Xylobolus subpileatus, a specialized basidiomycete

functionally linked to old canopy gaps

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ABSTRACT: Documenting succession in forest canopy gaps provides insights into the ecological processes governing the temporal dynamics of species within communities. We analyzed the fruiting patterns of a rare but widely distributed saproxylic macromycete, *Xylobolus subpileatus*, during the ageing of natural canopy gaps in oak forests. In one of the last remaining *Quercus ilex* old-growth forests (on the island of Corsica, western Mediterranean basin), we systematically recorded and conducted molecular analyses of *X. subpileatus* basidiomes in 80 dated natural canopy gaps representing a 45 year-long sequence of residence time of tree logs on the forest floor. *X. subpileatus* fruited exclusively on *Q. ilex* logs. The probability of fruiting of *X. subpileatus* significantly increases during the process of wood decomposition to reach its maximum in the oldest gaps, ca. 40 years after treefall. In contrast, the abundance and the richness of saprobic and ectomycorrhizal fruitbodies decrease as canopy gaps age. Our results emphasize the high ecological specialization of *X. subpileatus*. They also highlight the imperative need to conserve the last patches of old-growth Mediterranean forests to secure the persistence of endangered and functionally unique macromycetes whose presence is highly dependent on old wood in advanced stages of decomposition.

Keywords: old-growth stand, Mediterranean forest, coarse woody debris, natural canopy gaps, conservation
RÉSUMÉ : L'étude des successions écologiques ayant lieu dans les ouvertures de canopée forestière offre l'opportunité de mieux comprendre les processus écologiques gouvernant la dynamique temporelle des espèces à l'intérieur des communautés. Nous avons analysé la distribution des fructifications d'une espèce – rare mais largement distribuée – de macromycète saproxilique, *Xylobolus subpileatus*, au cours du vieillissement de trouées naturelles dans les forêts de chênes. Dans l'une des dernières forêts anciennes de chêne verts (*Quercus ilex* ; île de Corse, ouest du bassin méditerranéen), nous avons échantillonné systématiquement et analysé moléculairement les basidiomes de *X. subpileatus* dans 80 trouées datées qui représentent une chronoséquence de 45 ans de temps de résidence au sol des troncs d'arbres. *X. subpileatus* ne fructifie que sur les troncs de chêne vert (*Q. ilex*). Sa probabilité de fructification est positivement corrélée à l'âge des trouées et atteint un maximum dans les plus vieilles trouées (environ 40 ans après la chute de l'arbre). A contrario, l'abondance et la richesse des fructifications de champignons décomposeurs et ectomycorhiziens décroissent avec le vieillissement de la trouée. Nos résultats soulignent la spécialisation écologique poussée de *X. subpileatus* et mettent en avant le besoin impératif de préserver les dernières parcelles de forêts anciennes méditerranéennes afin de protéger cette espèce de macromycète fonctionnellement originale dont la présence dépend fortement de la complétion du processus naturel de décomposition.

Mots clés: parcelle forestière ancienne, forêt méditerranéenne, débris ligneux grossiers, ouverture naturelle de canopée, conservation
INTRODUCTION

Identifying the factors that determine the tremendous concentration of biological diversity in old-growth forests is a pivotal question for conservation ecologists, but also an inspiration for forest managers to implement integrated management in cultivated ecosystems. During the past decades, this issue has particularly interested mycologists, who have documented the diversity and specificity of fungal guilds characterizing old-growth forests (e.g. Smith et al. 2002 and Junninen et al. 2006 for ectomycorrhizal and wood-decaying fungi, respectively). Mycologists have also addressed the broader issue of historical legacies of human practices on the diversity of fungal communities in forests. In this regard, recent studies showed that anthropic disturbances alter the dynamics of fungal communities at short (e.g. Paillet et al. 2010, Halme et al. 2013) and longtime scales (Diedhiou et al. 2009).

Whatever ecological guild (plant mutualists, decayers) is considered, old-growth forests accommodate particularly rich fungal diversity in temperate and boreal biomes. From a belowground perspective, these ecosystems are dominated by long-lived ectomycorrhizal (hereafter ECM) tree species (e.g. Fagaceae, Pinaceae) that accumulate species-rich (Goodman and Trofymow 1998) and dynamic (Izzo et al. 2005) ECM fungal communities, which tend to differ in composition from those in managed ecosystems (Smith et al. 2002). Furthermore, because they accumulate large-diameter woody debris in all stages of decay, old-growth forests are considered as unique in the amount of suitable habitats for rich communities of wood-inhabiting fungi (Brazee et al. 2014), including species that are infrequent (Stokland and Larsson 2011) or particularly demanding in terms of their ecological requirements (Berglund et al. 2011).

In old-growth forests, biotic (e.g., insect outbreaks, root-pathogenic fungi, dwarf mistletoe; Worrall et al. 2005) and abiotic (e.g., wind and snow; McCarthy et al. 2001) disturbances provoke canopy openings of various sizes, by causing the fall of trees, singly or in groups (review: McCarthy et al. 2001). The physiognomy of forest ecosystems driven by canopy gap dynamics is thus typically
characterized by complex mosaics of small-scale patches of vegetation that are in different stages of response to disturbance (Worrall et al. 2005; Oldeman 2012). From its creation to its complete closure, a canopy gap is the scene of marked shifts in abiotic conditions (light availability, temperature and moisture; Rajala et al. 2012). These changes, taken together with the existence of contrasted life-history strategies of fungi adapted to the different stages of wood decomposition, drive the succession of organisms within gaps, from early- to late-successional species (Jönsson et al. 2008). For mycorrhizal fungal guilds, gap-driven dynamics have so far been poorly investigated. In contrast, the succession of saproxylic fungi on fallen logs has been accurately characterized, particularly in boreal and temperate forests. During the process of wood decay, composition shifts occur within these communities, from early- to late-successional assemblages successively dominated by ascomycete and basidiomycete species (e.g. Lindblad 1998; Rajala et al. 2012), with succession scenarios partially determined by log size and architecture (Heilmann-Clausen and Christensen 2003).

The wood-inhabiting stereoid basidiomycete *Xylobolus subpileatus* (Fig. 1a) is a widely distributed macromycete recorded from 14 countries from all continents but Africa and Antarctica (Global Biodiversity Information Facility, 12 July 2016). This fungus is rare throughout Europe, and in some countries is considered by mycologists to be endangered (Austria, Hongrie; Papp 2011) or critically endangered (Czech Republic; Kotlaba 1986). Although knowledge about this rare species is still fragmentary, it is known to be ecologically specialized. First, *X. subpileatus* has been recorded only on dead oak wood (*Quercus* spp.), with a marked preference for trees long dead (Long 1915; Parmasto 2001). Second, *X. subpileatus* selectively hydrolyses lignin, provoking characteristic heart-rotting honeycomb-like patterns of decay (Fig. 1b), where medullary ray parenchyma and early-wood vessels are not readily decayed and remain between pockets of degraded material (Blanchette 1984). The ecological specialization of *X. subpileatus*, and the
consequences of this specialization for its fine-scale spatiotemporal distribution in forest ecosystems, have never been investigated.

In the Mediterranean basin, remaining primary vegetation covers less than 5% of its original extent (Myers et al. 2000). Old-growth stands are extremely rare in this region because of millennia of intensive and uninterrupted human use of forest (Quezel and Médail 2003). In the mountainous island of Corsica (Fig. 2a), human activities over ca. 5 000 years have dramatically reduced both the area and the quality of natural forests (Reille 1992) and likely altered the occurrence of wildfires (Leys et al. 2014), which are the main large disturbance in Mediterranean forests. However, only few small and quasi-inaccessible patches of old-growth forests persist, where both the long-term continuity of forest cover and the absence of logging at the large scale of the complete forest cycle (Panaïotis et al. 1998) are attested by historical maps and textual archives on forests. The most emblematic old-growth forest, that found in the Fango (Fig. 2b), has been continuously monitored for macromycetes since 1999 (Richard et al. 2004). In this exceptionally well-preserved Quercus ilex old-growth stand, it is possible to accurately date forest canopy gaps using the understory shrub Phillyrea latifolia as a disturbance marker based on its capacity to spontaneously re-sprout immediately after the canopy opening (Panaïotis et al. 1995). In this study, we took advantage of the discovery of X. subpileatus in the Fango permanent transect during the British Mycological Society field trip in Corsica (2013) to determine the ecological requirements of this species. Unique opportunities for such a study are offered by this site, where a large variety of usable woody substrates co-occur, originating from various species, with wood of various ages and dimensions remaining on the forest floor until its complete decay.

Using a synchronic analysis performed on 80 natural canopy gaps of known age, we described the fine-scale distribution of X. subpileatus basidiomes on the basis of (i) the identity of the plant forming the colonized log, (ii) the dimensions of wood fragment and (iii) the age of the corresponding canopy gap (time since treefall). We hypothesized that the ecological specialization
of *X. subpileatus* results in preferential fruiting on *Q. ilex*, on the largest logs of this species and in the oldest canopy gaps. We finally compared the temporal patterns of *X. subpileatus* fruiting with those of ECM and saprobic fungal communities in the same gaps, using the dataset in Richard et al. (2004). We predicted that the abundance and the richness of ECM and saprobic fruitbodies in gaps decrease as gaps age, while *X. subpileatus* basidiomes show the opposite pattern. We aimed at testing the role of canopy gaps, from their initiation until the very end of the process of succession, in providing suitable habitat for highly specialized and rare organisms in forest ecosystems little disturbed by humans.

**MATERIAL AND METHODS**

**Study site**

The study site is situated in the Fango forest (42°20′N; 8°49′E), in the northwestern part of the island of Corsica (Fig. 2). The Fango valley has been a Man and Biosphere (MAB) reserve since 1973, as it contains rare stands of old-growth *Quercus ilex* (holm oak) forests (Panaïotis et al. 1995; Quézel and Médail 2003). This forest covers a 4 318-ha area on Hercynian granite with enclaves of volcanic rhyolites. Soils are alocrisols (Richard et al. 2009) with mull humus overlying (i) a thick organic layer with a slightly acidic pH ranging from 5.7 to 6.4 and a C:N ratio ranging from 24 to 28 and, (ii) a poorly fissured rhyolitic bedrock (Richard et al. 2009). The climate is subhumid with a mean annual rainfall of 750 mm and an average annual temperature of 14.6°C at 192 m above sea level (asl). Temperatures range from 3.5°C (mean January minima) to 29.9°C (mean July maxima).

In Corsica, a high diversity of wood-decaying fungi develop in *Q. ilex* wood, and commonly fruit on standing logs (e.g. *Hyphodontia quercina*, *Inonotus dryadeus*, *Phellinus erectus*), branches (e.g. *Abortiporus biennis*, *Hexagonia nitida*, *Meriulopsis corium*, *Polyporus cf. tuberaster*) and fallen logs (e.g. *Hyphodontia quercina*, *Abortiporus biennis*; Pieri and Rivoire 1992, 1994).

**Characteristics of the *Q. ilex* old-growth stand**
This study was carried out within a district of about 1 500 ha, named Perticato, extending from 90 to 1 619 m asl (Capu a u Ghjallichiccia; Fig. 2b), and where a permanent transect was established in 1994, devoted to the study of forest dynamics (Panaïotis et al. 1995). The vegetation at the study site is a complex mosaic of tall shrubby patches dominated by evergreen Mediterranean species (‘macchia’) and of old-growth forest stands, mainly located in the lower parts of valleys. In forest patches, large Q. ilex trees dominate a 7-m-high, species-poor and dense understory layer that persists under the oak canopy. This understory is composed of ‘macchia’ species, i.e. Phillyrea latifolia, Erica arborea, Arbutus unedo and scattered individuals of Fraxinus ornus, Cistus salviifolius and C. monspeliensis (Richard et al. 2009).

Textual forest archives unambiguously attest that the district of Perticato has not been exploited for wood and livestock (cows and goats) since 1827 (Panaïotis et al. 1995). For this reason, the long-term expression of forest dynamics generated a high density of natural canopy gaps of about 100 m\(^2\) each, which occur when old Q. ilex stems (170 ± 46 years) break and fall down (Panaïotis et al. 1997).

**Dating of canopy gaps**

Canopy gaps are small-scale disturbances involving either a part of a single tree (hereafter partial canopy gaps), the entire canopy of a single tree (hereafter single canopy gaps), or falls of several adjacent individuals (hereafter multiple canopy gaps; McCarthy et al. 2001). Our study included falls of either trees (Q. ilex) or large shrub individuals (Phillyrea latifolia, Erica arborea, Arbutus unedo and Fraxinus ornus).

The residence time of tree stems on the ground, i.e. the dating of fall events, was estimated using the synchronic method developed by Panaïotis and coauthors (1995) based on the high sprouting speed and rate of Phillyrea latifolia, which produces new aerial and basal stem sprouts during the first spring after canopy opening. In each sampled canopy gap, we then accurately dated the age of
Phillyrea latifolia stem sprouts to assess the date of the tree fall event (Panaïotis et al. 1995 and Fig. 1c-e). In 11 of the 80 dated gaps, the dynamics of *P. latifolia* re-sprouting attested to a succession of canopy-opening events and made uncertain the assignation of the different parts of fragmented logs to a given event. All analyses were carried out with and without these 11 gaps (Table S1 and Fig. S1). As the results are consistent whether or not these gaps are taken into account, we included these gaps in the final analysis presented here, and their age was set using the date of the most recent event recorded (conservative dating regarding the observed dynamics of *X. subpileatus*).

**Sampling of Xylobolus subpileatus basidiomes**

The survey was conducted in April 2015 in a 25-ha area where a 6 400-m² (160 m × 40 m) permanent transect was established in 1994 (Panaïotis et al. 1997; Fig. 1c-e and Fig. 2). All canopy gaps present in the 25-ha area were visited and dated using the method of Panaïotis et al. (1995), while the appearance of basidiomes of *Xylobolus subpileatus* and *Stereum hirsutum* was systematically recorded. In each gap where *X. subpileatus* was suspected to be present, basidiomes morphologically assigned to *X. subpileatus* were collected and stored for subsequent microscopic description, molecular analyses and final identification. We also collected two *S. hirsutum* specimens for molecular verification of their taxonomic identification.

In addition, in all *Q. ilex* canopy gaps located in the permanent transect of 6 400 m² (Fig. 2c), supplementary measurements were performed to refine the determination of the ecological requirements of *X. subpileatus*. On each *Q. ilex* stem, we measured the surface covered by *X. subpileatus* basidiomes, and the dimensions (length, diameter at each tip, and volume) of the stem.

**Taxonomic assignment of X. subpileatus basidiomes**
In order to confirm the taxonomic identity and homogeneity of the collected fungal material, 16 voucher collections assigned to *Xylobolus subpileatus* were randomly selected out of 22 colonized logs, microscopically analyzed, and compared to five reference collections assigned to genus *Xylobolus* by expert field mycologists (T. Læsøe, Denmark; G. Trichies, France; J.-P. Vidonne, France). Two voucher collections of the phylogenetically related species *Stereum hirsutum* were also sampled for taxonomic verification and comparative purposes. All samples are kept in the personal herbarium of J-M Bellanger at the CEFE (Montpellier, France) and one representative collection of the Corsican *X. subpileatus* is deposited at the LIP herbarium (Faculté des Sciences Pharmaceutiques et Biologiques, Université Lille 2, France), under the code LIP0401115.

All collections were then subjected to molecular-genetic analyses. Briefly, DNA was extracted and the internal transcribed spacer (ITS) locus was amplified from dried specimens as described by Loizides et al. (2016). PCR amplification and sequencing were performed with the primers ITS1F and ITS4 or ITS4B (Gardes and Bruns 1993). We obtained 15 validated sequences (14/22 sequences for *X. subpileatus* and 1/2 sequences of *S. hirsutum*). Sequences were deposited in GenBank under the accession numbers listed in Table 1. The analyzed dataset includes these sequences as well as a subset of publically available ITS sequences of *Xylobolus* spp. (*i.e. X. subpileatus, X. frustulatus, X. apricans* and *X. annosus*) and other Stereaceae in GenBank, selected by BLAST. Phylogenetic analyses were all performed online at www.phylogeny.lirmm.fr (Dereeper et al. 2008) and on the CIPRES Science Gateway, as described in Loizides et al. (2016). The tree depicted in figure S1 corresponds to the 50% majority-rule consensus phylogram resulting from the Bayesian Inference (BI) of phylogeny. A Maximum Likelihood (ML) analysis was also conducted. Branch supports were assessed using the Bayesian posterior probabilities (BPP, as percentages) and the Shimodaira–Hasegawa version of the approximate likelihood-ratio test (SH-aLRT), respectively.

*Analysis of fruiting patterns in canopy gaps of the permanent transect*
To compare the temporal dynamics of *X. subpileatus* occurrence with the general fruiting patterns of fungal communities in canopy gaps, we re-analyzed the dataset presented in Richard et al. (2004). In the aforementioned study performed at the same site, basidiomes of soil macromycetes, including decomposers and ectomycorrhizal fungi, had been mapped at a 0.1-m accuracy during the period 1999-2003 in ten gaps included in the permanent transect (Richard et al. 2004). Here, we used this dataset to describe the temporal dynamics of basidiome production and fungal species richness in ten dated canopy gaps of the permanent transect. These gaps ranged from 3 to 23 years in age. Briefly, basidiome abundances and species richness were calculated in a circle of 5 m in radius around the fallen tree trunk where the area covered by *X. subpileatus* basidiomes was assessed.

**Statistical analyses**

The effect of the age of canopy gaps on the presence of *X. subpileatus* and *S. hirsutum* basidiomes was tested using a General Linear Model (function `glm`, R-Development Core Team, 2016). Confidence intervals were calculated using the function `confint.glm` from the MASS R packages (Venables and Ripley, 2002). Correlation between gap ages and basidiome richness and numbers were tested using Pearson correlation tests.

**RESULTS**

**Taxonomic assignment of analyzed basidiomes**

The phylogenetic analysis of our ITS sequence dataset indicates that, as currently defined, *Xylobolus* is polyphyletic, with three distinct lineages nested within Stereaceae (marked by asterisks in Fig. S2): *X. apricans*, *X. spectabilis*, and a weakly supported clade encompassing almost all the other *Xylobolus* sequences, including those of our two reference collections of *S. hirsutum*, type species of that genus. The latter lineage is labeled “*Xylobolus*” in figure S1. However, a taxonomic revision of the genus, clearly beyond the scope of the present work, would be necessary to stabilize
names, species limits and phylogenetic boundaries in *Xylobolus*. Nevertheless, all 14 samples attributed to *Xylobolus* that we collected in Corsica nested within that lineage, as a strongly supported monophyletic subclade that was previously not represented in public databases. Collections in this clade are 100% identical at the ITS locus, and include LIP0401115, a sample collected in the same area during the 2013 annual foray of the British Mycological Society, and taxonomically assigned to *X. subpileatus* by the specialist of our consortium T. Læssøe. Because this species apparently displays a wide distribution and as no morpho-anatomical features observed in Corsican collections contradict the original diagnosis, we thus apply this binomial to the subclade, at least provisionally, even though no sequence from the North American type material is currently available to support our interpretation. The analysis also attested the identity of two collections of *Stereum hirsutum* (Fig. S2, AT44 and AT67), a common and widespread species with broad ecological and morphological ranges, often confused with other Stereaceae species.

**Characteristics of analyzed canopy gaps**

In all, we sampled 86 canopy gaps of about 100 m² each (Panaïotis et al. 1995) within a forest patch of 25 ha in area (Fig. 2c). Our sampling included 4, 48 and 34 multiple, single and partial multiple treefall canopy gaps, respectively, with a large majority of gaps created by *Q. ilex* (n = 65, 76%). The analysis also included canopy openings in ‘macchia’ patches, and mostly logs of *Arbutus unedo* (11, 13%) and *Erica arborea* (8, 9%), with very few individuals of *Phillyrea latifolia* (1) and *Fraxinus ornus* (1).

The date of treefall could be estimated in 80 (93%) of the 86 analyzed canopy gaps, of which 59 were created by *Q. ilex* treefalls. Our dataset included openings ranging from 1 (tree fallen in 2014) to 45 years old in age, with 14.6 years on average (Fig. 1c-e and Fig. S3). The temporal distribution of treefall events in the 25-ha area showed three main periods of gap creation corresponding to [1-5], [12] and [30-40] year-old logs (see Fig. S3).
Distribution of *Xylobolus subpileatus* on plant species

Basidiomes of *X. subpileatus* (Fig. 1a) were detected in 22/80 dated canopy gaps and only in gaps made of *Q. ilex* logs (22/59). The typical honeycomb-like decomposition pattern of decayed wood was frequently observed in the vicinity of basidiomes (Fig. 1b). *Xylobolus subpileatus* fruited in gaps from 7- to 40-years old (mean: 23.5 ± 10.5; Fig. 3a). Basidiomes were found in 11% of canopy gaps less than 20 years old, whereas 67% of gaps ranging from 20 to 45 years old contained logs colonized by this species. At the end of the wood-decaying process (40-year-old canopy gaps), *X. subpileatus* basidiomes were almost systematically present (84% of sampled logs). The fruiting probability significantly increased with age of gaps, gaining 7% of its value per year of log residence on the ground (P < 0.05 by one-way ANOVA; Table 2), and ranged from 18.5 to 83.4% in 7- and 40-year-old gaps, respectively (Fig. 3a).

Within the permanent transect of 6 400m², we found 35 logs distributed across 16 *Q. ilex*-created gaps. The analysis of the fine-scale distribution of basidiomes on *Q. ilex* logs revealed the presence of the fungus on logs of diameters from 15 to 80 cm (median: 27 cm), of length from 1.25 to 14 m (median: 5.91 m), and of volume from 0.02 to 2.36 m³ (median: 0.21 m³; Table S2). The log volume was not significantly correlated with the occurrence of *X. subpileatus* basidiomes (p = 0.84 using glm) or with the surface covered by basidiomes (p = 0.72 using lm). On the contrary, both occurrence (p = 0.037 using glm) and surface of *X. subpileatus* (p = 0.036 using lm) basidiomes were correlated with the age of the gap. The surface covered by *X. subpileatus* basidiomes varied from 0.13 to 1.01 m² (median: 0.20 m², Fig. S4). The fungus covered from 0.24 to 18.25% (median: 7.00%) of the log surface (Table S2).

In contrast, basidiomes of *Stereum hirsutum* were recorded in *Q. ilex* logs in 6/80 canopy gaps of recent origin (mean: 8.3 ± 3.1 years; Fig. 3b). By contrast with *X. subpileatus*, the probability of *S. hirsutum* basidiome occurrence tended to decrease with increasing gap age (Fig. 3b). However,
this tendency was not significant, probably owing to the low number of records of this species (Table 2).

**Dynamics of abundance and diversity of ectomycorrhizal and saprobic basidiomes within gaps**

In our analysis, the abundance of both ectomycorrhizal and saprobic basidiomes (dataset: cf. Richard et al. 2004) in canopy openings significantly decreased with gap age (Fig. 4, all p < 0.05 using Pearson correlation tests). The production of basidiomes is high in the youngest canopy gaps colonized by *X. subpileatus* (41.4 and 29.8 basidiomes per 100 m$^2$ for ECM and saprobic macromycetes in 7-year-old gaps, respectively; Fig. 4). On the contrary, at the average residence time of *X. subpileatus* (23.5 years), the production of basidiomes is very low (with 18 and 0.4 basidiomes per 100 m$^2$ for ECM and saprobic macromycetes in 23-year-old gaps, respectively; Fig. 4a).

Ectomycorrhizal species richness markedly decreased during gap ageing (Fig. 4b). Relating to the patterns of occurrence of *X. subpileatus*, species richness decreased from the youngest canopy gaps colonized by *X. subpileatus* (with 20.9 and 7.3 species per 100 m$^2$ for ECM and saprobic macromycetes in 7-years old gaps, respectively; Fig. 4b) to the average time of residence of colonized logs (with 9.6 and 0.4 species per 100 m$^2$ for ECM and saprobic macromycetes in 23-year-old gaps, respectively; Fig. 4b).

**DISCUSSION**

In this study, we used a unique experimental design to characterize the ecology of *X. subpileatus* along a 40-year-long sequence of forest canopy gap dynamics. In accordance with previous studies (Papp 2011), *X. subpileatus* fruited exclusively on oak logs in the Fango forest.
Using the dating of canopy openings, we showed that the fruiting of *X. subpileatus* was tightly correlated with the age of gaps, but not with the size and volume of logs laying on the ground.

**Xylobolus subpileatus preferentially reproduces in closed canopy gaps**

Our analysis of the temporal distribution of *X. subpileatus* basidiomes provided evidence of the fruiting of *X. subpileatus* on late stages of decay of fallen logs. Fruitbodies were never detected on logs within the seven years following treefall, and the probability of presence increased by 7% per year of residence on the ground until the complete decaying of the substrate (Fig. 3a). The strong affinity of *X. subpileatus*, at its sexual stage, for highly decayed wood sheds some light on the ecological niche of this rare species. Its increased probability of fruiting as gaps age may be a response to changes in both biotic and abiotic environments at ground level in old-growth forest patches (Oldeman 2012). In terms of ecological strategies, the pattern of *X. subpileatus* fruiting occurrence may reflect niche specialization toward highly decayed logs, exceptional competitive abilities, or both.

First, the temporal pattern of *X. subpileatus* fruiting may indicate its affinity for deep shade environments. From the time of the treefall to the complete canopy recovery by adjacent trees and understory shrubs, the amount of light entering into the forest gap progressively decreases, inducing changes in the microclimatic conditions (moisture, temperature, daily variability, etc.) for organisms living on logs (Messier et al. 1999; McCarthy et al. 2001). Sun exposure affects composition of saproxylic fungal communities in temperate forests, with some species fruiting in fully exposed large gaps, while others require canopy-closed, multilayered openings (Brazee et al. 2014). In the context of the seasonally dry and variable Mediterranean climate, sun exposure in new gaps and dry microclimatic conditions may contribute to restricting the fruiting of *X. subpileatus* to logs in deep shade.
Second, the temporal distribution of *X. subpileatus* basidiomes might reflect a high degree of functional specialization towards decomposition of lignin to complete its biological cycle (Blanchette 1984). Indeed, during the decay process, physical and chemical properties of wood gradually change. In particular, water and lignin contents increase with loss of cellulose and with decrease in density (Rajala et al. 2012), as a result of the functional succession of saproxylic species on the log. During this process, early dominant soft-rot ascomycetes are progressively replaced by white- and brown-rot species of basidiomycetes (Rayner and Boddy 1988). Regarding the catabolic abilities of the corresponding species, this species succession reflects the turnover from cellulose decayers to organisms that are efficient in degrading polyphenols, including lignin (Daniel and Nilsson, 1998). From this perspective, the very late dominance of fruiting *X. subpileatus* on *Q. ilex* logs may primarily be a positive response to the emergence of substrates with high concentrations of lignin in canopy gaps at the end of the decay process. It is likely that increasing lignin concentration acts as a chemical filter for many saproxylic fungi, but not for *X. subpileatus* (Otjen and Blanchette 1984), and as a driver of the dynamics of ecological succession on logs.

Third, the dynamics of *X. subpileatus* sexual reproduction may reflect a highly competitive strategy regarding ecological succession (Grime et al. 1977; Last et al. 1987). Indeed, fungal interspecific interactions become more complex during the wood-decay process. Based on a large corpus of basidiome records in temperate and boreal forests, but also using the sequencing of mycelia in decaying wood, it has been established that species richness increases during the decaying process (Kubartová et al. 2012), and interspecific fungal interactions increase with time within guilds (i.e. among saproxylic species) and among guilds (i.e. between saproxylic and ectomycorrhizal species) of fungi (Rajala et al. 2012; Ottoson et al. 2015). In other words, in late phases of decay, white-rot fungi not only compete among themselves, but also with rich communities of ectomycorrhizal delignifying species, that become the most dominant ecological strategy in wood (Rajala et al. 2012). In the system we studied, it is likely that the temporal pattern of *X. subpileatus* occurrence in
gaps reflects its high competitive abilities in particularly rich fungal communities (Richard et al. 2004). The use of next-generation sequencing methods in this exceptional set of dated gaps should provide complementary insight not only into the ecological niche of this *Xylobolus* species, but also into that of ectomycorrhizal communities in Mediterranean ecosystems.

**Implications for the conservation of fungal diversity**

In the present study, we compared the temporal fruiting pattern of *X. subpileatus* to (i) that of the related species *Stereum hirsutum* (Fig. 3) and (ii) those of the ECM and saprobic fruiting communities of macromycetes in the same study site (Fig. 4; Richard et al. 2004).

We found evidence for contrasted requirements of *X. subpileatus* and *S. hirsutum* regarding the age of canopy gaps enabling their fruiting. The two species never co-occurred on any wood logs investigated here. However, our dataset does not provide evidence of strict niche segregation with respect to gap age. Although *S. hirsutum* was not detected in gaps > 12 yrs old, *X. subpileatus* was recorded to fruit in gaps as young as 7 years, allowing a 5-year-long overlap (Fig. 3). The different optima of the two species may reflect their preferences not only for wood at different stages of decay but also for different parts of the decaying log (e.g. sapwood vs heartwood). Before concluding about possible mechanisms of niche differentiation between these two close relatives, additional work is needed to accurately describe the occupancy pattern of their respective mycelia in logs throughout the period from treefall to the end of the wood-decay process.

From a conservation perspective, and taking into account only basidiome occurrence, our findings highlight the complementarity of canopy gaps of uneven age in our study site in maintaining populations of these two species. Further, our results support the exceptional capacity of old-growth forests to allow the expression of contrasted ecologies at very fine scales, due to natural dynamics of gaps (Fournier et al. 2012). Indeed, in old-growth forests more than any other environment, the stochasticity of disturbance regimes governs the temporal distribution of treefall hazards, allowing
the persistence of all stages of wood decay at the scale of forest patches, which in turn allows a continuous beginning of new ecological successions (Wilson 1999).

The probability of occurrence of *X. subpileatus* basidiomes on the one hand, and the fruiting patterns of both litter saprobic and ectomycorrhizal macromycetes on the other hand, show opposite patterns during gap closure (Fig. 4). Comparable case studies are lacking, particularly in the Mediterranean area, mostly because long-term surveys based on permanent plots are scarce (Quézel and Médail 2003). In the best-documented study of succession of wood-inhabiting fungi, early peaks of species richness were observed a few years after treefall (Lindhe et al. 2004), but no clear pattern emerges from the available reports that consider the entire sequence of wood decay (e.g. Berglund et al. 2005). In contrast, the occurrence of red-listed species of wood-inhabiting fungi have been reported to increase with time in northern European forests (Heilmann-Clausen and Christensen 2005). Our results provide supplementary evidence, in the Mediterranean bioclimatic region, that species richness is an unsatisfactory proxy for the presence of the most threatened elements of fungal diversity, in the same guild (Heilmann-Clausen and Christensen 2005), or in different guilds (Fig. 4). The studies that examine this question, both the previous studies we cite and our own, are all based on fruitbody records. One remaining question is thus whether (and to what degree) the observed fruiting patterns reflect the distribution of resident mycelia. A perfect match is unlikely, in particular for mycorrhizal fungi. In a previous study conducted at the same site, we showed that reproductive (fruitbodies) and vegetative (soil mycelia) mycorrhizal communities differed markedly in structure and composition (Richard et al. 2005).

One important implication of the present study is the demonstration that it is possible to accurately date *X. subpileatus* fruiting with respect to the entire forest cycle. A fine-scale analysis of patch dynamics (Panaïotis et al. 1997, 1998) showed that oak individuals that created natural treefalls averaged 170 ± 46 years in age. In this context, *X. subpileatus* shows a maximum of occurrence 40
years after canopy openings, *i.e.* about 210 (170 + 40) years since the beginning of the long period without human intervention affecting the forest stand. In the Mediterranean basin, such temporal continuity of ‘naturalness’ is particularly rare because of the ancient and particularly high human pressure in the region, and we may predict that potentially suitable habitats for *X. subpileatus* are limited to very few locations (Quezel and Medail 2003) where the species could be sought within multi-secular old-growth forest.

From a management perspective, the ecological requirements of *X. subpileatus* highlight the high value of the remaining old-growth *Q. ilex* forests for macromycete conservation. The long-term persistence of this ecologically unique species requires implementing management practices that allow (i) the long-term continuity of forest cover in *Q. ilex* stands and (ii) the accumulation and the residence on forest soil of large woody debris until its complete decay. Such scenarios rarely occur in Mediterranean *Q. ilex* forests, which are mostly managed as short-rotation coppices to produce fuelwood.

**Conclusion**

Because they are among the most threatened organisms in Europe, saproxylic fungi have fueled an intense debate about how they should be conserved. In Mediterranean forest, saproxylic biota constitute a major conservation issue, not only because they depend on the most important forestry product, *i.e.* dead wood, but also because forest managers consider their preferred habitat to engender high fire risk (large woody debris are the main fuel to sustain fire during days). We here demonstrate that the conditions for fruiting of *Xylobolus subpileatus* are associated with forests long preserved from human exploitation, where large woody debris take decades to be mineralized by long and complex chains of organisms. The optimal conditions for this rare species occur only at the end of this long process. In these old-growth stands, only large *Quercus ilex* logs are suitable habitats for this late-successional fungal species, because oak logs last long enough on the forest.
floor to enable the accomplishment of a complete turnover of the composition of wood-decaying fungi. Therefore, management practices that preserve old-growth stands and their natural dynamics from fire risk are essential to secure the regional persistence of this endangered and functionally unique species, whose current distribution is restricted to a few hectares of preserved forest patches in Corsica.

Author Contributions

AT and FR originally formulated the idea, developed the methodology and performed statistical analyses. AT, FR, CP, TL and AC generated data. J-MB and P-AM performed phylogenetic analyses. FR and AT wrote the initial manuscript. All authors contributed to the final version of the manuscript.

Acknowledgments

This study is dedicated to the memory of Justin Smith, who accompanied the discovery survey in summer 2013 and died from sudden arrhythmic death syndrome, on March 3, 2014. We sincerely thank Basile Zorabiniu for his precious help during field survey. We are grateful to Doyle McKey for checking the English. This study was funded by the Observatoire de REcherche Méditerranéen de l’Environnement (OSU OREME, UMS3282 CNRS and UMS223 IRD.) All the experiments performed in this study comply with the current French regulations.
References


Table 1: GenBank accession numbers of ITS sequences generated for the present work.

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Table 2: Summary of the generalized linear model of the presence of basidiomes of *Stereum hirsutum* and *Xylobolus subpileatus* as a function of gap age (n = 59 logs; 7 with *S. hirsutum*, 22 with *X. subpileatus*).

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Fig. 1: (a) Reproductive structure (basidiome) of *Xylobolus subpileatus* on *Quercus ilex* logs. (b) Typical honeycomb decomposition pattern of decayed wood. View of decaying logs from the forest edge in 1- (c), 5- (d) and 20-year-old (e) canopy gaps.

Fig. 2: Location of the study site in the northwestern Mediterranean (a), and more precisely on the island of Corsica (b), and detailed pattern of the sampling area in red (c). The green area in panel b delimits the Fango UNESCO Man and Biosphere reserve. Circles (◦) symbolize *Quercus ilex* logs and crosses (×) gaps created by other woody species. Dark grey, light grey and open circles respectively indicate the presence of *Xylobolus subpileatus*, the presence of *Stereum hirsutum* and the absence of both species on gaps made of *Quercus ilex* logs. The grey rectangle in (c) represents the area where surface of *X. subpileatus* fruitbodies and *Q. ilex* logs were measured.

Fig. 3: Probability of fruiting of (a) *Xylobolus subpileatus* and (b) *Stereum hirsutum* on tree logs as a function of the number of years since tree fall within the 25-ha area (n = 59; 22 logs with *X. subpileatus*, 7 with *S. hirsutum*). The distribution of presence/absence is represented by violin plots: the white dot represents the median, the black rectangle the interquartile range and the grey envelope is a kernel density estimation of the distribution. Black lines depict the linear model and the associated grey shades delimit 95% confidence intervals of the probability of fruiting according to time since treefall.

Fig. 4: Number of fruitbodies (a) and species richness per 100 m$^2$ (b) of saproxylic (in red) and ectomycorrhizal fungi (in green) as a function of the age of the 10 canopy gaps. Black lines represent the linear model of the probabilities (grey shades 95% CI) of presence of *Xylobolus subpileatus* (cf. Fig. 3a).
Fig. 1: (a) Reproductive structure (basidiome) of Xylobolus subpileatus on Quercus ilex logs. (b) Typical honeycomb decomposition pattern of decayed wood. View of decaying logs from the forest edge in 1- (c), 5- (d) and 20-year-old (e) canopy gaps.
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