Received: 9 February 2020 | Revised: 27 August 2020 | Accepted: 3 September 2020

DOI: 10.1111/efp.12634

Forest Pathology WILEY-BLACKWELL WILEY

ORIGINAL ARTICLE

Decay fungi associated with cavity excavation by a large South American woodpecker

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Editor: H. Tuğba Doğmuş-Lehtijärvi

Abstract

In temperate systems of the Northern Hemisphere, wood-decay fungi are known to facilitate cavity excavation by woodpeckers. For South America, woodpecker–fungi interactions have not been explored. The aim of this work was to identify wood-decay fungi associated with the process of cavity excavation by the Magellanic woodpecker (*Campephilus magellanicus*), a large South American picid that excavates on living trees. The survey was conducted in old-growth *Nothofagus pumilio* forests of Patagonia. For freshly excavated cavities, wood condition was assessed, adjacent basidiocarps were collected, and fungal cultures were obtained from wood samples taken to the laboratory. All cavities exhibited softened wood. Four Agaricomycotina were isolated in cultures: *Stereum hirsutum* was the most frequent, followed by *Postia pelliculosa*, *Nothophellinus andinopatagonicus* and *Aurantiporus albidus*. Basidiocarps around cavities were of two species that did not develop in cultures: *Laetiporus portentosus* and *Macrohyporia dictyopora*. Excavations were slightly more frequent in white rot colonized than brown rot colonized wood, but this may be an artefact of differential success in fungal isolation and culturing, since several cavities that showed visual symptoms of brown wood rots did not yield mycelia of those wood-decay fungi. As shown by research elsewhere, basidiocarps underestimated heart rot on cavity walls and revealed additional wood-decay species living on the same trees; therefore, assessments of fungal diversity in substrates used for cavity excavation should be based on culturing and/or DNA extraction. Because fungal communities in the southern Andes are poorly known, decay fungi and their roles in ecosystem development should be studied across different forest areas, where samples from non-cavity-bearing (control) trees should also be taken in order to determine excavation-site selection.

KEYWORDS

Agaricomycotina, *Campephilus magellanicus*, ecological facilitation, heart rot, Patagonia, polypores, wood condition, wood-decay fungi

1 | INTRODUCTION

Wood hardness has long been recognized as a relevant feature of trees that are excavated by woodpeckers (Aves, Picidae) for nesting and sheltering (Bednarz, Ripper, & Radley, 2004; Jackson & Jackson, 2004; Jusino, Lindner, Banik, & Walters, 2015; Lorenz, Vierling, Johnson, & Fischer, 2015; Matsuoka, 2008; Zahner, Sikora, & Pasinelli, 2012). Hardness depends both on structural

features intrinsic to tree species and on decay-promoting organisms, particularly wood-decay fungi, which are essential for the functioning of forest ecosystems (Boddy, 2001; Boddy, Frankland, & van West, 2008; Lonsdale, Pautasso, & Holdenrieder, 2008; Parks, 1998). These fungi belong to various orders within the Agaricomycotina (Basidiomycota) worldwide (Hibbett et al., 2014). Although all decay fungi digest wood causing it to lose density and strength, they differ in their capacity to attack the different cell wall components of wood. Brown rot fungi decompose almost exclusively carbohydrates, whereas white rot fungi degrade both carbohydrates and lignin. This functional difference reflects fungi-specific differences in enzymatic/oxidative repertoire, the residual products of decomposition from fungal action having variable pH, solubility and redox potentials (Aguiar & Ferraz, 2007; Cullen & Kersten, 2004; Lemaire & Beguin, 1996; Micales & Highley, 1989).

The role of wood-decay fungi as precursors for cavity excavation was verified for several woodpecker species in temperate systems of North America and Europe (Elliott et al., 2019). Yet woodpecker–fungi interactions remain poorly known for most Picid species, especially those that inhabit the Neotropics, Africa and Asia. The only study linking fungi and a suite of cavity-nesting species (woodpeckers included) in South America was conducted in a tropical forest site, and no taxonomic specificity was provided for fungi associated with woodpecker cavities (Cockle, Martin, & Robledo, 2012).

The cool temperate forests that stretch *ca*. 35–55°S along the southern Andes shared by Chile and Argentina host the Magellanic woodpecker (*Campephilus magellanicus*), a large picid (~45 cm) which excavates large (~9 × ~16 cm opening, >30 cm vertical depth) nesting and roosting cavities on native trees (Ojeda, 2004; Saavedra, Ojeda, Soto, & Galaz, 2011). The ecological roles of wood-decay fungi in these forests are poorly known, as most prior fungal research has focused on biodiversity (Greslebin & Rajchenberg, 2003; Rajchenberg, 2006; Wright & Deschamps, 1972) and pathology (Cwielong & Rajchenberg, 1995).

These forests are dominated by southern beech trees (Nothofagaceae). Among these, lenga (*Nothofagus pumilio*) is one of the most important because of its coverage, ecological functions and value as a timber resource (Donoso, 2006). Magellanic woodpeckers have been studied for decades in old-growth lenga stands of north Argentine Patagonia. Here, despite high deadwood availability, the trees selected for cavity building are living individuals with decline symptoms such as partial crown mortality and ring growth arrestment (Ojeda, Suarez, & Kitzberger, 2007). In such conditions, completion of most cavities is a lengthy process that takes months to several years (Ojeda, 2004; Ojeda & Chazarreta, 2014).

Taking advantage of a complete set of cavities inventoried during the long-term study in Argentine Patagonia, we aimed at assessing whether Magellanic woodpeckers benefit from wood-decay present in the trees selected for cavity construction. Softened wood chips eventually found at the tree base, along with macroscopic signs, such as mycelia, discoloured wood, and basidiocarps observed during cavity–nest inspections, were indications of the presence of heart rots (Ojeda, 2006; Appendix S1). The most common basidiocarps of xylophagous fungi were of *Laetiporus portentosus*, both on cavity and non-cavity trees (Ojeda, 2006; Figure a. & b. in Appendix S1).

Specifically, we (a) assessed the decay status of the wood (and wood remains) around recent (fresh) cavities, by close inspection, (b) identified the decay fungi present in the walls of these cavities by means of fungal culture morphology in the laboratory and (c) compared the results based on fungal cultures with those based on the basidiocarps present around the cavities.

2 | MATERIAL AND METHODS

2.1 | Study area

The study was conducted on the eastern (Argentine) slopes of the Patagonian Andes, 15 km south of Bariloche city. From west to east, north Patagonia includes the Andean cordillera (>2,000 m elevation), the lower foothills intersected by glacial lakes and valleys covered by forests, and the Patagonian plains at *ca*. 700 masl. The rain shadow effect of the Andes on the eastern slopes brings a sharp decline in precipitation of *ca*. 3,000 to *ca*. 500 mm, in only 70–80 km, which is paralleled by a west-to-east vegetation gradient. The eastern slopes are covered by stands of pure southern beech species (Nothofagaceae), or by the conifer *Austrocedrus chilensis*, with increasing aridity.

2.2 | Sampled forest and woodpecker population

Lenga is a deciduous broad-leaf tree forming extensive monospecific stands that range widely from 35–56°S, covering over three million ha (Veblen, Donoso, Kitzberger, & Rebertus, 1996). Lenga forests have proved suitable habitat for resident Magellanic woodpecker populations through their wide range, from montane stands in north Patagonia (e.g. Ojeda & Chazarreta, 2014; Vergara et al., 2017), to subpolar low altitude stands in Tierra del Fuego and adjacent islands (e.g. Soto, Pérez-Hernández, Hahn, Rodewald, & Vergara, 2017; Vergara & Schlatter, 2004).

We focused on a successful Magellanic woodpecker population that was monitored intensively since 1998 in the Challhuaco Valley (41°15′S, 71°17′W). Challhuaco is a rugged area (800–1,900 masl) located near the xeric limit of lenga distribution (Heinemann, Kitzberger, & Veblen, 2000), limited by the Patagonian steppe in the east, so the understory is open, and dominated by few shrubs and herbaceous species. Mountain slopes are covered by mature (>200 years) lenga forest that extends for approximately 2,400 ha and are contiguous to forests in adjacent valleys. The Challhuaco mature woodlands were targeted for long-term studies of Magellanic woodpeckers because old-growth stands are an important reference point for research in forest ecology, especially for little studied biotas (Heilmann-Clausen et al., 2017; Wirth, Gleixner, & Heimann, 2009).

Long-term monitoring of the Challhuaco woodpecker population revealed that territorial families (8–10, depending on the year) almost saturate the available forest (*ca*. 1 territory/100 ha, Ojeda & Chazarreta, 2014). Before and after the fungal sampling, members of these families were marked with coloured metal bands, and their nesting and roosting cavities geo-located and inventoried, including holes in preliminary stages of excavation (prospective nests and roosts). As a result, the age and excavation history of most cavities were known before the fungal assessment.

2.3 | Field procedures

Columnar rot in the bole of lenga trees is discontinuous and may derive from infections via different routes (Cwielong & Rajchenberg, 1995). Thus, for a description of the infections present at a specific height (i.e. around a cavity under excavation), direct sampling of the wood tissues, with further determination of the rots present, either by culturing or molecular analyses, are needed.

Sampling was conducted from late autumn through early winter 2006, coinciding with the fruiting phenology of most Agaricomycotina in the study area (Gamundi & Horak, 2002). Sampling included fully excavated nests and/or roosts (≥20 cm of vertical depth) that had been recently completed (<1.5 years before) and unfinished cavities (<20 cm of vertical depth) that were in advanced stages of excavation (i.e. entrance fully opened) at the time of sampling. The maximum age restriction was set to ensure that rots associated with the cavity excavation process by woodpeckers were studied (i.e. potential precursors), avoiding secondary colonizers as much as possible. Moreover, these criteria allowed us to exclude failed excavation attempts (cavity trials) that are sometimes found in woodpecker territories (Ojeda, 2006). Over 30 cavities in the study were examined in detail, of which 20 were randomly chosen, covering all woodpecker territories (usually 2 or more cavities/territory).

Fungal cultures from non-cavity-bearing trees (as for a fungal availability assessment) were not attempted due to time and logistic limitations. Partly overcoming this failure, we explored the variety of wood-decay fungi present in the study site by photographing and collecting basidiocarps from nearby non-cavity trees: all collected basidiocarps were deposited at BCRU herbarium (Bariloche, Argentina) (http://sweetgum.nybg.org/science/ih/).

Along with a detailed survey of conks present, for each cavity tree we measured: height (m), diameter at breast height (cm, hereafter DBH) and degree of crown dieback in three classes (AL, crown 100% alive; MD, moderate damage with crown dieback ≤25%; AD, advanced damage with crown dieback >25%, but still supporting some foliage). There were no dead trees in the sample.

Trees were climbed with ropes (Laman, 1995) or using ladders (Appendix S2). At cavity level, we measured: height above ground (m), cardinal orientation of the entrance (degrees, every 5°), and diameter of the supporting bole (DSB, hereafter). We surveyed the basidiocarps of wood-decay fungi from inside and around the cavities, taking pictures in the field and in the laboratory. Basidiocarps were identified to species with the aid of literature describing local wood-decay fungi (Greslebin, 2002; Rajchenberg, 2006).

Every cavity was evaluated visually with lights and mirrors, and the condition of the wood at all walls was assessed based on colours (wood streaks of different colour), presence of mycelia or other fungal structures, and any abnormal feature was noted; several photographs were taken (Appendix S3). Wood core samples up to 20 cm in length were extracted with sterilized increment borers (Haglöf 3-Thread 0.200 ", various lengths) from seven locations within each cavity, according to the protocol shown in Figure 1. The distance from the cavity entrance to each sampling location was chosen based on the mean dimensions of Magellanic woodpecker cavities and starts (Ojeda, 2004).

To avoid cross-contamination before extracting each sample, the operator's hands, the borer and other instruments eventually used, were dipped in 96% ethanol and left to dry naturally; in addition, the borer was passed through a flame. Inexpensive sterile storage devices were based on commercially available plastic straws that were closed at both ends with hot pliers immediately after unwrapping. Ends of straws were opened by cutting in the field, immediately before introducing a core sample. During the extraction process, core condition was evaluated on the basis of colour, structural integrity and ease of extraction. In this way, boring also served to assess wood hardness. After introducing a core sample, straws were closed using heat. Samples were taken to the laboratory on the same day as extraction and stored at 5°C for a maximum of 3 days before processing.

2.4 | Laboratory protocols

Laboratory procedures were conducted under aseptic conditions. In order to identify wood-decay fungi through cultures, four pieces of heartwood were extracted from each core, from portions not visibly rotten or decayed, to increase the chances of culturing pioneer decay fungi, and not only secondary colonizers of the wood (Rayner & Boddy, 1988). Four wood chips were placed at opposite sides of sterile 4 cm diameter Petri dishes on 2% malt extract agar with fungicide and antibiotic (0.05% benomyl and 2% streptomycin) added in order to reduce moulds and bacteria growth (Rajchenberg, 1997). Duplicate cultures were prepared from each core. Isolations were incubated at *ca*. 25°C and examined daily for mycelium development. Isolations were repeated from pieces of the original material if cultures were contaminated. Mycelia selected for further study were those considered to be Agaricomycotina based on either (a) clamp connections or (b) simple-septate hyphae that were >5 um in width, with no meander shape and/or oily contents (i.e. more likely to be Ascomycota). Except for mycelia that produced chlamydospores, asexual spore production was indicative of moulds. Mycelia

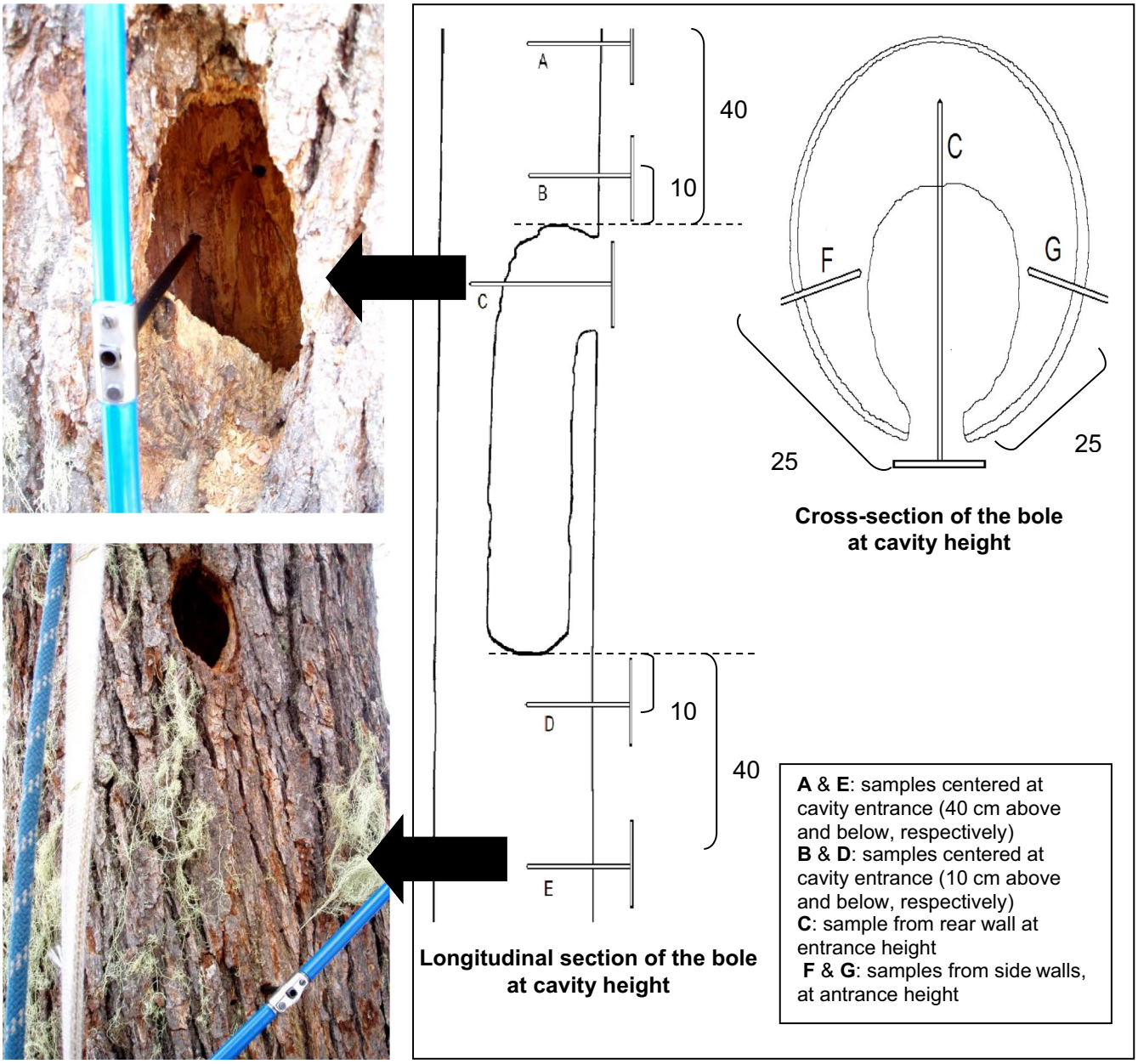

FIGURE 1 Schematic representation of the protocol for the extraction of core samples from freshly excavated Magellanic woodpecker cavities. The 'T' object represents the increment borer used for core extraction, which is shown in the images on the left. Dimensions are in centimetres

of interest were transferred to test tubes containing 2% malt extract agar, in triplicate. These tubes were maintained at 5°C for later analyses based on Nobles (1965).

Standardized culture medium was prepared according to Nobles (1965): malt extract agar Difco (AEM) 35 g, distilled water 1,000 ml. This medium was sterilized by autoclaving for 15 min at 121°C. Three Petri dishes were inoculated with each culture. Mycelia of interest were inoculated on one side of a 9 cm diameter Petri dishes. Cultures were grown under controlled conditions at 25°C in a lighted room. All working surfaces were previously disinfected with chloramphenicol and 96% ethanol. Each culture was studied for 6 weeks, with systematic analyses at weeks 2, 4 and 6.

2.5 | Identification of cultures

Determination of strains followed standardized protocols (Nobles, 1965, amended by Nakasone, 1990), as summarized for wood-rotting fungi of *Nothofagus pumilio* in Patagonia by Rajchenberg (1997, 2006) (Appendix S4). The main variables recorded were as follows: radial mycelial growth to the edge of the dish; shape and other attributes of the growth front; colour and texture of the hyphal mat; presence or absence of sporulating structures; changes in colour of the agar as derived from the growth of the mycelium and culture odour. Microscopic features of the mycelia were studied with preparations mounted in 1%

phloxine and 5% KOH. Along with the morphological analyses, oxidase tests were conducted to detect the production of laccases, on every culture. For these tests, mycelia were inoculated into a medium containing (per litre) malt extract agar (Difco) 35 g and gallic acid 5.0 g.

Photographs were taken to document morphological and physiological characterizations, and drawings of representative structures were prepared under an optical microscope (with a Leitz drawing tube) (Appendix S4). For the storage of post-fixation tissues, lactophenol cotton blue stain was used, as recommended for filamentous fungi (Hawksworth, 1974). Microscopes used were Olympus BX50 and Leitz Laborlux 11, both with transmitted light.

2.6 | Data treatment

The range and means ± *SD* for quantitative tree and cavity variables are presented. Given the exploratory nature of this research, raw data are presented in tables, which include the cultures that were identified in each cavity.

3 | RESULTS

3.1 | Characteristics of cavities and trees

DBH of cavity trees was 64 cm (±23.97 *SD*; range 41–148 cm), and height was 19.45 m (±2.33 *SD*; range 16–24 m). Almost all (95%) cavity trees exhibited signs of crown dieback; only a single tree was totally vigorous. While most trees exhibited only incipient dieback (i.e. a few dead or fallen apical branches), *ca*. 25% of cavity trees showed advanced dieback, with over one-quarter of the crown absent or dead.

Almost one-half of the cavities sampled ($n = 9$) were fully excavated (>20 cm vertical depth below the lower entrance rim) and had been used as nests; the rest were unfinished excavations at different stages, usually <20 cm deep below the lower entrance rim. Cavities were 6.7 m (±2.55 *SD*; range 2.4–11.5 m) above ground level, normally in the main trunk (DSB 57 cm ± 9.9 *SD*; range 42–83 cm), just below the crown. A cavity located much higher than the others examined was on a primary branch (a division of the main trunk, DBH 141 cm); nevertheless, the bole where the cavity was excavated was 56 cm in DSB, equivalent to most other cavity-bearing trunks.

One-half of the cavities were oriented southwards (91–269°), one third showed a northerly (271–89°) orientation and 10% (2 cavities) faced west (270°).

3.2 | Wood-decaying fungi

Assessment of structures present, colours and consistency of the cavity walls (Appendix S3) and of the appearance of the fresh cavity samples obtained indicated that all cavities were excavated in wood softened by wood-decay fungi. This conclusion was later confirmed by the identification of fungi obtained in cultures from the heartwood of the cavity walls.

A total of 127 wood samples were obtained from the cavities. For eight cavities, the seven intended samples were not obtained, due to constraints such as pockets of decay from an adjacent fallen branch. Sometimes, a branch or stub precluded use of the borer in the desired location. These limitations were extreme for one cavity, where only two wood samples were extracted.

Culture-based work enabled accurate fungal species identification in 17 cavities, where at least one species of a wood-decay fungus (Agaricomycotina) was isolated. For the remaining three cavities, cultures contained non-xylophagous species or no mycelium grew. Approximately one-half of the wood samples collected and examined in the laboratory developed mycelium of wood-decay fungi. Mucoromycotina (Order Mucorales) and deuteromycetes also developed from samples with and without polypores. No fungi developed in 5.5% of the wood tissue samples under culture conditions.

Four wood-decay species (Agaricomycotina) were isolated from the cultures (Table 1): *Stereum hirsutum* was the most common (11 of the 17 cavities with wood-decay fungi isolated), followed by *Postia pelliculosa*, present in 7 cavities. Less commonly isolated were *Nothophellinus andinopatagonicus* (3 cavities), and *Aurantiporus albidus* (one cavity). *Postia pelliculosa* was the only brown rot fungus isolated, present in 36% (18/50) of the wood-decay samples. The remaining samples (64%) developed white rot fungi. While most cavities contained a single decay species, two different species were detected in some cavities (*ca*. 18%); only one cavity contained three different wood-decay species (Table 2). Wherever multiple colonizations occurred, species were segregated into different cavity walls (and hence, different wood samples) and did not share the same heartwood portion (Table 1).

Along with species identified from cultures, basidiocarps of *L. portentosus* and *Macrohyporia dictyopora* were observed and collected from cavity trees (Table 1). In only one case was a basidiocarp found on a cavity tree that corresponded to a species later isolated from wood from the same tree (*P. pelliculosa*). As for non-cavity trees in the study site, fruit bodies of *L. portentosus* were by far the most abundant and visible.

Decay species identified from cavities and the surrounding tissues were apparently neither influenced by the cardinal orientation of the cavity opening, nor by their distribution in the study site (raw data in Table 2). In the same way, nest height was unrelated to the presence of a specific fungal species, except perhaps for *A. albidus*, but small sample size prevented analyses. Since most cavity trees were in the same crown dieback condition (moderate), there was no obvious relationship between this characteristic and specific fungal decay species found (Table 2).

4 | DISCUSSION

Fungal cultures and close inspection of cavity walls demonstrated that fresh cavities created by the Magellanic woodpecker in xeric

TABLE 1 Distribution of polypore species associated with Magellanic woodpecker cavities

Cavity		Location relative to cavity (see Figure 1)							
Start	Fully excavated	A	B	C	F	G	D	E	Basidiocarps present
1			SH		SH	SH			
2		PP	SH	-----	PP	PP		PP	
3						SH	PP	PP	PP
	4	PP	PP			PP	SH	SH	
	5				----- *				*Macrohyporia dyctiopora*
6			AA	AA		AA	AA		*Laetiporus portentosus*
7					SH			SH	
	8				PP		-----		
	9	SH	SH	SH				SH	
10						SH			
11		SH		SH					
12						SH			
	13	PP	PP						
14				SH		PP	NA		
15		PP		PP	----	PP	PP	PP	
16		SH				----			
	17	NA	NA	---- *	NA	NA	NA	NA	*Laetiporus portentosus*
	18						NA	NA	
	19			----- *				-----	PP
	20	-----	-----		----- *	----- *		-----	

Note: Initials are used for the four species isolated from cultures (full names given below). Empty cells indicate no wood-decay fungus was obtained from the cultures. Dashed lines indicate no sample was taken (e.g. due to a branch stub impeding manoeuvres). Asterisks along with a dashed line indicate the sample could not be obtained due to excessive rot.

Abbreviations: AA, *Aurantiporus albidus*; NA, *Nothophellinus andinopatagonicus*; PP, *Postia pelliculosa*; SH, *Stereum hirsutum*.

old-growth lenga forest were associated with white or brown rot that had softened the heartwood. The widespread presence of heart rot in the cavity walls was not paralleled by the presence of basidiocarps, which were scarce and mostly belonged to species not detected in the cavity walls (i.e. by culturing). Advanced crown dieback was not a necessary condition for trees to have heart rot, as most trees in our sample exhibited moderate dieback, in line with previous findings for this woodpecker (Ojeda et al., 2007). Although the selection of 'healthy' trees for nest or roost building by Magellanic woodpeckers, which is not the norm among woodpeckers (Cockle, Martin, & Wesołowski, 2011), may relate to stronger excavation abilities in large bodied picids, heart rots seem to be necessary preconditions for excavation.

The presence of basidiocarps misrepresented fungal presence and diversity in the cavity trees in our study area, as also recognized for the Northern Hemisphere (Jusino et al., 2015; Rayner & Boddy, 1988). Most species isolated from the cavities had not produced sporophores in the adjacent trunk regions at the time of the fieldwork (highest fruiting season; Gamundi & Horak, 2002), with the exception of one basidiocarp growing inside a cavity (Appendix S3, cavity #5). The total absence of basidiocarps of *S. hirsutum* is noteworthy, as it was the most common species found in the cavity tissues. A plausible explanation for this is that *S. hirsutum* is known to produce fruiting bodies on dead trees (Vasaitis, 2013); alternatively, colonization by this species may still be in an early phase in some trees, so the degree of wood degradation and/or the wood volume captured around the cavities was possibly too low. The formation of fruiting bodies requires that the fungal mycelia to have colonized a sufficient volume of wood in order to allocate energy resources to formation of fruiting structures (Moore, Gange, Gange, & Boddy, 2008).

Conks of two species were present, one of which was *L. portentosus*, a brown rot fungus typical of xeric lenga stands like Challhuaco and with conspicuous basidiocarps, growing on living trees (Cwielong & Rajchenberg, 1995; Rajchenberg, 1997). Based on its predominance among the basidiocarps observed through the site, its absence in the cultures was unexpected. Our work supports the proposition that collection of the tissues around the cavities is

TABLE 2 Attributes of Magellanic woodpecker cavities and cavity trees, from which wood-decay fungi were obtained in cultures ($n = 17$)

Wood-decay fungi present (# of cavities)	Cavity height (m)	Cardinal orientation (in quadrants)	Crown dieback	Territory[a]	
				Start	Fully excavated
Only *S. hirsutum* (7)	6.7	E	Moderate dieback	1	
	7.2	S	Moderate dieback		2
	2.4	S	Moderate dieback	2	
	9.9	E	100% live	3	
	6.5	N	Moderate dieback	3	
	8.7	W	Moderate dieback	3	
	6.2	N	Advanced dieback	4	
S. hirsutum-P. pelliculosa-N. andinopatagonicus (1)	9	S	Moderate dieback	4	
S. hirsutum-P. pelliculosa (3)	4.9	E	Moderate dieback	1	
	4.2	E	Moderate dieback	2	
	11.5	W	Moderate dieback		2
Only *P. pelliculosa* (3)	5.3	S	Moderate dieback	2	
	6.8	W	Advanced dieback		3
	6.6	E	Moderate dieback	5	
Only *A. albidus* (1)	3.4	N	Moderate dieback	3	
Only *N. andinopatagonicus* (2)	5.9	S	Moderate dieback		6
	6	S	Advanced dieback		7

[a]Arbitrary numbers were given to the different territories.

needed—using sterilized tools, for culturing (as in this study) and (ideally) for DNA extraction (e.g. Jusino, Lindner, Cianchetti, & Grisé, 2014), to determine which fungi are present.

Most wood-decay species isolated from cultures were exclusive to one cavity (i.e. not sharing the bole portion with another decay species), and whenever two or more species were obtained (<30% of the cavities), the isolates came from different parts of the heartwood (i.e. cavity walls). Such territorial occurrence is consistent with the formation of melanized demarcation lines between xylem tissues colonized by competing decay fungi (Heilmann-Clausen & Boddy, 2005). The largely most common species *S. hirsutum* (60% of cavities) is a cosmopolitan species that causes white rot. The prevalence of this corticioid species in this study was surprising, as it had not previously been isolated from the heartwood, but only from the sapwood of lenga trees growing in mesic and humid conditions, where it was not considered common (Cwielong & Rajchenberg, 1995; Rajchenberg, 1997). Probably, its high frequence in the present study was more associated with its ability to develop quickly and sporulate as a saprophyte on the debris of a large variety of forest species (perhaps with a ruderal colonizing strategy, Mirić, 2005), and a wound heart rot fungus (Vasaitis, 2013), given that woodpecker excavation into living trees serves as an entrance point to wood-decay fungi. It is interesting to note that we isolated this species almost exclusively from the recently exposed heartwood of cavities under construction.

The second most common species found was *P. pelliculosa*, a brown rot fungus, which was present in approximately 35% of cavities analysed. The relatively high prevalence of this species was not surprising, as it was already recognized as a dominant polypore in mesic and humid lenga stands of central and north Patagonia (Cwielong & Rajchenberg, 1995). The low frequency of *N. andinopatagonicus* matches its natural scarcity in xeric lenga forests across the region (Cwielong & Rajchenberg, 1995), as this fungus appears to prefer humid stands; otherwise, it is the main white heart rot agent of lenga. The only cavity colonized by *A. albidus* was 3.4 m above ground, which would be consistent with its erratic and variable presence along the stem, both in basal and high parts (Rajchenberg, 2006). This species might be especially limited in a xeric site such as Challhuaco, with extreme water deficit during the summer.

Little information has been published on wood-decay fungi present in dry lenga forests, possibly due to the poor regeneration potential of these trees (Heinemann et al., 2000), which, in turn, lowers the value of these stands for timber production. At more humid lenga sites, a reduced number of decay species (including *Postia pelliculosa*, *Postia dissecta*, *Serpula himantioides* and *Fistulina hepatica*, among the brown rots, and *N. andinopatagonicus* and *A. albidus*, among the white rots) represented 90.1% of the isolations from heartwood cultures (Rajchenberg, 1997). In line with this reduced number of wood-decay fungi, we identified

only four species involved in the softening of lenga heartwood for woodpecker excavation in Challhuaco (six species, including those identified as conks). Although we did not assess the availability of decay fungi at the site (i.e. by sampling control trees), lenga stands in dry conditions might be expected to contain lower diversity of wood-decay fungi than the humid and mesic stands that have been studied in more detail.

Analysing if woodpecker excavations are preferentially associated with white or brown rot fungi is relevant in terms of their adaptation to the Lenga forest environment, for these rots usually yield different substrata for excavating breeding and roosting cavities. While white rots derive light coloured fibrous chips (e.g. Cavities #18 and 20 in Appendix S3), brown rots form fine reddish dust (e.g. Cavity #19 in Appendix S3); for example, dust in large amounts can be detrimental for small nestlings if inhaled, but additional potential effects (both negative and positive) on breeding performance should be evaluated for each rot type. Our culture-based determination of taxa suggested that white rot was slightly more common compared with brown rot as a precursor of Magellanic woodpecker cavity excavation in dry mature lenga stands. This pattern should be interpreted cautiously, however, as it may be an artefact of differential success in culture. Several cavities that showed visual symptoms of brown wood rots (Appendix S1 and S3) did not develop mycelia in culture. In turn, despite not isolated from the cultures, basidiocarps of *L. portentosus* were very common in the Challhuaco forest site, and sometimes occurred adjacent to Magellanic woodpecker cavity starts (Appendix S1, photographs a and b). Broken trees in the study site also usually showed well-developed cubic brown rot (Appendix S1, photograph e) (Ojeda, 2006). Based on these inconsistencies, further sampling and alternative detection methods (such as fungal DNA barcoding) may be necessary to uncover the presence of brown rot fungi (particularly *L. portentosus*) in the wood tissues supporting Magellanic woodpecker cavities.

Studies in the Northern Hemisphere (e.g. Jusino et al., 2015; Parks, Bull, Filip, & Gilbertson, 1996; Parks, Raley, Aubry, & Gilbertson, 1997) showed that distinct fungal communities may act as key precursors of cavity excavation by woodpeckers, depending on cavity function and age, site conditions and other factors that include the picids themselves as vectors of fungal colonization of prospective cavity substrates (Jusino, Lindner, Banik, Rose, & Walters, 2016). Considering the wide area covered by lenga forests in the south Andean landscapes and their renowned value as woodpecker habitat (Ojeda & Chazarreta, 2014; Soto et al., 2017; Vergara et al., 2017; Vergara & Schlatter, 2004), most successful Magellanic woodpecker populations may occur in these forests, so the wood conditions that allow for their persistence should be explored in depth. Different factors constrain lenga stands along their wide latitudinal range, such as annual precipitation in the dryer, northern range and mean annual temperature in the southern, humid extreme (Lara et al., 2005). Moreover, stand dynamics differ greatly east of the Andes, from humid, to mesic, to xeric conditions (Heinemann et al., 2000). Accordingly, wood-decay fungi that are precursors of cavity excavation by Magellanic woodpeckers may vary across these gradients.

The present study is a first approach to understanding the decay fungi that might facilitate the process of woodpecker cavity construction in living trees by Magellanic woodpeckers, integrating ornithology and forest mycology, disciplines that are traditionally distant. Given its limitations (i.e. lack of control samples, and molecular tools for identification), further mycological research is still required to understand the links among birds and fungi in this region. Be it based on cultural procedures, molecular tools, or both, the fungal communities that facilitate excavation for Magellanic woodpeckers, as well as the fungal assembly in the surrounding forest, should be explored in different lenga stands. In particular, selectivity for brown or white rots depending on cavity function (i.e. roosts or nests) seems a promising line for future studies. Further research should also take into account stands dominated by other Nothofagaceae species used by Magellanic woodpecker for cavity building (see Saavedra et al., 2011), where potential precursors of cavity excavation are less known. Such mycological research would be relevant beyond Magellanic woodpeckers, as our understanding of the diversity and ecology of South American fungi remains limited.

ACKNOWLEDGEMENTS

We sincerely thank Drs. Laura Lorenzo and Gernot Vobis for kindly lending laboratory facilities at Universidad Nacional del Comahue and for their valuable assistance in the laboratory. Juan Karlanian provided key help in the field, both at cavity searching and monitoring, and for tree climbing. We thank especially two anonymous reviewers for their valuable comments on an earlier version of this manuscript. IDEA WILD (Fort Collins, CO, USA), and Birders' Exchange (Colorado Springs, CO, USA), donated equipment that was fundamental for this study. Field activities were mainly supported by CANON USA Inc. through its National Parks Science Scholars Program for the Americas to VO. Sampling in Nahuel Huapi National Park was conducted under permit 781 (2006) to CP and VO from Dirección Regional Patagonia Norte (DRPN-APN).

AUTHOR CONTRIBUTIONS

CP and VO conceived the general idea and discussed it with MR in search of a valid research approach. VO and CP led the field procedures. CP and MR led the laboratory procedures. All authors contributed critically to drafts and gave final approval for publication. MR and VO are members of CONICET (National Research Council of Argentina); CP is member of APN (National Parks Administration); and all are Argentine public institutions.

PEER REVIEW

The peer review history for this article is available at https://publons.com/publon/10.1111/efp.12634.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Pozzi C, Rajchenberg M, Ojeda V. Decay fungi associated with cavity excavation by a large South American woodpecker. *For. Path.*. 2020;00:e12634. https://doi.org/10.1111/efp.12634