by the specific sequence (5′-GC CTTGCCAGCCCGCTCAGGCATCGATGAAGAACGC AGC-3′).

For those 97 samples (54%, 24%, 21% and 6% of the samples in decay classes 1, 2, 3 and 4, respectively) that had a DNA concentration <1.43 ng μl^{-1}, the PCR reactions for both ITS1 and ITS2 regions were first done with primers lacking the A and tag sequences so that ITS1F and ITS4 were used in a shorter form, ITS1F (5′-CTTGGTCATTTAGAGG AAGTAA-3′) and ITS4 (5′-TCCTCCGCTTATTGATA TGC-3′). The obtained PCR reactions were then diluted and amplified like in the direct PCR using primers with tag sequences and A sequences for ITS1F and ITS4. We were unable to obtain PCR fragments for six samples.

All PCR reactions were separately purified with AMPure (Agencourt Bioscience Corporation, Beverly, MA, USA) and concentrations measured with Qubit (Invitrogen by Life Technologies) before dilution for the 454 emPCR. The sequencing was done on a Genome Sequencer FLX (454 Life Sciences, Roche, Branford, CT, USA) as described in Ovaskainen *et al.* (2010b). The DNA sequences are archived at NCBI SRA: SRX033126.

Molecular species identification

We used the BLAST-based algorithm of Ovaskainen *et al.* (2010b) for molecular species identification. The reference sequences, including 2826 specimens of 1290 species, originate from the UNITE database of ectomycorrhizal asco- and basidiomycetes (Kõljalg *et al.*, 2005), and a custom-made reference database SAF of spruce-associated fungi (Ovaskainen *et al.*, 2010b). At the identification stage, we treated the sequence data from the ITS1 and ITS2 regions

independently. As a preliminary filtering step, we excluded all sequence reads that were shorter than 150-bp long. We did not cluster the sequences to operational taxonomical units, but used a BLAST search to compare all sequences individually against the reference database. To assess the confidence of species identifications, we modeled the probability of correct identification (as in Ovaskainen *et al.*, 2010b) as a function of sequence similarity, the difference in sequence similarity between the best and the second best BLAST match, sequence length and the database (UNITE or SAF) to which the reference sequence belonged to.

To link the sequence data to the fruit body data, we selected for subsequent analyses those sequences for which the probability of correct species identification was at least 90%. This implies not only a high level (typically at least 98%) of sequence similarity between the environmental and reference sequences, but also that the best reference sequence is unique in the sense that there is no other species in the reference database with almost equally high sequence similarity.

Data preparation

For the present analyses, we use the individual logs as sampling units. We thus pooled the data on the basal and middle parts of each log, both for the fruit body data (by summing the hymenophore areas) and for the DNA data (by summing the sequence counts). We further pooled the DNA data based on the ITS1 and ITS2 sequences (by summing the sequences counts), and we pooled the fruit body data over the three surveys (by taking the average of hymenophore area). Thus, the data consist of the presence–absence and abundance of each species on each log, both as fruit bodies and as DNA. Relative DNA abundance was measured as the fraction of sequences identified to the focal species (out of all sequences, not only the identified ones). Absolute DNA abundance was measured by multiplying the relative abundance by an estimate of fungal DNA concentration. Fungal DNA concentration was estimated as the product of total DNA concentration and the fraction of fungal DNA out of all DNA. The latter was obtained from the qPCR measurements by a comparison with dilution series based on pure fungal cultures (for further details, see Supplementary Information). All abundance measures were $\log_{10}$-transformed (Table 1).

Comparisons among fruit bodies and DNA are meaningful only for those species that could have been observed using both methods. We thus defined the target community as the set of species that we could have observed as fruit bodies and as DNA, independently of whether they were actually observed or not. A species was included in the target community if at least one sequence of the species was included in the reference database, and if the species was considered in the fruit body inventory (all polypores, corticioids and hydnoid species, and particular ascomycete species). The target community consisted of 561 species (Supplementary Information).

Species-level analyses

We first computed seven species-specific population dynamical parameters (P1–P6, with two versions of P3; Table 1) based on the DNA and fruit

Table 1 Species-specific parameters derived from the fruit body and sequencing data

Parameter	*Explanation*	*Criteria*	N
P1: DNA prevalence	Fraction of logs in which the species was observed as mycelia (detected from DNA)	—	561
P2: fruit body prevalence	Fraction of logs on which the species was observed as fruit bodies	—	561
P3R: mean relative DNA abundance	$\log_{10}$ (fraction of sequences belonging to the target species), averaged over all logs in which the species was detected as DNA	DNA prevalence at least 0.02	99
P3A: mean absolute DNA abundance	$\log_{10}$ (total amount of DNA belonging to the target species), averaged over all logs in which the species was detected as DNA	DNA prevalence at least 0.02	99
P4: mean fruit body abundance	$\log_{10}$ (hymenophore area), averaged over all logs on which the species was present as fruit bodies and the hymenophore area was measured	Fruit body prevalence (with hymenophore area measured) at least 0.02	32
P5: fruiting rate	Among the logs in which the species was detected as DNA, the fraction of logs on which the species was observed as fruit bodies	Prevalence as DNA at least 0.02	99
P6: time delay in fruiting since colonization by mycelia	The difference between decay class of mycelial appearance (as detected from DNA) and the decay class of fruit body appearance	Prevalence both as fruit bodies and as DNA at least 0.05, and the number of logs with fruit body observations but no DNA observations at most 0.5 times the number of logs with DNA observations	20

Criteria gives the data requirement for computing the parameter values, and *N* shows the number of species within the target community for which the parameter was computed.

body data. We then performed a set of species-specific analyses for those species for which prevalence both as DNA (P1) and as fruit bodies (P2) was at least 0.05 ($\geqslant$5 occurrences on the 100 logs). For each species, we used Fisher's exact test to examine if the occurrences as DNA and as fruit bodies were more often than by random on the same logs. We applied $P = 0.05$ as the threshold value for significance in all tests in this paper. We then selected those logs in which the species was present as DNA, and used logistic regression to explain the presence–absence of fruit bodies by the relative (P3R) and absolute (P3A) abundances of the species as DNA. Finally, we selected those logs in which the species was present both as DNA and as fruit bodies and used linear regression to test if fruit body abundance (P4) was explained by relative (P3R) or absolute (P3A) abundance as DNA. As it is evident that a species can produce fruit bodies only if it is present as mycelia, these tests were partly motivated as a data quality check. In the case of fruit body data, the main source of uncertainty is related to seasonal and year-to-year variation in the occurrence of fruit bodies (Halme and Kotiaho, 2012), whereas in the case of DNA, the main sources of uncertainty include sampling and PCR biases, uncertainty associated with identification of the obtained sequences to the species level, and the fact that presence of DNA does not necessarily imply the presence of viable mycelia.

We defined the fruiting rate (P5) for each species as the fraction of logs on which the species was found as fruit bodies out of those logs in which the species was detected as DNA. As we did not have time-series data, we inferred the decay class in which the species typically first appeared as DNA (or as fruit bodies) by comparing prevalences among different decay classes. We used this information to infer the average time delay (P6) from mycelial colonization until fruit body appearance using the formula given in Supplementary Information.

To examine how DNA abundance (relative or absolute) and fruit body abundance develop over the course of decay, we fitted second-order regressions with decay class as an explanatory variable. The second-order term models the possibility of a maximal abundance at an intermediate decay stage. If this term was not significant, we dropped it and performed a linear regression.

Community-level analyses

As discussed in the Introduction section, relationships between species occurrences and their ecological and life history characteristics have been studied earlier mostly based on fruit body occurrence data. The main purpose of this study was to bring information on mycelial occurrence (as measured by DNA) into such analyses. More specifically, our aim is to test the hypotheses illustrated in Figure 1, which ask how life history variation (Table 2) shape population dynamic feedbacks in the fungal life cycle. Underlying our hypotheses, H1–H5 is the r/K selection theory (MacArthur and Wilson, 1967) according to which species can be characterized as r selected ('colonizers') or K selected ('competitors') based on their life history strategies. The r selected species allocate a higher share of their resources to reproduction than K selected species, which are in turn often more long-lived and more combative.

We hypothesized that DNA prevalence (P1) increases with the rate of spore deposition on suitable unoccupied logs. This is in turn expected to increase with the total rate of spore production at the forest stand level, which is a product of fruit body prevalence (H1a), fruit body abundance (H1b) and rate of spore production per unit area and per season (H1c). The last of the above-mentioned factors is expected to be higher for fruit bodies, which are perennial and more robust in their structure (di- or trimitic hyphal system). The role of spore size is dual (H1d), as smaller spores can be produced in greater quantity but they are likely to be less tolerant to environmental stress during dispersal. Spore size may further influence

Table 2 Species-specific parameters relating to life history and phylogenetic position

Parameter	*Explanation*	*Type*	N
E1: ecological specialization	High for species that produce fruit bodies only on certain kinds of logs or forest stands. Taken from Nordén *et al.*, 2013.	C	46
E2: specificity in decay class	High for species that produce fruit bodies only at a specific decay stage. Log-transformed version of the same measure in Nordén *et al.*, 2013.	C	46
E3: fruit body shape	Pileate (including fruit bodies with a stipe, puffballs and discs) or resupinate (including effused-reflexed) fruit body	D	175
E4: fruit body thickness	$\mathrm{Log}_{10}(_{\max}(1\text{ mm}, \text{fruit body thickness}))$	C	177
E5: fruit body duration	Annual or perennial fruit body	D	177
E6: fruit body hyphal system	Monomitic or dimitic (including trimitic)	D	176
E7: spore size	$\mathrm{Log}_{10}(V)$, where spore volume $V = \text{length} \times \pi \times (\text{width}/2)^2$	C	177
E8: order	Fungal order (Karl-Henrik Larsson, personal communication)	D	177

For type, C stands for continuous and D for discrete (each with two categories, except fungal order with 24 categories) variables. *N* shows the number of species for which the parameter was available (and could be classified within the listed categories) among those 177 species detected in our study either as DNA or as fruit bodies.

population dynamic processes through dispersal to either direction because of the costs and benefits of local vs long-range dispersal (for example, North *et al*., 2011). Species with specialized resource requirements may be able to establish only on certain types of logs (H1e). Fruit body shape (thickness and resupinate vs pileate shape) can correlate with spore production and thus with DNA prevalence but we did not have *a priori* expectation of the direction of the effect (H1f).

As fruit body prevalence (P2) is by definition DNA prevalence (P1) times fruiting rate (P5), we did not associate a hypothesis for factors influencing fruit body prevalence.

We expected that DNA abundance (P3) is high for species, which produce costly fruit bodies because such species are likely to require a large amount of mycelial resources to reproduce (H3a). Species with specialized resource requirements can be expected to be competitive under conditions in which they occur, leading to high mycelial abundance. However, such species may be present with low mycelial abundance in resource units, which are not suitable for them, and thus we did not formulate an expected direction for hypothesis H3b.

Concerning fruit body abundance (P4), we hypothesized that species that are abundant as mycelia are able to produce large hymenophore areas (H4a). For a given amount of mycelial resources, fruit body abundance is expected to decrease with the cost needed to produce a unit area of fruit body hymenophore (H4b). Fruit body duration has a double role, as perennial fruit bodies are supposedly more costly to produce, but the size of a perennial fruit body can accumulate over a number of years (H4c).

We hypothesized that fruiting rate (P5; fraction of logs on which the species occurs as fruit bodies out of all logs where it is detected as DNA) decreases with increasing time delay in fruiting (H5a), and increases with the species' ability to gain a dominating position within the mycelial community (H5b). We expected fruiting rate to decrease with the cost of fruit body production (H5c) and with the degree that the species is specialized to produce fruit bodies only on particular types of logs (H5d). Fruit body duration again has a double role, as perennial fruit bodies are supposedly more costly to produce than annual ones, but they also remain longer on the log. Thus, we did not specify an expected direction for the hypothesis H5e.

We hypothesized that species that obtain a high mycelial abundance are able to produce fruit bodies with a short time delay (H6a). We expected time delay to increase with the cost of fruit body production (hypothesis H6b), which cost we assumed to be the highest for thick, perennial fruit bodies with skeletal and in some species also binding hyphae (di- or trimitic hyphal system; compare with only generative hyphae, monomitic hyphal system). We hypothesized that time delay in fruiting is higher for those species, which are specialized to produce fruit bodies at a particular decay stage (H6c).

We tested the above community-level hypotheses using data on those 157 species, which were observed in our study at least once (either as DNA or as fruit bodies), and considered each species in these analyses as one data point. We computed a mean abundance for each species by averaging over the log-specific abundances, and log-transformed the DNA and fruit body prevalences.

We addressed each of the five hypotheses with the help of a linear mixed model. The fixed effects included in the models stem directly from the hypotheses (Figure 1), and are indicated in Table 3. We first constructed the full model consisting of the fixed effects and the random effect of the

Table 3 Results from linear mixed models testing the hypotheses of Figure 1

Hypothesis	*H1*	*H3*	*H3*	*H4*	*H4*	*H5*	*H5*	*H6*	*H6*
Weighting	*U*	*U*	*W*	*U*	*W*	*U*	*W*	*U*	*W*
Variable									
P2	0.45***	NA	NA	NA	NA	NA	NA	NA	NA
P3R	NA	NA	NA	0.87**	0.71**	0.16***	0.16***		
P4		NA	NA	NA	NA	NA	NA	NA	NA
P6	NA	NA	NA	NA	NA			NA	NA
E1	0.49*			NA	NA	−0.13*	−0.12*	NA	NA
E2	NA	NA	NA	NA	NA	NA	NA		
E3		0.26*	0.28**		0.77*				
E4									−0.37*
E5	−1.18**		−0.38*						−1.0*
E6	1.07**	−0.63*	−0.37*			−0.32***	−0.33***		
E7		NA	NA	NA	NA	NA	NA	NA	NA
E8									

Shown are the coefficient estimates of those variables that were included in the final model. The coefficients for categorical variables show the effect of being (E3) resupinate (pileate as reference), (E5) annual (perennial as reference) or (E6) monomitic (dimitic or trimitic as reference). Significance is indicated as $*P \leqslant 0.05$, $**P \leqslant 0.01$, $***P \leqslant 0.001$. For each model, version U is based on unweighted analysis, and version W on weighted analysis (see Materials and methods section). NA indicates that the variable was not part of the hypothesis and thus not included in the model selection procedure.

fungal order, which was included to correct for phylogenetic dependence. If the random effect was not significant ($P > 0.05$ in a likelihood ratio test), we dropped it. We then performed backward model selection until all the remaining fixed effects were significant at the 0.05 level. All analyses were performed with the nlme R-package (Pinheiro *et al.*, 2012).

For models related to hypotheses H3–H6, we considered two versions of each model, where in version U we weighted all species equally, whereas in version W we weighted the species by the amount of data (square root of DNA prevalence for H3 and H5, square root of fruit body prevalence for H4, and the square root of the minimum of DNA and fruit body prevalences for H6). For models where some of the explanatory variables had especially many missing values (time delay in fruiting, fruit body abundance, ecological specialization, specificity of decay class), we performed model selection in such a way that these covariates were excluded from the initial set of explanatory variables. After model selection with the remaining variables, these variables were added to the model if significant at the 0.05 level.

Results

Numbers of sequences and species

The sequencing resulted in a total of 638 357 sequences with length at least 150 bp, out of which we identified 488 902 sequences (77%) to the genus level with at least 90% probability of correct identification. Out of these, 222 142 sequences (35% of all sequences) were identified to the species level with at least 90% probability, representing altogether 198 different species (Figure 2). The three fruit body surveys resulted in observations of a total of 137 species. Among the target community, that is, species that we could have detected both as DNA and as fruit bodies, we identified 133 species from DNA (represented by 209 239 sequences) and 99 species from fruit bodies. The species that were detected as DNA but excluded from the target community were ectomycorrhizal asco- and basidiomycetes that were included in the reference sequence database but not considered in the fruit body inventory, whereas species observed as fruit bodies but excluded from the target community were species for which no reference sequences were available.

The mean number of species (per individual log) detected as DNA was 16.0, with a range from 3 to 40 (for the target community, mean 11.2, with a range from 2 to 26). The mean number of species observed as fruit bodies in any of the three surveys was 9.2, with a range from 0 to 20 (for the target community, mean 7.6, with a range from 0 to 18). The number of species detected from DNA increased monotonously with decay class whereas the number of species observed as fruit bodies peaked at the third decay class (Figure 3). Data on fruit body and DNA prevalence and abundance for all species are presented in the Supplementary Information.

Physicochemical properties of the wood and qPCR results

Wet weight was much greater in decay class 4 than in the other decay classes, the concentration of nitrogen increased more than fivefold as decay class increases from 1 to 4, whereas the concentration of carbon stayed stable (Supplementary Information). Technical

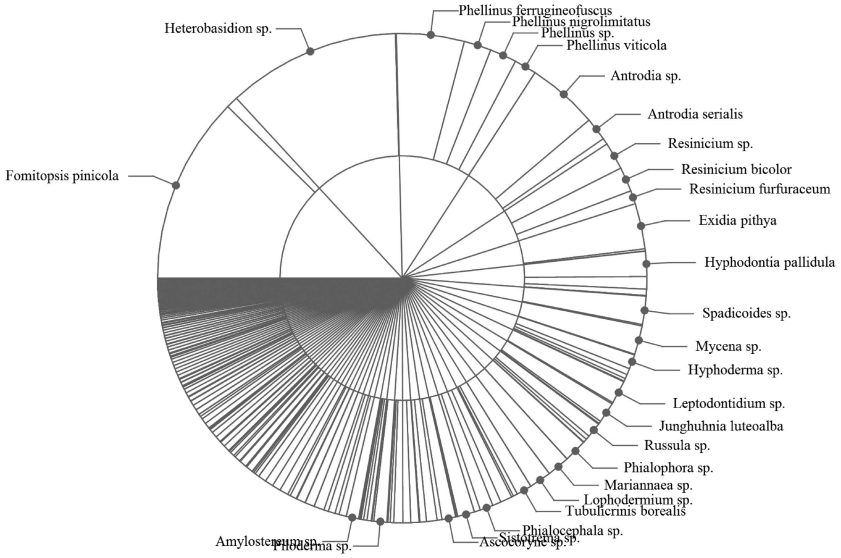

Figure 2 Species and genera of fungi identified from 454-sequencing data in 100 Norway spruce logs. The wheel shows the distribution of those sequences that were identified either to the genus level or to the species level with at least 90% probability of correct identification. The inner part of the wheel represents the genera and outer part the species. For the sake of readability, species names are shown only for those cases, which yielded at least 4000 sequences. For the data on all species, see Supplementary Information.

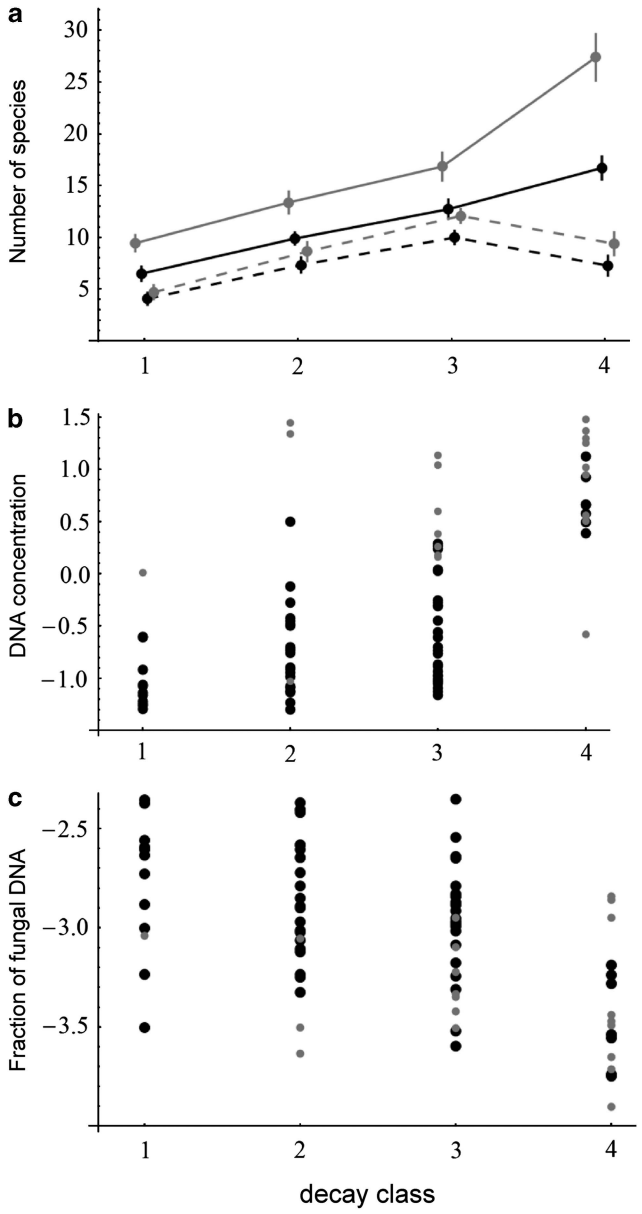

Figure 3 Patterns of mycelial (as detected from DNA) and fruit body occurrence. (**a**) Shows the mean number of species detected as DNA (continuous lines) or as fruit bodies (dashed lines), either for the target species community (black) or for all observed species (grey). The error bars show ±1 s.e. (**b**) Shows the total DNA concentration and (**c**) the fraction of fungal DNA out of all DNA (both $\log_{10}$ transformed) across decay classes and samples for which PEG treatment was applied (grey dots) and was not applied (black dots). Treating decay class as a nominal variable (with three degrees of freedom), the influence of decay class is highly significant in both panels ($P<0.001$), as is the influence of PEG ($P<0.001$ in **a** and $P=0.001$ in **b**).

variability among the qPCR measurements was much lower than biological variability (Supplementary Information). The total DNA concentration increased with decay class whereas the fraction of fungal DNA out of all DNA decreased with it (Figure 3). The PEG treatment was associated with increased DNA yield and decreased fraction of fungal DNA out of all DNA (Figure 3). To examine the robustness of the results against the PEG treatment, we performed all analyses related to DNA abundance both with including and excluding the samples with the PEG treatment.

Species-level analyses

Figures 4 and 5 exemplify the species-level analyses for two species. Out of these *Fomitopsis pinicola* shows a very high fruiting rate and virtually no time delay from mycelial establishment (as inferred from DNA presence) until fruit body formation (Figure 4). *Phellinus nigrolimitatus* shows a contrasting pattern, that is, a low fruiting rate explained by a long time lag from mycelial colonization until fruit body formation (Figure 5). For similar graphs for those 28 other species with sufficient data for producing the graphs, see Supplementary Information.

Out of the 30 species for which the number of occurrences was at least five both as fruit bodies and as DNA, for 24 cases the species was significantly ($P<0.05$) more often present as fruit bodies on those logs in which it was detected as DNA than on those logs in which it was not detected as DNA, whereas the remaining six cases showed nonsignificant associations. However, as expected because of the very limited size of the wood sample, fruit bodies were observed also on logs in which the species were not detected as DNA. For 6 out of the 30 species, a significant ($P<0.05$) positive relationship was found between DNA abundance and fruit body occurrence, whereas the remaining 24 cases showed nonsignificant relationships. The absolute and relative DNA abundances resulted in the same six species with a positive effect, the association being stronger (in terms of smaller *P*-value) for relative DNA abundance in five cases and absolute DNA abundance for one species. For example, *F. pinicola* produced fruit bodies with 33%, 67% and 89% probability if it represented 0.1%, 1% or 10% of the DNA data, respectively (Figure 4c). Excluding the logs with PEG treatment resulted in three significant relationships both for relative and absolute DNA abundances.

For five out of the eight species for which the data allowed regressing fruit body abundance against relative DNA abundance, a positive relationship was found (see Figures 4d and 5d and Supplementary Information), the remaining three species showing nonsignificant associations. Using the absolute DNA abundance instead of relative DNA abundance, a positive result was found for four out of eight species. Out of these four species, the association was stronger (in terms of smaller *P*-value) for relative DNA abundance for two species and for absolute DNA abundance for two species. Excluding the logs with PEG treatment decreased the number of species with a significant association: three species showed a positive relationship, both for fruit body abundance versus relative DNA abundance and for fruit body abundance versus absolute DNA abundance.

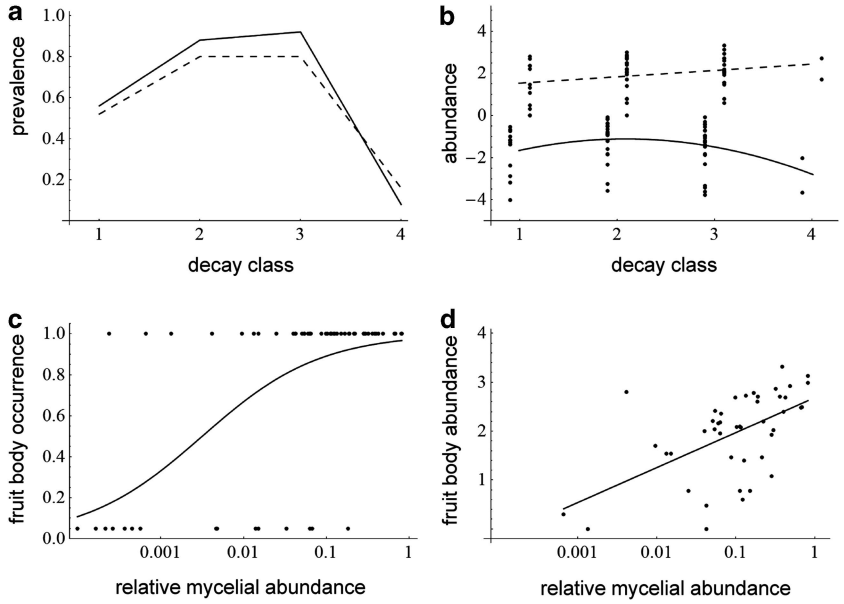

Figure 4 Patterns of mycelial (as detected from DNA) and fruit body occurrence for *Fomitopsis pinicola*. (**a**, **b**) Show prevalence and abundance (relative abundance for DNA), respectively, as a function of the decay class. In these panels, continuous lines refer to DNA and dashed lines to fruit body data. (**c**) Shows the probability of fruit body occurrence as a function of relative DNA abundance, and (**d**) the relationship between fruit body abundance and relative DNA abundance. The lines in **b**, **d** show linear regressions to the data, whereas the line in **c** shows logistic regression.

Among the 63 species for which we could model DNA abundance as a function of decay class, six species (for absolute DNA, denoted below by A) and four species (for relative DNA abundance, denoted below by R) yielded positive and 1 (A) and 4 (R) negative relationships, whereas relationships with intermediate maximum were found for 1 (A) and 6 (R) and relationships with intermediate minimum for 2 (A) and 2 (R) species. If excluding the logs with PEG treatment, among those 47 cases where we could model absolute (relative) DNA abundance as a function of decay class, we found 8 (5) positive and 0 (1) negative relationships, and relationships with intermediate maximum for 0 (1) and intermediate minimum for 0 (1) cases. Among the 16 species for which we had sufficient data on fruit body abundance, we found that fruit body abundance increased with decay class for *F. pinicola* (Figure 4b), it peaked at an intermediate decay class for *Antrodia serialis*, whereas for the remaining 14 species no pattern was found.

The time delay between the appearance of mycelia (as inferred from DNA data) and the appearance of fruit bodies was measured for 20 species. It varied from no observable delay (*F. pinicola* in Figure 4a shows a delay of 0.0 decay classes) to a considerable delay (*P. nigrolimitatus* in Figure 5a shows a delay of 1. 7 decay classes), with a mean of 0.7 decay classes.

Community analyses

The results from the community-level analyses are shown in Table 3 and summarized by the colors indicating positive, negative or mixed responses in Figure 1. As was the case for the species-level analyses, also the community-level analyses show strong links between the DNA and fruit body occurrences, as species for which we recorded a high DNA abundance also had a high fruiting rate and a high fruit body abundance. Further, DNA prevalence was high for species with high fruit body prevalence. Conversely, and as expected solely by our definitions, fruit body prevalence was high for species with high DNA prevalence and high fruiting rate ($P<0.001$ for both effects).

The influence of species traits (Table 2) on the feedback loops of Figure 1 was in line with our predictions only in some of the cases: species with specialized resource use requirements had an especially low fruiting rate and resupinate fruit bodies reached high fruit body abundance. For some cases, we found evidence to opposite direction compared with our prediction, as species with di- and trimitic fruit bodies had a higher fruiting rate than species with monomitic fruit bodies, and as mycelial prevalence increased with resource use specialization.

For three of the hypotheses that were derived from variation in fruit body type, we found evidence both supporting and contradicting our hypotheses. According to our prediction, time delay in fruit body production was higher for species with perennial than for annual fruit bodies, but contrary to our prediction it was higher for species with thin than thick fruit bodies. According to our prediction, DNA abundance was the highest for species with perennial and di- and trimitic fruit bodies, but contrary to our prediction it was higher for species with

resupinate than pileate fruit bodies. Finally, according to our prediction DNA prevalence was higher for species with perennial than annual fruit bodies, but contrary to our prediction it was higher for species with monomitic than di- or trimitic fruit bodies.

Discussion

In spite of much research on fungi inhabiting dead wood, the exact mechanisms behind their spatio-temporal population dynamics and distributional patterns are still poorly known. This is partly because of the dichotomy between the types of studies, which have been feasible in the past: field-based research has been largely restricted to fruit bodies, whereas laboratory studies have almost solely focused on mycelia. In recent years, mycelia of fungi inhabiting dead wood has been studied *in situ* with the help of molecular methods (for example, Lindner *et al.*, 2011; Rajala *et al.*, 2011; Vetrovsky *et al.*, 2011), including high-throughput sequencing (Ovaskainen *et al.*, 2010b; Kubartova *et al.*, 2012). In this paper, we have contrasted mycelial occurrences with fruit body occurrences, revealing a number of ecologically important patterns that would not have been possible to identify if either of these research methods would have been used in isolation.

In our study, fruit bodies were predominantly found on logs from which we recorded the species also as DNA, and often on logs in which the species was present with a high DNA abundance. Although such a result is to be expected from biological grounds, it demonstrates that high-throughput sequencing provides a robust signal both at the levels of presence–absence and relative abundance, despite the sampling and PCR-related biases and the fact that the presence of DNA does not necessarily imply the presence of viable mycelia. We found strong links between the mycelial (as detected from DNA data) and fruit body occurrences also in the community-level analyses. Most importantly, species that are able to obtain a dominating position in the mycelial community possess a high fruiting rate, produce abundant fruit bodies, and have a high prevalence both as fruit bodies and as DNA, suggesting a positive feedback-loop (Figure 1).

We applied two measures for DNA abundance: relative and absolute. Although the absolute measure is likely to be biologically more relevant, we found in many analyses a stronger signal for relative DNA abundance. This is likely to be the case because the measurement of absolute DNA abundance involves two additional measurements (total DNA concentration and the fraction of fungal DNA out of all DNA), both of which have the potential of bringing additional measurement noise. In this study, we chose to use qPCR to quantify fungal biomass mainly for the reason of making both species identifications and biomass quantification based on the same type of molecules, that is, DNA. We, however, note that alternative methods such as those based on ergosterol and phospholipid fatty acids would also have been possible. These biomarkers have recently been considered more reliable than qPCR in quantifying fungal biomass because the ribosomal DNA copy number per genome varies between species and among strains of the same species (Baldrian *et al.*, 2013). Difficulties in applying exactly the same procedures to all DNA samples, for example, the need for PEG treatment in some samples of much decayed wood, provides an additional level of uncertainty. However, as our results were qualitatively consistent whether or not the logs with PEG treatment were included or excluded, we conclude that a sufficiently strong biological signal may override the effects of such biases.

For some species, such as the generalist species *F. pinicola*, DNA and fruit body prevalences follow almost identical patterns as a function of the decay stage (Figure 4a). As the fruiting probability of *F. pinicola* greatly increases as a function of its DNA abundance, the formation of fruit bodies in this species seems to be triggered by its internal condition, that is, the size of its mycelium. In contrast, other species such as the specialist species *P. nigrolimitatus* have a long time delay from DNA establishment until fruit body formation (Figure 5a). As the DNA abundance of *P. nigrolimitatus* was not associated with its fructification probability, the fruiting of this species seems to be triggered by external conditions, such as the physicochemical properties of the wood or changes in the abundances of the species interacting with *P. nigrolimitatus*. An interesting avenue for future research would be to examine what makes some species wait even decades until they form fruit bodies, and what triggers fruit body production. As the fitness of an individual mycelium occupying a single log is closely related to its life-time rate of spore production, a trade-off between survival, growth and early formation of fruit bodies is likely to have a key role. Such trade-offs between survival and reproduction are known to be common especially under harsh conditions, for example, in zooplankton (Kirk, 1997) and birds (Martin, 1995) at times of starvation.

Species that produce perennial fruit bodies were on average more abundant as DNA than species with annual fruit bodies. This result is likely to reflect the differential investment needed for producing long-living and robust perennial fruit bodies compared with short-living and soft annual fruit bodies. The trade-off here resembles that of K and r strategies (MacArthur and Wilson, 1967). An example of a species with K selected characteristics is *P. nigrolimitatus*, which produces fruit bodies only long after mycelial colonization. Those fruit bodies can be large, especially if the species has achieved a dominating position in the mycelial community, and they may remain reproductive for many years, possibly even for decades. The species *Postia caesia*

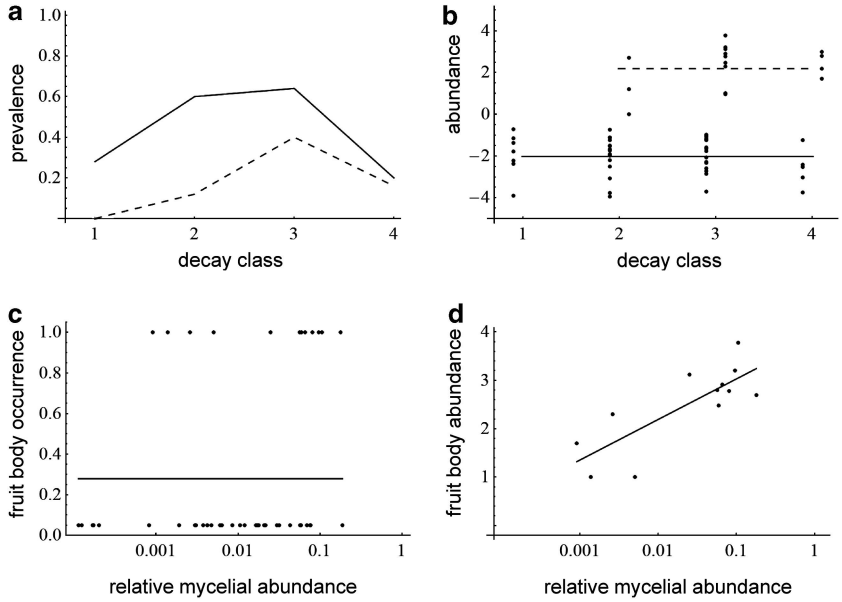

Figure 5 As Figure 4, but for *Phellinus nigrolimitatus*. Nonsignificant regression models are shown as horizontal lines. For corresponding figures for 28 other species, see Supplementary Information.

exemplifies the r strategy. This common generalist species has small annual fruit bodies, and it never reaches a high total hymenophore area on a log. In this study, *P. caesia* was found from 21 logs as fruit bodies, but only from three logs as DNA (Supplementary Information). A small mycelial mass is probably sufficient for *P. caesia* to produce fruit bodies, and it thus represents a strategy with fast growth and fruit body production, which properties enable it to escape competition.

Life history-based approaches can be helpful in explaining how species respond to land use change (Verheyen *et al.*, 2003). As in many other species, groups such as butterflies (Kotiaho *et al.*, 2005; Charrette *et al.*, 2006) and birds (Owens and Bennett, 2000), also in fungi inhabiting dead wood especially those species with specialized resource requirements have responded negatively to habitat loss and fragmentation, to the extent that a large fraction of these species have been red-listed (Hottola *et al.*, 2009; Nordén *et al.*, 2013). We found that species with highly specialized resource requirements have a lower fruiting rate than generalist species. This result is interesting, as it shows that the often red-listed highly specialized species (Hottola *et al.*, 2009; Nordén *et al.*, 2013) are not as rare as mycelia as they are as fruit bodies. Thus, an especially critical phase in the life history of the highly specialized species is the formation of the fruit body rather than the establishment of the mycelium. How this feature interacts with habitat fragmentation would require acquiring the kind of data reported here from multiple sites with contrasting land use histories.

Conflict of Interest

The authors declare no conflict of interest.

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