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Landscape scale environmental DNA sampling for a rare fungal species: Implications for forest management

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ABSTRACT

Surveys for conks on noble fir trees (*Abies procera*) have suggested that the fungus *Bridgeoporus nobilissimus* is rare in the Pacific Northwest. As a result, the fungus has been managed for several decades as a rare old-growth forest species on federal lands. We undertook a randomized landscape sampling approach to determine the distribution of *B. nobilissimus* in an 88,200 ha study area in the northern Oregon Cascade mountains. We used molecular tools to test for the presence of fungal DNA in wood cores from living and dead trees. We sampled 58 plots from a system of permanent forest plots. Of these, 25 (43%) produced positive detections though no plots had visible *B. nobilissimus* conks. We estimate that *B. nobilissimus* occurs throughout 41% (±11%) of the northern Oregon Cascade mountains. *Bridgeoporus nobilissimus* presence was not related with land use status (reserve vs. non-reserve) or stand age. The use of molecular DNA technology with environmental samples changed our perception from a rare species present at a few sites, to a widely distributed species that produces few fruit bodies.

1. Introduction

Conservation initiatives have grown in scope to include lesser known, less charismatic, and more cryptic taxa such as fungi (Heilmann-Clausen et al., 2015). For example, for several decades fungi have been managed for conservation in the Pacific Northwest of the USA. As part of the federal government's 1994 Northwest Forest Plan, 234 fungal species were identified as associated with late-successional (>80 years old) or old-growth forests (at least 180 to 220 years old) and in need of extra protection through a mitigation measure known as the Survey and Manage Program (SM) (Molina, 2008; Marcot et al., 2018). Under Survey and Manage, a system of reserve and non-reserve land use allocations was established across the range of the northern spotted owl with reserve lands intended to provide the backbone for conservation for old-forest associated species. SM mitigation required species' surveys prior to federal agency projects that were "habitat-disturbing," regardless of land use allocation, and the protection of any sites (individuals) found by buffering from 10's to 100's of meters.

Under the Northwest Forest Plan late-successional forest is generally defined as mature forests older than 80 years and old-growth forests are generally defined as being stands at least 180 years of age with specific structural characteristics (USDA and USDI, 2001). Presumed association with old-growth forest was a main criterion for listing species under the SM program. The fungal species *Bridgeoporus nobilissimus* was one such species, with required pre-disturbance surveys and site protection. In addition the Oregon Natural Heritage Program (now the Oregon Biodiversity Information Center) added this species to their list in 1993 (as *Oxyporus nobilissimus*) making it one of the first non-lichenized fungal species to be added to a rare species list (Lizon, 1995). It has also been a Sensitive species for both the U.S. Bureau of Land Management (BLM) and U.S. Forest Service (USFS) in Region 6 in Oregon and Washington. These federal agencies use the NatureServe Global and State ranks for rarity and imperilment as criteria to list species as "Sensitive" under agency policy manuals. The goal of these policies is to conserve species and prevent their listing under the federal Endangered Species Act.

Bridgeoporus nobilissimus produces distinctive, often large, perennial basidiocarps (i.e., conks) on noble fir (*Abies procera*) trees and stumps. A phylogeny based on the ITS and partial LSU regions indicates that *Bridgeoporus* forms a strongly supported clade in the Hymenochetales (Wu et al., 2017). The only other described species in the genus is *B. sinensis*, which has only been collected in China (Wu et al., 2017). However, several samples collected near the central California coast in recent years may represent a new species for the genus (D. Arora,

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personal communication, 2015).

Bridgeoporus nobilissimus' ecological role is unknown. Species in the Hymenochetales are generally wood decomposers, but a small number are biotrophic (Korotkin et al., 2018). *Bridgeoporus nobilissimus* has been considered a 'brown-rot' fungus although Redberg et al. (2003) hypothesized that it could be a 'white-rot' fungus based on its genetic lineage. Multiple efforts to culture *B. nobilissimus* have been unsuccessful (Cooke, 1949; Burdsall et al., 1996; Redberg et al., 2003) including a 2008 effort by the Forest Service Region 6 Westside Forest Insect & Disease Service Center (D. Hildebrand, personal communication, 2009).

Bridgeoporus nobilissimus produces perennial fruit bodies known as conks that continue to grow and produce spores for many years. Because conks are persistent, surveys were considered practical under the SM program (USDA and USDI, 1994a; USDA and USDI, 2001). Once detected, temporary protection of 242 ha around the conk is required until a complete census survey can be conducted and a Site Management Plan is completed (USDA and USDI, 1997). Site Management Plans generally provide direction to maintain habitat conditions by buffering the host tree, minimizing changes to microsite conditions, and enhancing conditions for large-diameter host species to grow within occupied stands (USDA and USDI, 1997). Federal agencies have surveyed ~ 7,000 ha for *B. nobilissimus* conks since 1994 at a cost of >$250,000. In conservation terms, *B. nobilissimus* has been one of the most protected fungal species on federal lands in the Pacific Northwest.

Abundance of fungal species is difficult to measure. Fungi can fruit irregularly, infrequently, or only when their resources are depleted (Peay et al., 2008; Molina et al., 2011; Molina, 2008), so determining which fungal species are truly rare is challenging. Generally, the sporocarps (fruit bodies) of fungi are more conspicuous than the vegetative bodies, and measures of species abundance are often based on fruit body abundance. This is a problem because fruit body abundance does not necessarily represent species abundance (Lodge et al., 2004). Likewise, lack of a fruit body does not necessarily indicate species absence. Previous efforts undertaken by the USFS and BLM to understand the size and ecological requirements of *B. nobilissimus* populations have been based exclusively on fruit body observations.

To test if *B. nobilissimus* mycelium is actively growing inside a host tree before a fruit body is produced, we developed an environmental DNA (eDNA) detection technique that used a species-specific molecular marker to identify *B. nobilissimus* mycelia in wood cores taken from host trees. We first used this method to map *B. nobilissimius* presence in stands with at least one conk present (Gordon and Van Norman, 2015). We found that species occurrence patterns indicated scattered presence throughout four sampled forest stands with 6% to 39% of live and dead *Abies* trees showing *B. nobilissimus* mycelium (Gordon and Van Norman, 2015). Despite testing over fairly large areas (18 to 37 ha), we did not detect the "edge" of the fungal populations, nor did sampling indicate an "infection center." Additionally, we found that *B. nobilissimus* mycelium could colonize three other conifer genera, previously unknown to host the fungus. These results indicate that *B. nobilissimus* may be more prevalent and more widely distributed than suggested by conk abundance. A better assessment of species' abundance and distribution, insights into life history, and appropriate conservation actions would be assisted by a more accurate understanding of mycelial presence.

In this study we use the same eDNA method (Gordon and Van Norman, 2015) to describe the distribution of *B. nobilissimus* in the area considered the core of its range, the noble fir zone of the northern Oregon Cascade mountains. We tested the hypothesis that *B. nobilissimus* is present across the study area and more prevalent than indicated by conk abundance. Secondly, we sought to assess if *B. nobilissimus* is differentially associated with reserve lands or stand age as defined under the Northwest Forest Plan (USDA and USDI, 1994b). An overarching goal of our study was to apply the use of new molecular DNA sampling technologies to better assess species' presence and habitat requirements, and to contribute to our understanding of the conservation status of this *Bridgeoporus* species.

2. Methods

2.1. Study area

The study was conducted in the northwestern Oregon Cascade mountains, which is the center of the known distribution of *B. nobilissimus* (Lebo, 2007). The Western Cascades ecological zone is comprised of dense, mesic temperate coniferous forests characterized by the large size and longevity of many of the tree species (Franklin and Dyrness, 1973). Early successional tree species, noble fir and Douglas-fir (*Pseudotsuga menziesii*), form the dominant forest canopy. The elevation for noble fir in this geographic area ranges from approximately 750 to 1600 m. Much of the western slope of the Cascade mountains is public forest land managed by the USFS and BLM. The topography in the study area is steep, highly dissected, and underlain by volcanic terrain. The climate is strongly maritime and receives an orographic effect; moist air blows in from the Pacific Ocean approximately 120 km to the west and much of the moisture precipitates on the west side of the Cascade mountains as the air mass rises and cools. Winters are cool and wet with snow generally persistent at the upper elevations. Summers are warm and dry. The natural disturbance regime is characterized by a mixed severity fire regime with a fire return interval of >200 years. In these fires, anywhere from 20% to 70% of the overstory trees or basal area are killed by the sum of all fire effects (Agee, 1993).

Three administrative units of these public lands were selected for our study. The study area was defined as all forest land containing noble fir in the Sweet Home and Detroit Ranger Districts of the Willamette National Forest and the Cascades Resource Area of the Salem District BLM (now Northwest Oregon District BLM; Fig. 1). The total area of these

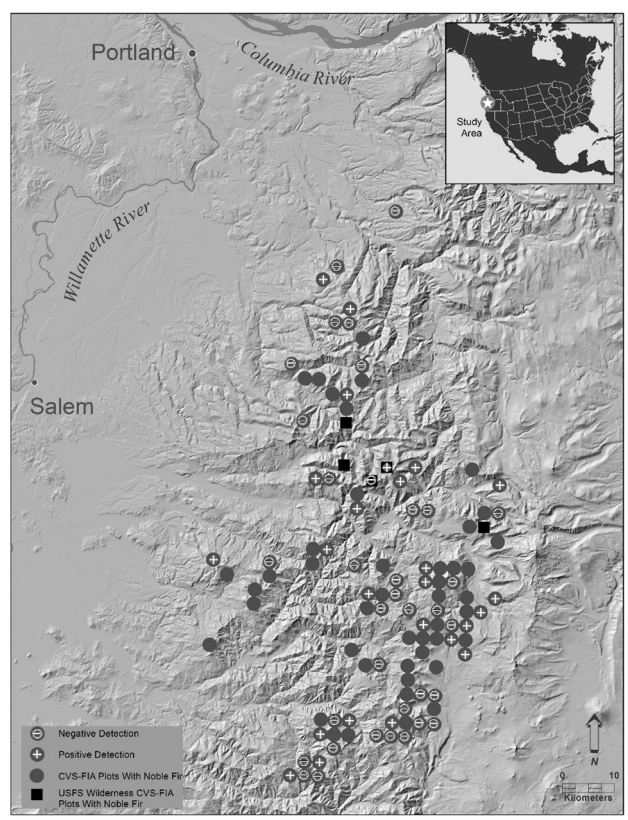

Fig. 1. Sample population of all CVS-FIA plots with noble fir presence within the study area represented by filled gray circles and black squares for the USFS Wilderness plots. Randomly selected plots that were sampled are shown with detection results as either positive with a white plus sign or negative with a white minus sign.

three administrative units is about 248,000 ha. Historically and currently this landscape is managed for multiple uses, including timber harvest, although the highest elevations of the study area are reserved as Wilderness (48,000 ha, approximately 18%) where human disturbance is minimized.

To determine where noble fir was established in this region, we used data collected by the Current Vegetation Survey-Forest Inventory Analysis (CVS-FIA) program (Max et al., 1996). In the CVS-FIA program permanent plots were established on a randomly started systematic grid, generally with a 2.7 km spacing between plots, across forested federal lands. All CVS-FIA plots are assigned a stand birth year, based on tree ring data. The plots are inventoried every decade to collect forest data such as tree species, size, and live/dead status.

We queried the most recent survey data from each plot and found 108 plots within the three administrative units that contained noble fir (26 BLM, 82 USFS). However, after data collection, 5 of the USFS plots were found to have been established on a 5.4 km grid in USFS Wilderness areas rather than the 2.7 km grid used elsewhere, and thus were removed. The 88,200 ha area covered by the 103 remaining plots formed our study area.

2.2. Sample population

Plot locations were selected using a double sampling design (Cochran, 1977). The first phase utilized the CVS-FIA grid and the selection of noble fir areas outlined above. In the second phase, we defined two strata within our study area based on the management system established in the Northwest Forest Plan: reserve and non-reserve land use allocations. Reserve lands include Congressionally designated areas such as Wilderness and land allocations designated under the Northwest Forest Plan including Late-Successional Reserves and Riparian Reserves to protect and enhance conditions of late-successional and old-growth (LSOG) forest ecosystems (USDA and USDI, 2001) versus non-reserve land allocations where management actions are allowed. A random sample of 30 plots from each of these strata was selected for testing for *B. nobilissimus* presence in trees. These plots were selected, sampled, and tested before the difference in the grid size in USFS Wilderness areas was recognized. Two of the selected reserve status plots were in USFS Wilderness and, since all USFS Wilderness plots were removed from the study area, these plots were removed from the data set, reducing the number of reserve status plots in our analysis to 28. Although the USFS Wilderness plots were not used in the plot level analysis, tree data from these plots were used in the tree level analysis.

2.3. Survey plot definition and sampling

To minimize impacts to the permanent CVS-FIA plots, "survey plots" were located adjacent to the permanent plots. Using a Geographic Information System, the 58 permanent plot center points were mapped. A survey plot corner was then mapped by projecting 61.0 m at a randomly selected azimuth from each permanent plot center. The geographic coordinates of the other three corners were calculated and a file of the survey plot corner coordinates was copied to GPS units for field use. The square-shaped survey plots were 0.8 ha.

Within each survey plot, wood cores were taken from the 20 largest trees whether alive, dead, or stumps. Stumps were defined as any dead tree below 1.37 m. Trees to be sampled were determined as follows until the 20 largest trees were sampled:

1. Sample the 20 largest noble fir trees > 25 cm diameter,
2. If not available, sample the largest of all other *Abies* species > 25 cm diameter,
3. If not available, sample the largest *Abies* stumps with a top diameter > 25 cm,
4. If not available, sample the largest of any other conifer species > 25 cm diameter,
5. If not available, follow the same criteria order for trees and stumps 12.7–25 cm diameter until the 20 largest trees and stumps total are sampled.

The entire plot was searched before moving to the next criterion. At each tree a unique tree number, tree species, diameter-at-breast-height or approximate stump diameter, live/dead status, and decay class was recorded. Cores were extracted manually with increment borers (minimum length 40 cm). The number of wood cores per tree (4), location at the tree base, and handling of wood cores followed the methods developed by Gordon and Van Norman (2015).

2.4. Lab analysis

Tree core samples were delivered to the lab on ice, and then refrigerated until processing. The contents of each bag, containing the four cores from one tree, were ground for 20–30 s in a high speed 1500 W blender, and 0.25 to 0.30 g of the ground wood was added to a microcentrifuge tube. DNA extraction and cleanup then followed the procedure of Gordon and Van Norman, 2015. Every extraction batch included at least one positive control and one blank. Each cleaned DNA sample was run at least once in a PCR reaction with the *B. nobilissimus*-specific primer Bn215-118 (ATGTGCTCGTTGCCCTTGAC) (Redberg et al., 2003) and the universal reverse primer r4 (TTCATCGATGCGAGAGCC) (Gordon and Van Norman, 2015). Reactions were 25 µl in volume and contained 0.5 U of OneTaq Hot Start DNA polymerase (New England BioLabs), 1X of buffer, 200 µM each dNTP, 400 nM each primer, and 5.0 µg Bovine Serum Albumin (Biotechnology Grade, Amresco). Reactions were subjected to 94 °C for 1.5 min, followed by a touchdown PCR routine, where the anneal temperature decreased from 64 °C to 61.6 °C for the first seven cycles, and then remained at 61 °C for 40 cycles. Anneal time was 35 s. Extension was 68 °C for 60 s, and denaturing was at 94 °C for 18 s. Products were visualized on an agarose gel. Samples that showed a distinct product at 238 bases were scored as positive. Every PCR batch contained at least one negative control and one positive control.

The *B. nobilissimus* ITS1 product contains an AvaII restriction site, and this enzyme was used to confirm each positive PCR. Amplicons that appeared to be positive for *B. nobilissimus* were subjected to restriction digest with AvaII (New England Biolabs) according to the manufacturer's directions. Digestion products were visualized in a 2.1% agarose gel. Two restriction products at 105 bases and 136 bases confirmed the presence of the *B. nobilissimus* ITS1 region in the original amplicon. A subset of positive samples were sequenced to confirm the species as *B. nobilissimus*.

2.5. Statistical analysis

Descriptive statistics were calculated for tree level detections. In addition to the overall detection rate, the detection rate for each of the six tree species sampled was calculated. The detection rates for live trees and dead trees were calculated and compared using a chi squared test.

We interpreted a detection of *B. nobilissimus* on any of the 20 trees sampled in a plot as a positive 0.8 ha plot, and estimated the area of noble fir habitat colonized by *B. nobilissimus* across the 88,200 ha noble fir study area. To calculate the overall proportion of positive plots, we used weighted estimates to account for the overrepresentation of reserve plots in our original population. The means and standard errors of the proportion of positive plots in the reserve and non-reserve areas were generated using bootstrapping (RStudio, version 0.98.1103). Three thousand replicates were used in the bootstrap process. We tested the null hypothesis of no difference in positive plot proportion between the two land use designations using a *t*-test.

For the noble fir study area and land use allocations, the proportion of permanent plots with a presence of noble fir was calculated and multiplied by the total area to estimate the area of forest land available

for colonization by *B. nobilissimus*. We then multiplied the result by the measured proportion of plots that were positive for *B. nobilissimus* (0.41) to obtain our estimate of the area occupied by *B. nobilissimus*.

To designate study plot age, we used CVS-FIA stand birth year data, which was confirmed with satellite imagery. Plot age was divided into three classes (<80, 81–180, >181), and 2 classes (<80 and ≥ 81) based on the stand age in the year 2011 to test whether *B. nobilissimus* presence varied with forest stand age class. The null hypothesis of no difference in frequency of detection of *B. nobilissimus* in the different age classes was tested using a chi-squared test. For all statistical tests we set $\alpha = 0.05$.

3. Results

3.1. Tree level analyses

Sampling occurred between September 2011 and June 2013. In total, core samples were taken from 1200 live and dead trees in 60 survey plots. The final list of tree species in order of number of trees sampled included: noble fir (427), Douglas-fir (396), Pacific silver fir (*Abies amabilis;* 260), western hemlock (*Tsuga heterophylla;* 61), grand fir (*Abies grandis;* 29), western redcedar (*Thuja plicata;* 24) and unknown species (3). The greatest number of *B. nobilissimus* detections in descending order were noble fir (31 trees with positive detections; 7.3% detection rate by species), Douglas-fir (20; 5.1%), Pacific silver fir (11; 4.2%), western hemlock (2; 3.3%) and western redcedar (1; 4.2%). The grand fir and unknown tree samples had no positive detections. Of 1200 trees tested, 65 had *B. nobilissimus* detections for a 5.4% detection rate. PCR amplicons from eleven of the 65 positive samples were sequenced and all had sequences close to *B. nobilissimus* sequences from conks. The sequences were deposited in GenBank (MW133861- MW133871). Within the 25 plots with detections, the number of trees with *B. nobilissimus* ranged from one to seven (20 plots had 1–3 positive trees while 5 plots had 4–7 positive trees), with an average of 2.6 detections per positive plot.

Of the 1200 trees tested, 198 were dead, and seven tested positive for *B. nobilissimus*, for a detection rate of 3.5%. In comparison, the set of live trees had a 5.8% detection rate (58 out of 1002). We found no significant difference in *B. nobilissimus* detection rates of live versus dead trees, χ^2 (1, 1200) = 1.638, p = .20.

3.2. Plot level analyses

Of the 103 permanent plots used to estimate the area of noble fir habitat available for colonization by *B. nobilissimus,* we sampled 28 plots in reserve and 30 plots in non-reserve allocations. We found *B. nobilissimus* in 10 of the reserve plots and 14 non-reserve plots. Bootstrapped estimates (+/- SE) of *B. nobilissimus* DNA presence were 41% (±6%) of the random plots overall, 36% (±9%) of the reserve plots, and 47% (±9%) of the non-reserve plots (Fig. 1). We found no difference in the mean proportion of positive plots between land use designations (p = .399).

The estimated area occupied by *B. nobilissimus* is 36,175 ha within the 88,230 ha noble fir study zone; 23,180 ha in reserve lands and 12,995 ha in non-reserve.

Stand ages ranged from 12 to 801 years with a median of 107 years for all plots. The youngest stand with *B. nobilissimus* detected was 15 years old while the oldest was 801 years old. We found no significant difference in the proportion of *B. nobilissimus* detections among the three age classes, χ^2 (2,58) = 3.86, p = .145. Collapsing age into two classes (<80 and > 81), we again found that there was no significant difference in *B. nobilissimus* detections between age classes, χ^2 (1,58) = 1.72, p = .19.

4. Discussion

Despite the investment of many hours of visual survey work over two decades, our understanding of *B. nobilissimus* in its noble fir habitat was still incomplete. By using a series of plots based on a random systematic design and novel eDNA methodology, our findings are much different than the picture provided by visual conk surveys. Specifically, we found *B. nobilissimus* mycelium to be well-distributed in the noble fir zone of the northern Oregon Cascade mountains. Our study indicates that *B. nobilissimus* occurs throughout the 88,200 ha noble fir sample area such that 41% of 0.8 ha plots will contain at least one tree with *B. nobilissimus*. Approximately 5% of trees that met our testing had detectable *B. nobilissimus*. That is, the species occurs throughout the sample area, but at the forest stand scale it occurs in a dispersed, scattered pattern. We found no evidence of increased presence of *B. nobilissimus* in reserve land versus non-reserve land, nor did we find any association of *B. nobilissimus* presence with stand age.

4.1. Detectability and eDNA tools

Prior to our study there had been <200 conks found over approximately 25 years despite great effort. Using eDNA, we detected *B. nobilissimus* in 65 trees including non-Abies, none of which had a *B. nobilissimus* conk. After two eDNA studies, one random and one in conk areas (this study and Gordon and Van Norman, 2015), we sampled almost 1700 trees and found *B. nobilissimus* in 182 trees (10.7%). Though we have not calculated a detection rate for conk surveys, it is drastically lower than using eDNA. Thus, we conclude that it is not justified to conduct pre-disturbance surveys as prescribed by Survey and Manage guidelines for *B. nobilissimus* conks (USDA and USDI, 2001; Marcot et al., 2018) to successfully detect the species when present.

When a species is cryptic and occurs in a substrate such as the wood of a tree trunk, there is the potential for false negatives, even when using molecular methods. Our tree sampling process involved taking four wood cores distributed evenly around the base of a tree and pooling these cores to represent one tree. We previously found that this method gave excellent detection results when testing trees hosting a *B. nobilissimus* conk. In fact, we should be able to reliably detect as little as 4 mg of colonized wood in a typical 18 g sample, which is the typical weight of four wood cores (Gordon and Van Norman, 2015). It is likely that some of our negative samples represented false negatives, owing to the small proportion of the tree volume sampled. Also because of this, we suspect that we do not detect *B. nobilissimus* in the early stages of colonization.

eDNA studies have become an important tool for studying other fungal species. For example, using high throughput sequencing to determine fungi present in wood cores from decaying logs Ovaskainen et al., (2013) identified more species present as mycelium in the cores than they did in three fruit body surveys over two years. Several other similar molecular studies have detected more of the fungal wood-inhabiting community than was detected by fruit body surveys (Ovaskainen et al., 2010; Parfitt et al., 2010; Lindner et al., 2011; Rajala et al., 2012). Parfitt et al., (2010) found three fungal species that were thought to be rare based on conk surveys in Great Britain were actually latent in angiosperm sapwood. Ovaskainen et al., (2013) noted that species with specialized resource requirements are rarely seen fruiting and are often classified as IUCN red-listed. Similar to our results, they found that some wood-inhabiting species were more abundant as mycelium in wood than would be expected from their occurrence as fruit bodies.

Using eDNA methods to sample trees is expensive and labor intensive. In the future new technologies may present more efficient testing methods, based on groups of fungi that inhabit similar habitats. With next generation sequencing techniques, hundreds of thousands of barcode sequences covering the entire fungal community can be extracted from a single environmental sample. So, for example, with a random sampling study design dead wood could be sampled over an area and all fungi that use that resource can be identified, quantified, and assessed for rarity.

4.2. Life history and habitat insights

Our study adds to our understanding of the ecology of *B. nobilissimus* while raising more questions. Based on conk surveys, the amount of dead wood at a site can be a strong determinant of polypore species richness (Junninen and Komonen, 2011). In surveys for *B. nobilissimus* conks, agency botanists found it most frequently associated with large, old, and mostly dead noble fir. eDNA studies present a very different picture of the vegetative mycelium. Dead trees were no more likely to be infected than live trees, and *B. nobilissimus* mycelium was detected in both small diameter trees and in live trees (Gordon and Van Norman, 2015).

B. nobilissimus is considered rare because at a given time only a very small proportion of the mycelium are fruiting. This may indicate an organism that lives a long time in its host and rarely invests energy in fruit body production, or an organism that depends mainly on asexual reproduction or clonal growth. It is unknown what triggers conk production.

Within a stand containing a *B. nobilissimus* conk, infected trees are just as likely to be at the edges of the stand (up to 0.5 km from a conk) as they are within 7 m of a conk (Gordon and Van Norman, 2015). Potentially *B. nobilissimus* could move from tree to tree through root contact or the production of rhizomorphs. Wind dispersed spores germinating on tree wounds or wood boring insects carrying fungal propagules from tree to tree are propagation mechanisms that would lead to a dispersed presence or vertical transmission through infected seeds (Gange et al., 2019). Bark beetles are known to act as fungal dispersal agents (Paine et al., 1997; Krokene and Solheim, 1996; Persson et al., 2011), and PCR-based metabarcoding has been developed to determine fungal communities associated with bark beetles (Miller et al., 2016). Mature infected trees found at seemingly great distances from any known conk could have been infected from spores generated many years ago by a nearby conk that is no longer present.

Agency botanists have noticed some *B. nobilissimus* conks occur in disturbed areas such as noble fir stumps in regenerating stands that are approximately 40 years old (T. Fennell, personal communication, 2007). Other conks have been found near trails, roads, or forest edges next to clear-cuts. For this reason, forest managers have hypothesized that *B. nobilissimus* produces conks in response to disturbance in order to produce spores and maintain presence, which could be experimentally tested. If true, as long as infected *Abies* trees are present in suitable habitat, conks will be produced over the landscape as trees age and die or die of human caused disturbances. Why *B. nobilissimus* does not produce conks on non-*Abies* hosts is not known.

The observation of *B. nobilissimus* conks growing on stumps and snags and the detection of *B. nobilissimus* mycelium within dead wood indicates that this fungus has the potential to have a saprotrophic lifestyle. However, 129 live trees with a positive detection of the fungus have been found (out of 1385 tested in this study and Gordon and Van Norman, 2015). We saw little to no evidence of rot in these live trees. These findings raise the possibility that *B. nobilissimus* adopts an endophytic lifestyle in its living host. Endophytic fungi have the ability to live within the tissue of a plant host in a quiescent state for long periods (Schulz and Boyle, 2005; Parfitt et al., 2010). Over time these fungi can take on biotrophic roles ranging from mutualism to pathogenicity (Sieber, 2007; Rodriguez et al., 2009; Alvarez-Loayza et al., 2011), and can change roles and become primary decomposers of host tissue after host tissue death (Korkama-Rajala et al., 2008). It may be that as a *B. nobilissimus*-infected *Abies* tree weakens, the previously inactive fungus takes on a pathogenic role, or perhaps adopts a saprotrophic role as tree tissues die.

What role or roles that *B. nobilissimus* plays within its hosts is unknown, but the fact that the fungus can be detected by testing a relatively small volume of tree biomass indicates that it builds up a significant presence in these trees, while the trees themselves remain vigorous.

4.3. Rarity

The concept of rarity exists along a continuum from almost non-existent to abundant. This has been referred to as the commonness-rarity continuum (Rey Benayas et al., 1999; Rosenberg, 2019). Rarity does not necessarily imply extinction likelihood though it is a main criterion (Mace and Kershaw, 1997; Gaston and Fuller, 2008; Rosenberg, 2019). Assigning a rareness threshold (e.g., 5% of survey sites occupied) to a species is a subjective exercise (Edwards et al., 2004). Typically, a number of factors are used to assess rarity; including range, distribution, threat, occupancy, abundance, abundance trend, and habitat condition (Flather and Sieg, 2007). To determine rarity, NatureServe has quantitative categories for range of extent, area of occupancy, number of occurrences, population size, and environmental specificity (Master et al., 2009), which is based on Rabinowitz's rarity classification scheme (Rabinowitz, 1981). When determining whether a species is "rare" or "uncommon", the SM 2001 Record of Decision provided a similar list of seven relative rarity factors (USDA and USDI, 2001).

Based on our findings, our knowledge has shifted for five of the seven SM rarity factors (Table 1). We found that *B. nobilissimus* is less rare than previously thought. We found that it is present at a higher number of sites than was previously known, and that these sites are well-distributed over the landscape, though the species is not densely abundant at sites. The previous understanding of its host requirement of noble fir trees has expanded considerably to include additional *Abies* species and three other conifer genera of all sizes. Thus *B. nobilissimus* habitat is not as highly specialized as previously thought. We did not measure population trends in this study nor the reproductive characteristics of this fungal species. Yet its reproductive strategy appears to be successful in maintaining a vegetative mycelial presence over our study area. We expanded our understanding of the potential habitat where *B. nobilissimus* occurs and now recognize that the species occupies a wider ecological amplitude.

The Oregon Biodiversity Information Center (ORBIC) uses the well-defined NatureServe rank methodology (Faber-Langendoen et al., 2009). Because of our findings, ORBIC initially reassessed the rarity and imperilment of the species and dropped it from their publication of Rare, Threatened and Endangered Species of Oregon (ORBIC, 2016).

However, the situation is more complicated when considering rarity for fungi such as *B. nobilissimus* that appear to rarely produce a fruit body. We can assume that finding a mycelial mass indicates an individual capable of reproducing, though we have not genetically tested *B. nobilissimus* to determine how large a single individual is. A single individual could be associated with one tree or one clonal individual encompassing many hectares similar to *Armillaria ostoyae* (Ferguson et al., 2003). Nevertheless, at this point we interpret our results to mean that finding a population of *B. nobilissiumus* mycelia distributed over a large landscape, in a variety of tree species, indicates a healthy

Table 1
Qualitative comparison of the seven SM "relative rarity" factors (USDA and USDI, 2001) for *B. nobilissimus* prior to this study and after.

FACTOR	Visual surveys only/ prior knowledge	This study
Limited distribution?	No	No
Low number of sites or individuals per site?	Yes	No
Highly specialized habitat requirements?	Yes	No
Declining habitat or population trends?	Unknown	Unknown, but less likely
Reproductive characteristics that limit population growth rates?	Unknown	Unknown, but less likely
Restricted distribution pattern relative to range or potential habitat?	No	No
Narrow ecological amplitude?	Yes	No

population.

Bridgeoporus nobilissimus conks are thought to be long-lasting on the order of decades. Field biologists have recently reported known *B. nobilissimus* conks dying, which may or may not indicate a problem with the species' reproduction, though relatively few new conk surveys have been conducted in the past 15 years. Most *B. nobilissimus* sites have only been known for 30 years or less, and we do not yet have enough information about the longevity of *B. nobilissimus* conks. To err on the side of caution because of the perceived conk decline and unknown reproductive success, the species has recently been reassessed in Oregon and raised in conservation priority to "S2" meaning that at the state level it is thought to be imperiled and "List 3" meaning it is under review (ORBIC, 2019). As noted by others, once a species has been included as deserving special management, it may be difficult to change that designation (Rosenberg, 2019).

4.4. Association with late-successional/old-growth forests

One of the basic criteria for inclusion in Survey and Manage is that the species is associated with late-successional/old-growth forests (LSOG; USDA and USDI, 2001). After testing 1160 trees in stands ranging from 12 to 801 years old, we found no association of *B. nobilissimus* with older stands. It is not more abundant in LSOG forests than non-LSOG forests, and we found no evidence that it requires habitat components contributed by LSOG forests to persist.

LSOG forests are in both reserve and non-reserve land allocations, but are more abundant in reserves. The reserve land allocations under the Northwest Forest Plan were set aside to provide for reasonable assurance of the taxon's persistence. We found that the species is not preferentially associated with either the reserve or non-reserve status lands. Previous conk surveys mainly focused on non-reserve lands as part of timber management projects so there were few surveys in the reserve lands. Within the core northern Oregon Cascades species' range, we found there is a high likelihood of sites and habitat in reserves as well as non-reserves, and that the species is well-distributed in both areas with a wider ecological amplitude than previously understood.

CRediT authorship contribution statement

Kelli J. Van Norman: Conceptualization, Funding acquisition, Project administration, Investigation, Methodology, Resources, Data curation, Writing - original draft. **Matthew Gordon:** Investigation, Methodology, Validation, Formal analysis, Data curation, Resources, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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