Cellobiase Activity as an Indicator of Fungal Decay in the Wood of Woodpecker Nest Cavities in the Pacific Northwest.

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Abstract

Woodpeckers require trees and snags with decayed wood in order to excavate nest and roost cavities, and interior wood hardness is considered an important factor determining where a woodpecker can create a cavity. In most ecosystems, saprophytic fungi are responsible for the decay and softening of wood, and are thought to be important in providing soft wood for woodpecker cavity excavation. We conducted a study of cellulose-degrading enzymes in the wood surrounding woodpecker nest cavities. We measured wood hardness, percent wood density loss (PWDL), and activity of cellobiase (an extracellular fungal cellulase that degrades cellulose) within wood surrounding the nest cavities of the northern flicker (*Colaptes auratus*), black-backed woodpecker (*Picoides arcticus*), white-headed woodpecker (*Dryobates albolarvatus*), and hairy woodpecker (*D. villosus*) in Oregon and Washington. We found that mean wood hardness was significantly lower, and cellobiase and PWDL were significantly higher, at nest cavities than controls for each woodpecker species. Wood hardness was higher and cellobiase lower at nests of black-backed woodpecker than northern flicker, but did not differ among the other woodpecker species. Our results suggest that increased amounts of cellobiase result in softer wood due to the increased decay caused by higher fungal enzyme activity and that measuring cellobiase can be used to estimate wood decay without directly measuring wood hardness. All four woodpecker species selected nest substrates with softer wood and higher fungal enzyme activity than controls. This supports findings from previous studies of the importance of saprophytic fungi for woodpecker cavity excavation.

Keywords: cellobiase, fungal enzymes, wood hardness, woodpeckers, cavity excavation

Introduction

Primary cavity excavators, particularly woodpeckers, remove wood from decaying trees in order to create nest and roost cavities. Past studies have often used the external appearance of snags/trees to understand how woodpeckers select a substrate for cavity excavation. Results from these studies suggest that variables such as cavity orientation (Inouye 1976, Korol and Hutto 1984), selection for large-diameter substrates (Lehmkuhl et al. 2003, Bagne et al. 2008), and visual signs of decay (e.g., fungal fruiting bodies, sloughing bark, broken top; Harestad and Keisker 1989, Ganey and Vojta 2004, Kozma 2012) are important correlates for this selection process. However, woodpeckers are ultimately limited to the choice of nesting sites by their own morphology and excavating ability. Thus, interior wood hardness is considered a critical factor determining where a woodpecker can create a cavity and why few woodpeckers are able to excavate cavities in living trees with sound wood (Schepps et al. 1999, Jackson and Jackson 2004, Lorenz et al. 2015).

In order for most woodpeckers to excavate a cavity, the interior wood of dead or live trees needs to be softened, but not extensively rotted (Jackson and Jackson 2004). This task is accomplished by saprophytic fungi in many ecosystems. Srivastava et al. (2013) proposed that the vast majority of these are basidiomycetes that degrade both the sapwood and heartwood, although more recent studies have found woodpecker cavities containing

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hundreds or even thousands of taxa (e.g., Jusino et al. 2015), representing both Basidiomycota and Ascomycota. Actual surveys of saprophytic fungi at woodpecker nests in most forest communities have not been conducted because decay fungi are difficult to detect; they do not reliably produce fruiting bodies (i.e., sporocarps) and require advanced molecular methods to identify. Other decay classification methods (e.g., broken tops, branches, or degree of sloughing bark) have been used in an attempt to qualify the extent of wood decay (Cline et al. 1980, McClelland and McClelland 2000, Bevis and Martin 2002). However, softened wood may be present in trees without visible evidence of decay (Lorenz et al. 2015), resulting in a need for alternative methods of assessing fungal activity.

To explore alternative methods of assessing fungal activity, we conducted a study of cellulose-degrading enzymes in the wood surrounding woodpecker nest cavities. Cellulose is one of the main components of wood and is composed of long chains of glucose, where β-glycosidic bonds link each glucose to the next. Cellulose is degraded by extracellular fungal cellulases (Vaheri et al. 1979), such as cellobiase, that cleaves the final β-glycosidic bond and produces two glucose molecules that fungi use as a food source. Because saprophytic fungi produce enzymes that degrade plant cell wall polysaccharides, such as cellulose (Rytioja et al. 2014, Payne et al. 2015), we predicted that cellobiase would be present in higher amounts in wood samples obtained from dead trees with a greater degree of wood decay. We measured wood hardness and cellobiase activity within wood surrounding the nest cavities of four woodpecker species that represent a range of excavating capabilities: northern flicker (*Colaptes auratus*), black-backed woodpecker (*Picoides arcticus*), white-headed woodpecker (*Dryobates albolarvatus*), and hairy woodpecker (*D. villosus*). We chose these species because they are common in our study area (Kozma 2012, Lorenz et al. 2015), and they are important creators of cavities used by other species (Martin and Eadie, 1999, Kozma 2014). Our objectives were to determine if cellobiase activity is an indicator of decay activity (e.g., wood hardness) and if cellobiase activity and/or wood hardness at cavity sites differs between the woodpecker species. To aid land managers who are tasked with providing suitable snags for woodpeckers, we also explored if cellobiase activity matches visual indicators of decay (e.g., percent bark loss, percent top loss). We hypothesized that the amount of cellobiase will be negatively correlated with wood hardness because we expected higher fungal activity in sites with well-decayed wood. We also hypothesized that wood hardness and cellobiase activity will differ between nest cavities of the different woodpecker species because the species differ in their excavation abilities, which are linked with foraging specializations. Specifically, black-backed and hairy woodpeckers forage primarily by excavating in dead wood, and thus we predicted they would excavate in snags with lower cellobiase activity compared to white-headed woodpecker and northern flicker, which forage primarily by gleaning and should excavate in softer snags with higher cellobiase activity.

Methods

Study Area

We conducted our study in 2017 at two locations in the Pacific Northwest, USA (Figure 1). One site was in central Oregon at Pringle Falls Experimental Forest on the Deschutes National Forest (lat 43.79794, long –121.67821). The second site was in central Washington on the Naches Ranger District, Okanogan-Wenatchee National Forest and surrounding state-managed lands (lat 46.79941, long –121.06985). Both sites are located along the eastern slope of the Cascade Range and were dominated by ponderosa pine (*Pinus ponderosa*), with Douglas-fir (*Pseudotsuga menziesii*) co-dominant at higher elevations and north aspects. Other tree species occurred less frequently and included grand fir (*Abies grandis*), western larch (*Larix occidentalis*), and in Oregon, sugar pine (*Pinus lambertiana*). Elevation ranged from 1,340 to 1,800 m in Oregon and 700 to 1,460 m in Washington. Fire and timber harvest were the most common disturbances at these sites. While we searched for woodpecker nests in both burned and

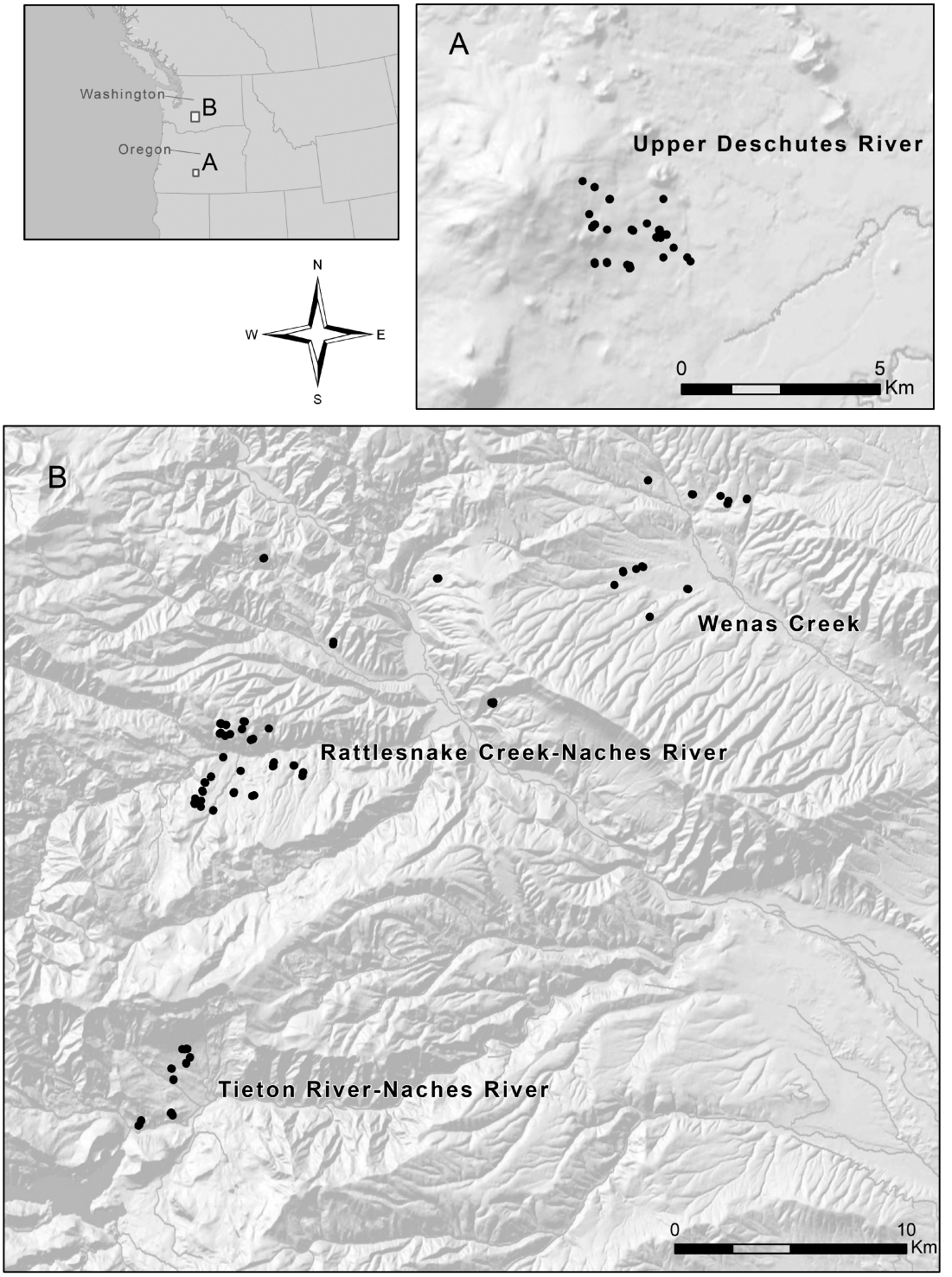

Figure 1. Location of woodpecker nest and control pairs in 2017 used to sample cellobiase levels and percent wood density loss in A) central Oregon, and (B) central Washington.

unburned forests, most were found in forests that had burned by either prescribed fire or wildfire.

Locating Woodpecker Cavities

We searched for woodpecker nests from March through July. We focused on the four most common woodpecker species on our sites: black-backed woodpecker (BBWO), hairy woodpecker (HAWO), northern flicker (NOFL), and white-headed woodpecker (WHWO). These species represent both specialists (e.g., BBWO in burned forests, WHWO in pine-dominated forests) and generalists (e.g., NOFL and HAWO) in regards to habitat preferences, and exhibit a range of life history strategies and presumed excavation capabilities. We searched for nests in both live and dead trees. To make finding nests easier, we used playbacks of calls and drumming to locate adult birds (Johnson et al. 1981) and then followed adults until we located their nest cavities. We considered nests occupied if we heard or saw nestlings or if adult behavior indicated that incubation or nestling feeding was underway (Jackson 1977), and we marked the locations of all occupied nests using portable global positioning system (Garmin CSX60, Olathe, KS) units. Because woodpeckers may reuse cavities among years, we looked for fresh wood chips on the ground surrounding cavities to verify current-year excavations to avoid sampling reused cavities.

We paired all nest substrates with a nearby randomly selected tree or snag (hereafter substrate), or control, in order to compare the amount of cellobiase, wood hardness, and percent wood density loss (PWDL) between used and unused substrates. Control substrates matched attributes of nest substrates but lacked cavities. We selected controls by walking > 75 m from nests in a random orientation until we encountered a suitable substrate within 10 m of the bearing. Following Bonnot et al. (2009), we assumed that nesting territories were within a 250-m radius centered on the nest cavity. Therefore, if no substrate was encountered within 250 m of a nest site, we returned to the nest and selected another random orientation. For controls, we included only those with a diameter at breast height (DBH) larger than 20 cm for the large-bodied NOFL and 15 cm for the other woodpeckers, which was the smallest DBH used for nesting by woodpeckers in our study area (Lorenz et al. 2015).

Vegetation Sampling

We measured vegetation characteristics and collected wood samples (see below) at nest sites in August and September after nesting had ceased. We measured cavity and substrate height with a clinometer, and substrate DBH with a diameter tape. We noted the presence of sporocarps on the bole of nest substrates, visually estimated the percentage of bark lost and top missing from each nest substrate, and estimated percent of the bole blackened by fire. We did not use a decay classification system because many snags on our study sites did not fit neatly into a decay class. For example, snags with an intact top but that were missing branches or bark were difficult for us to classify in any of the commonly used systems.

Wood Sampling and Wood Hardness

We collected a sample of wood from all nest substrates and controls. We obtained samples by drilling into each substrate with a cordless drill, with the drill bit positioned parallel to the ground. For safety reasons, we omitted nests from sampling if they were too high to be accessed by climbing ladders (> 12 m; $n = 3$) or if the snag appeared unstable ($n = 2$). We used a 9-mm wood drill bit, which we sterilized before taking each wood sample using a 20% sodium hypochlorite bath, 70% isopropyl alcohol spray, and flame sterilization, to aseptically collect wood shavings from the bole of each nest substrate 2 to 5 cm above the nest cavity opening. We sampled 2 to 5 cm above the cavity opening because measures of wood hardness by Matsuoka (2008) indicate that sampling wood in this location should be fairly representative of hardness in the entire cavity. Initially we attempted to measure hardness below the cavity chamber similar to Matsuoka (2008), but we were never confident whether we were measuring unexcavated wood or if we were drilling into a small pile of compressed wood shavings that sometimes line the bottom of woodpecker cavities. Our method

was also meant to be nondestructive because woodpecker cavities are valuable to wildlife; we wanted to avoid drilling multiple holes into nest cavities in an attempt to locate the bottom of the chamber. By measuring once above the cavity opening, we were ensured of obtaining a representative sample (Matsuoka 2008) without disturbing the integrity of the cavity.

To obtain wood samples, we first drilled approximately 3 cm deep into each nest substrate and discarded this sample because it represented the sill region and not the wood excavated from the main nest chamber. We then collected a sample of wood as we drilled from 3 to 10 cm deep, as this depth is within the range of cavity widths reported by Lorenz et al. (2015). We collected a sample from the bole of controls at the same height and orientation as the paired nest cavity. We transferred wood shavings into sterile 50-mL tubes and placed them on ice until returning to the field station, where they were then frozen at –18 °C until lab work was conducted in fall 2017.

We used every precaution to avoid contaminating sites and samples with wood shavings that were not naturally occurring at the site. We wore disposable nitrile gloves while sampling and sterilized gloved hands with 70% isopropyl alcohol or sodium hypochlorite between every sample. We did not touch cavities or substrates with any non-sterilized item, including gloved hands, until after a sample had been obtained. Sites were remote, only accessible by unimproved forest roads, and were not typically visited by humans for recreation or other purposes.

We non-destructively measured wood hardness at each cavity and control following Matsuoka (2000) and Lorenz et al. (2015), where wood mass density is proportional to the torque required to spin an increment borer into a pre-drilled hole. We took wood hardness measurements, using the hole we drilled for the wood samples, at 1-cm increments between 3 and 10 cm deep using a torque wrench to measure the torque in newton meters (N·m) at the point the increment borer begins to spin (Matsuoka 2000). We then averaged the wood hardness measurements to obtain a single estimate for each nest cavity.

We converted the estimate of wood hardness to wood density (g cm^{-3}) using equations from Matsuoka (2000) and also estimated PWDL for each sample, or the loss in wood density due to decay. To do this, we first needed an estimate of the initial wood density of dead trees before they started to decay. The density of wood in trees can vary regionally, and therefore we used different estimates for our Washington and Oregon samples. To estimate the average initial wood density of snags in Washington, we used estimates of wood density from 58 undecayed ponderosa pine and Douglas-fir snags in autumn 2016. We collected these data at trees killed within 3 months in recent burns and timber harvests on four locations in our study area. We restricted this sampling to snags with a DBH within the interquartile range of woodpecker nest substrate DBH (32 to 54 cm), estimated from 259 snags sampled in Lorenz et al. (2015), and we sampled all snags at the same height as the median cavity height (2.35 m) from Lorenz et al. (2015). We chose a random orientation for the sample on the snag bole because WHWO and NOFL orient nest cavities in our study area non-randomly (Kozma, unpublished data). From this sample of 58 recently killed trees, we estimated that the mean wood density of undecayed snags in our Washington study site was 0.38 g cm^{-3} for ponderosa pine and 0.46 g cm^{-3} for Douglas-fir. For Oregon, we obtained estimates of undecayed ponderosa pine wood (0.41 g cm^{-3}) from Bouffier et al. (2002). We did not sample Douglas-fir snags in Oregon. Overall, our estimates of wood density for undecayed wood are nearly identical to those reported in Harmon et al. (2008) for undecayed wood for these two tree species; 0.38 g cm^{-3} and 0.45 g cm^{-3} for ponderosa pine and Douglas-fir, respectively.

We estimated PWDL by subtracting each sample's estimate of wood density from estimated values of undecayed wood for the species. These estimated values used in the calculations were 0.38 g cm^{-3} for ponderosa pine samples from Washington, 0.41 g cm^{-3} of ponderosa pine samples from Oregon, and 0.46 g cm^{-3} for Douglas-fir samples from Washington only. We assumed PWDL was zero for ponderosa pine samples with wood density > 0.38 and > 0.41 g cm^{-3} from Washington and

Oregon respectively, and for Douglas-fir samples with wood density > 0.46 g cm^{-3}.

Because we only measured wood hardness at one small spot on control trees, we acknowledge that our sample scheme could not detect all pockets of rot within control trees. Control trees may have had soft wood in places on the bole that we did not measure. We therefore use the term control "sites", rather than control "trees". Additionally, our inferences are restricted to limiting extrapolation of hardness to the entire bole of control trees.

Cellobiase Activity

To assess the amount of cellobiase within each wood sample, we first ground all samples using a Wiley Mill into a fine dust. We then added 0.1 g of wood dust from each wood sample to 2 mL of extraction buffer to extract cellobiase. We centrifuged the resulting extract, collected three 250-µL samples of the supernatant, and added 0.6 mL of 1.5 mM p-nitrophenyl glucopyranoside, a molecule with the same β-glycosidic bond as cellobiase, to each sample. We allowed the p-nitrophenyl glucopyranoside and enzyme extract to react for 48 hours, and then stopped the reaction by adding 600 µL of 0.2-molar sodium carbonate buffer (pH 9.5). This stops the reaction by denaturing the enzymes and deprotonating the p-nitrophenol, resulting in a yellow color. We placed 250 µL of this solution into a microplate well and measured absorbance at 400 nm. To subtract the intrinsic color of the wood sample supernatant, we created a blank for each sample by adding 500 µL of sodium carbonate, 462 µL of p-nitrophenyl glucopyranoside, and 38 µL of supernatant. We then subtracted the absorbance of each sample from its blank. We averaged absorbance for the three extracts from each sample to determine our final estimate of absorbance for each wood sample. Finally, we converted absorbance measurements into nanomolar (nM) of p-nitrophenol using a standard curve. Higher concentrations of liberated p-nitrophenol only result from cleavage by cellobiases in the sample, which indicates cellulase activity. Because free glucose is the ultimate purpose of cellulose digestion by fungi and bacteria, cellobiase activity in a sample should correlate with the amount of cellulose degradation. We expect all fungal cellulolytic pathways to ultimately go through a final step to liberate glucose for metabolism, and therefore our method should index and track the effect of all cellulases.

Statistical Analysis

We used *t*-tests to compare wood hardness and an index of cellobiase amounts between nests and controls. Because cellobiase data were not normally distributed, we applied a square root transformation. We used sign tests to compare PWDL between nests and controls because PWDL data were not normally distributed and could not be transformed. We used ordinary least squares regression to investigate the relationship between wood hardness and cellobiase levels. We determined whether mean wood hardness and the amount of cellobiase were different among the four woodpecker species by comparing if 95% confidence intervals overlapped, a conservative approach for identifying significant differences between groups. Values are reported as means ± standard deviation, and all analyses were conducted in Microsoft Excel (2016) and R Studio (RStudio Team 2020).

Results

We sampled wood hardness and cellobiase activity at 78 woodpecker nest cavities: 19 BBWO, 17 HAWO, 16 NOFL, and 26 WHWO nests. Almost all nest cavities were in snags (n = 74), and most were located in ponderosa pine (n = 61), followed by Douglas-fir (n = 16) and sugar pine (n = 1). Likewise, almost all controls were snags (n = 75), and 61 were ponderosa pine, 16 were Douglas-fir, and 1 was sugar pine. Mean height of nest substrates was 10.36 ± 11.35 m (range 0.43 to 52.92 m), mean DBH of nest substrates was 42.31 ± 18.29 cm (range 16.80 to 107.00 cm, n = 77), and mean height of nest cavities was 2.22 ± 1.34 m (range 0.32 to 7.50 m). We found a significant negative association between the amount of cellobiase and wood hardness (r^2 = 0.129, P = < 0.001, n = 156), but cellobiase levels did not explain much of the variation in

TABLE 1. Wood hardness in Newton meters (N·m), percent wood density loss (PWDL), and the amount of cellobiase (nM of liberated p-nitrophenol) for black-backed woodpecker (BBWO; $n = 19$), hairy woodpecker (HAWO; $n = 17$), northern flicker (NOFL; $n = 16$), and white-headed woodpecker (WHWO; $n = 26$) nest cavities and control snags in Oregon and Washington. Values presented as means ± standard deviation. Results of statistical tests shown where: [a] indicates a significant difference between nests and controls at $P = < 0.001$; [b] indicates a significant difference between nests and controls at $P = 0.031$; and [c] indicates a significant difference between nests and controls at $P = 0.009$.

Woodpecker nest sites	Hardness (nests)	Hardness (controls)	PWDL (nests)	PWDL (controls)	Cellobiase (nests)	Cellobiase (controls)
All sites	1.67 ± 1.18	8.41 ± 3.39	0.48 ± 0.08	0.13 ± 0.14	0.02 ± 0.04	0.004 ± 0.005
BBWO	2.27 ± 1.51[a]	8.05 ± 3.37[a]	0.46 ± 0.11[a]	0.17 ± 0.12[a]	0.01 ± 0.01[b]	0.005 ± 0.006[b]
HAWO	1.41 ± 0.81[a]	9.11 ± 3.75[a]	0.51 ± 0.07[a]	0.12 ± 0.12[a]	0.02 ± 0.02[c]	0.001 ± 0.003[c]
NOFL	0.96 ± 1.05[a]	8.08 ± 4.64[a]	0.53 ± 0.07[a]	0.17 ± 0.19[a]	0.05 ± 0.06[a]	0.004 ± 0.003[a]
WHWO	1.84 ± 0.94[a]	8.42 ± 2.19[a]	0.46 ± 0.07[a]	0.10 ± 0.12[a]	0.02 ± 0.02[a]	0.005 ± 0.006[a]

wood hardness at nests ($r^2 = 0.041$, $P = 0.076$, $n = 78$) and controls ($r^2 = 0.061$, $P = 0.597$, $n = 78$). Mean wood hardness was significantly lower, and cellobiase and PWDL were significantly higher, at nest cavities than in controls for each of the woodpecker species (Table 1; Figure 2).

We recorded the highest wood hardness levels at BBWO nests and the highest amounts of cellobiase at NOFL nests (Table 1). As indicated by non-overlapping confidence intervals, wood hardness was higher and the amount of cellobiase was lower at nests of BBWO than NOFL, but did not differ among the other woodpecker species (Figure 3). In contrast, PWDL did not differ among the woodpecker species (Figure 3). We found sporocarps on only 12% of nest substrates, and we found a high degree of overlap in the amount of cellobiase detected in nest and control substrates based on the percentage of blackened bark, top broken off, and bark remaining on snags (Figure 4).

Discussion

We predicted that cellobiase activity would be negatively associated with wood hardness. Our results support this prediction, because as cellobiase increased, wood hardness declined. This suggests that higher amounts of cellobiase result in softer wood due to the increased rate of decay caused by higher fungal enzyme activity and that measuring cellobiase can be used to estimate wood decay without directly measuring wood hardness. However, cellobiase was a poor indicator of the variation observed in wood hardness when we investigated this relationship in nest and control sites separately. Two limitations in our methodology may account for the poor fit between wood hardness and enzyme activity within these samples. First, our method did not measure degradation of lignin, which is another important component of wood, especially softwoods like conifers (Rytioja et al. 2014). Saprophytic fungi, in addition to breaking down cellulose, are the most effective decomposers of lignin (Arantes et al. 2010). Lignin binds the cells, fibers, and vessels that make up wood, and it must be broken down in order for cellulases, such as cellobiase, to work effectively (Blanchette 1991). Lignin decay could be occurring at varying degrees within the wood samples we collected, resulting in mismatches between wood hardness and cellobiase enzyme activity. Second, when samples had no or exceedingly low amounts of cellobiase activity, as typically seen in control samples, the resulting variability in measurements was likely due to variation in the coloration and clarity of blanks used to zero each sample rather than variation in enzyme activity. However, we do not feel that the poor relationship between cellobiase and wood hardness is the result of this last limitation because we followed standard protocol to minimize measurement errors.

Research indicates that most woodpeckers require decayed or softened wood in order to excavate a nest cavity (Conner et al. 1976, Schepps et al. 1999, Lorenz et al. 2015). However, most woodpecker nest studies do not measure wood hardness directly because it is time consuming and accessing high cavities can be difficult.

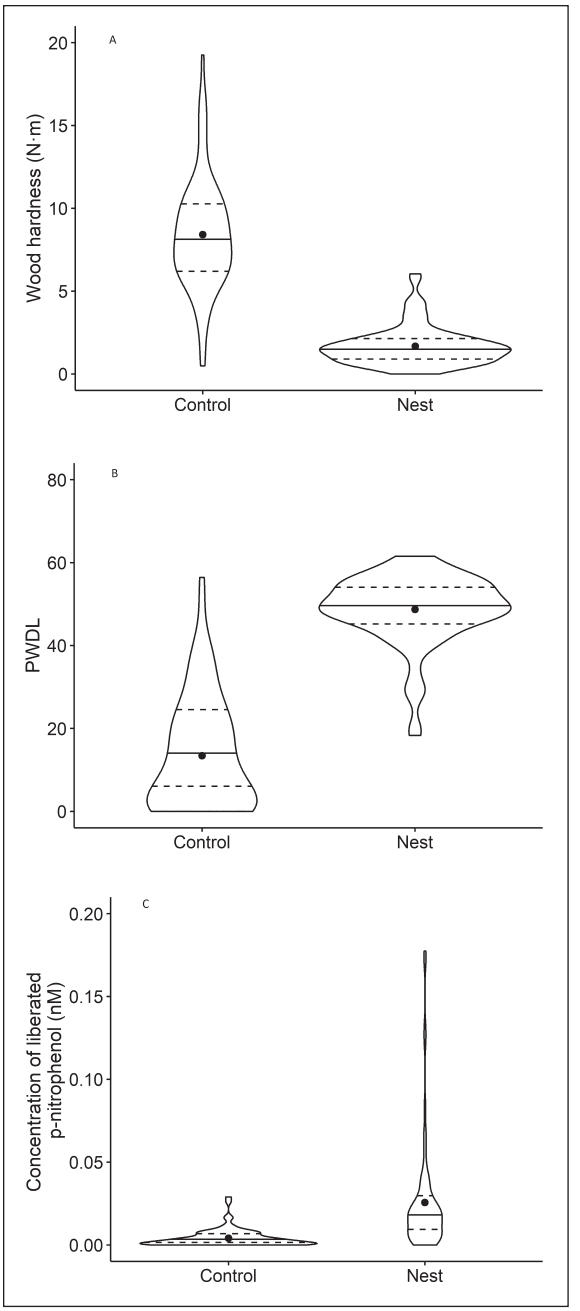

Figure 2. Violin plots showing the distribution of A) wood hardness, B) percent wood density loss (PWDL), and C) amount of p-nitrophenol liberated (i.e., amount of cellobiase) for nest substrates and controls measured in Oregon and Washington in 2017. The solid horizontal line in each violin represents the median, dashed lines represent the first and third quartiles, dots represent means, and the width of each curve corresponds with the approximate frequency of data points in each of those regions.

Thus, despite previous studies showing that variables such as cavity orientation to optimize thermoregulation (Inouye 1976, Butcher et al. 2002), cavity height to deter terrestrial predators (Joy 2000, Kozma 2011), and DBH of the nest substrate (Melletti and Penteriani 2003, Ganey and Vojta 2004) influence where woodpeckers excavate a nest cavity, all studies that have included wood hardness conclude that woodpeckers strongly select for nest cavities where the interior wood is sufficiently soft for excavation, which is a small subset of all potentially available sites. Our results support this supposition. We found wood hardness was lower, and cellobiase and PWDL were greater, in the interior wood of woodpecker nest cavities compared to controls. Few control sites were as soft as nest sites. Likewise, there were few control sites that had the high amounts of cellobiase enzyme we found at nest sites. Moreover, all four woodpeckers we studied selected nest substrates with softer wood and higher fungal enzyme activity than controls, even species predicted to have strong excavation morphology such as BBWO (Kirby 1980). Although one single factor most likely does not influence where woodpeckers excavate a cavity (Korol and Hutto 1984), our findings support previous conclusions on the importance of wood decay and saprophytic fungi for nest cavity excavation (Kilham 1971, Connor 1976, McClelland and McClelland 2000, Jusino et al. 2016), and the selection of nest substrates with softer wood than random substrates in the woodpecker species we studied (Schepps et al. 1999, Lorenz et al. 2015).

We found some differences in the hardness of substrates excavated by the different woodpecker species. The BBWO excavated nest cavities in harder substrates than NOFL, although BBWO did not select harder substrates than HAWO and WHWO. This provides weak support of findings from previous research that classify the BBWO as a strong excavator that is capable of excavating cavities in relatively firmer wood than other woodpeckers (Kirby 1980, Raphael and White 1984, Saab and Dudley 1998, Edworthy et al. 2012)

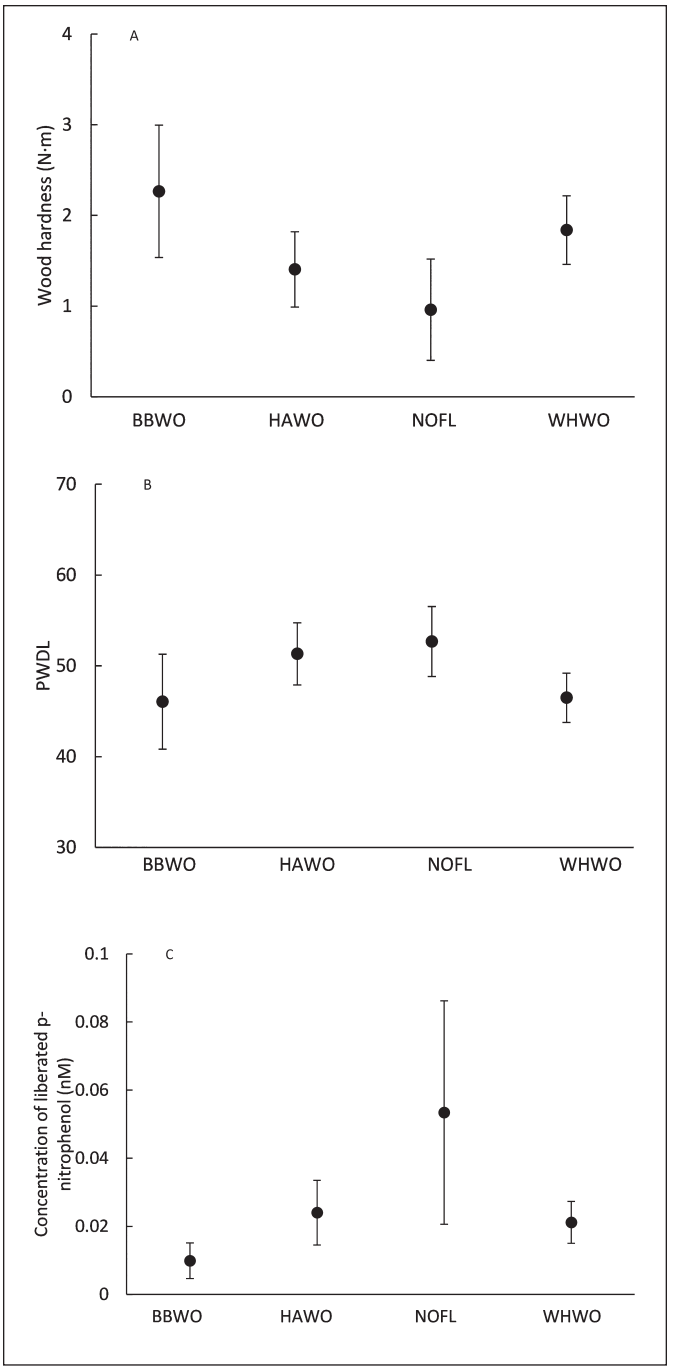

Figure 3. Mean and 95% confidence intervals of A) wood hardness, B) percent wood density loss (PWDL), and C) amount of p-nitrophenol liberated (i.e., amount of cellobiase) at black-backed woodpecker (BBWO), hairy woodpecker (HAWO), northern flicker (NOFL), and white-headed woodpecker (WHWO) nest cavities in Oregon and Washington in 2017.

and the NOFL as a weak excavator that selects soft, or well-decayed, substrates to excavate a cavity (Raphael and White 1984, Wiebe et al. 2007, Wiebe 2009, Kozma 2012). However, our findings do not support some other generalities from past studies. For example, like the NOFL, the WHWO is often referred to as a weak excavator because of its selection of short snags with broken tops for cavity excavation and because it forages primarily by probing the bark and foliage of conifers for insects rather than routinely digging into wood for insects (Ligon 1973, Raphael and White 1984, Milne and Hejl 1989, Kozma 2012). Our results show that WHWO excavated cavities in substrates with similar hardness to HAWO, a species considered a strong excavator (Spring 1965), and in substrates that were twice as hard as those selected by NOFL. This suggests that even though WHWO and NOFL nest most frequently in broken-topped conifer snags (Milne and Hejl 1989, Bevis and Martin 2002, Kozma 2012), WHWO are selecting firmer substrates to excavate cavities than NOFL. Woodpeckers should excavate nest cavities in the firmest wood they can in order to limit predation, yet we found considerable overlap in the use of softer substrates among the woodpeckers we studied. This suggests that categorizing woodpeckers based on excavating ability can be problematic and that woodpeckers are perhaps more limited to where they can excavate a cavity based on the availability of suitable substrates (Lorenz et al. 2015).

Past researchers have developed protocols to estimate decay classes based on visual characteristics (e.g., percent bark, top breakage, sporocarps, percent limb loss, etc.) as a

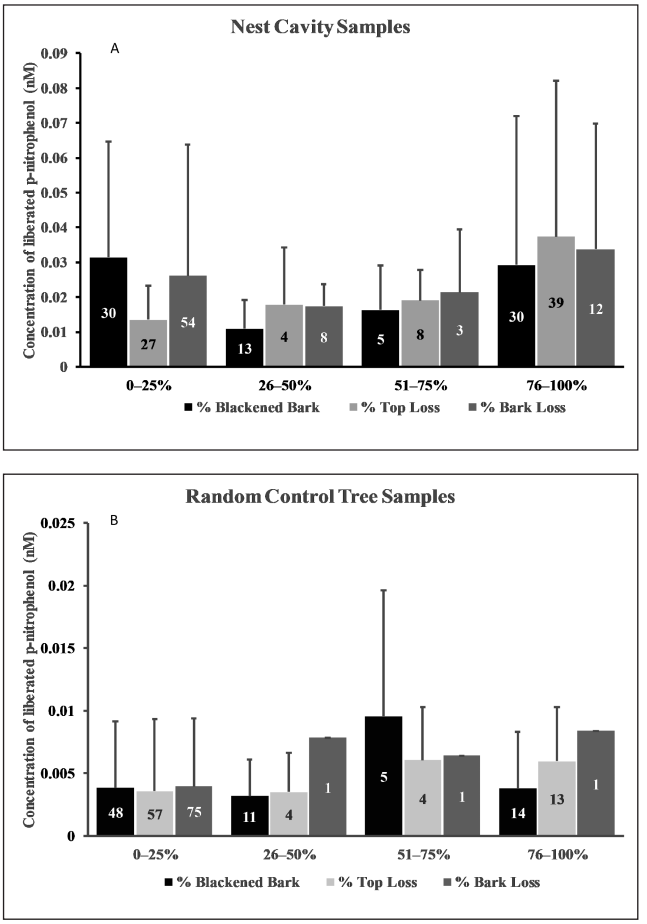

Figure 4. Mean and standard deviation (upper line) of p-nitrophenol liberated (amount of cellobiase) from A) nest sites, and B) control sites, for a range of percentages of blackened bark, top loss, and bark loss for four woodpecker species in Oregon and Washington in 2017. The number in each column is the sample size.

way to classify snags (e.g., Cline et al. 1980, Bull et al. 1997). These protocols have been used extensively by natural resource professionals as a way to characterize snags used by nesting woodpeckers in order to manage snags on the landscape (Schreiber and deCalesta 1992, Lehmkuhl et al. 2003, Ganey and Vojta 2004, Bagne et al. 2008). With the development of techniques to directly measure wood hardness, research has demonstrated that visual characteristics may be insufficient for identifying snags that can be excavated by woodpeckers (Schepps et al. 1999, Lorenz et al. 2015). While we acknowledge that we only measured one spot on controls, our results corroborate these past findings. For example, we found that sporocarps were not common on woodpecker nest substrates. Most of the sporocarps we did observe were those of the sapwood fungus *Cryptoporus volvatus*, which is visible on dead trees primarily 1 to 2 years after tree death. This is an insufficient period for conifer wood deterioration to permit woodpecker excavation; thus, the presence of these sporocarps was not a good indicator of decay in our wood samples. Similarly, we found that the percentage of bark lost, top broken, and bole blackened from fire were poor indicators of cellobiase activity. While decay may have been present on control substrates in locations we did not measure, our results considered together support the idea that visual cues are fairly unreliable indicators of internal wood decay. Cellobiase and PWDL were higher, and wood hardness lower, at nest substrates compared to controls. Therefore, even if controls contained soft wood in some spot that we did not measure, soft wood is clearly quite rare compared to hard wood in controls, regardless of decay class. The converse was also true in our study; woodpeckers excavated nests in soft wood in substrates exhibiting various stages of deterioration. Even in live trees, cavity excavation sites were on average more decayed than controls. Thus, our findings add to the growing body of literature suggesting that visual cues are poor at assessing decay stage and wood hardness of standing dead trees. Future examination of wood hardness and cellobiase over a larger portion of nest trees and snags could help clarify if relationships between external snag features and internal wood rot are present. Future studies should also focus on identifying the fungal taxa responsible for wood decay in order to better understand the relationship between fungi, wood decay, and woodpecker nest cavity excavation in conifer forests.

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