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SHORT COMMUNICATION

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Dead wood characteristics influencing macrofungi species abundance and diversity in Caspian natural beech (*Fagus orientalis* Lipsky) forests

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Abstract

Aim of study: This study aimed to examine the dead wood inhabiting macrofungi communities occurring on dead beech and hornbeam trees in Caspian forests.

Area of study: The Kheiroud forest in the north of Iran.

Material and Methods: Data from 205 sampling dead tree were analyzed by means of Generalized Linear Models (GLM) to test the effects of decay stage, DBH, Length or Height on macrofungi diversity. Additionally, tree species, dead wood size, log position, decay stage were used as predictor factors for the number of sporocarps species (NSS) as a fungal species richness and diversity in each dead log using analysis of variance

Main results: The number of sporocarps species (NSS) varied in different dead wood size and decay classes. The different stages of decay and the different size classes of dead wood had significantly different species richness of macrofungi. Deadwood in the high-decayed stages contained the highest diversity of fungi. Most of fungi identified on both logs and snags belonged to Basidiomycetes and Ascomycetes. The highest value for richness and evenness indices calculated in large diameter dead wood in decay class III. The results indicated the size and decay class of dead wood describe the greatest variance of the model that means the highest number of sporocarps species inhabited on the large dead wood in advanced stage of decaying.

Research highlights: Macrofungi diversity varied significantly across pieces of dead wood with downed logs, larger pieces, and wood in later stages of decay having the highest macrofungi diversity.

Keywords: Caspian forest; coarse woody debris; down woody debris; Iran.

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Introduction

An important ecological feature of old-growth forests is that they possess high volumes of dead wood in various stages of decay (Harmon *et al.,* 1986; Jenkins *et al.,* 2004). Dead wood is an important stabilizing component of most forest ecosystems because it serves as a substrate for plants and fungi, facilitates nutrient cycling and energy flows, and maintains hydrology and soil retention capacities (Harmon *et al.,* 1986; Huntington & Ryan, 1990; McAlister, 1995).

Dead wood has been identified as the most important, manageable habitat to increase plant, fungi, and animal biodiversity in forests. Dead wood provides a home for birds, mammals, insects, mites, collembolans, nematodes, bryophytes, lichens, fungi slime moulds, and bacteria. Of all of these groups, fungi and insects have the richest species diversity (Siitonen, 2001) and because fungi are the principal agents of wood decay in terrestrial habitats they develop the dead wood habitat for a host of other living organisms (Boddy, 2001).

Decay stage appears to be the most important variable for predicting bryophyte and fungal community diversity within dead wood (Ódor & Standovár, 2001; Ódor & van Hees, 2004; Penttilã *et al.,* 2004). Different dead wood qualities including decay stage and dead wood size highly affect dead wood-inhabiting fungi presence and diversity (Juutilainen *et al.,* 2011; Küffer & Senn-Irlet, 2005). Chronosequence studies focusing

on one forest type typically show a compositional change of fungal species along a temporal continuum of dead wood decay (Heilmann-Clausen & Christensen, 2004; Ódor & van Hees, 2004); thus, as the structure of the dead wood changes, the species that inhabit the dead wood change. Wood-inhabiting fungi can be classified according to the growing patterns to main groups, for example some of them prefer dead wood in a certain stage of decomposition without being specific about the diameter of the dead wood piece (Abrego & Salcedo, 2013).

Other important attributes include tree species, tree size, microclimatic conditions, cause of death, piece orientation, and stand management practices (Lindblad, 1998; Sippola *et al.,* 2001; Heilmann-Clausen & Christensen, 2004; Ódor & van Hees, 2004). This continuous presence of deadwood may be important for the sustainability of the forest communities. In northern Iran, standing dead beech (*Fagus orientalis* Lipsky) and hornbeam (*Carpinus betulus* L.) create a sheltered environment that improves the successful establishment of seedlings (Sefidi *et al.,* 2008).

This is a particularly timely subject because the economic value of bryophytes and fungi has been gaining attention. It is widely recognized that there is an enormous potential for finding important new medicines among all the species including bryophytes and fungi that have not been tested for their pharmacological traits. Medicines are one of the favorite topics of biodiversity advocates because so many of our drugs are derived from a diverse array of organisms. Management of these non-timber forest products requires a better understanding of their diversity and biology to ensure sustainable management (Peck *et al*., 2008). Moreover the one of the aim of forestry plans is maintaining biological diversity and ecosystem health. The management of forest in the north of Iran progress to the ecological forestry based on new theories in the silviculture that required conserving ecosystem integrity and health. The first step to reach the given goal is maintaining biological diversity. Thus, the goal of this study was to examine the dead wood inhabiting macrofungi communities occurring on dead beech and hornbeam trees in Caspian forests. The specific objective was quantifying differences in macrofungi diversity by dead wood position (downed logs, leaning snags, or standing snags), piece size, and decay class. The results of this research will supplement existing studies that have identified the biodiversity of macrofungi species within the Caspian beech forests (Hallenberg, 1977; Niemala & Uotila, 1977; Gharizadeh *et al.,* 2007; Ghobad-Nejhad *et al.,* 2008), by focusing upon on the specific substrate structural preferences for macrofungi growing on dead wood.

Material and methods

Study area

The study area is in the University of Tehran's Experimental Forest at Noushar on slopes of the Alborz Mountains at an elevation of 200-2200 m in northern Iran (south of the Caspian Sea) (Figure 1 and 2). The climate of the area is temperate with an annual mean temperature of 8.5° C and a total annual precipitation of 885 mm. Forests occupy plateaus on moderately

Figure 1. The distribution of Caspian forests in Iran (modified according to Knapp *et al*., 2005).

Figure 2. The closer view of the studied forest stands in the north of Iran.

inclined slopes with limestone bedrock and largely free of rock outcrops. Caspian forests occupy an approximate area of 2,000,000 ha and are dominated by oriental beech. These forests are characterized by natural, unevenaged structures with gap dynamics typical of old-growth forests (Marvie-Mohadjer, 2001). Forests at middle and upper elevations are primarily composed of beech with hornbeam, alder (*Tilia daststyla* Steven subsp. *caucasica* (V.Engl.) Pigott), and Persian maple (*Acer velutinum* Boiss.) as important minor species (Marvie-Mohadjer, 1976). Beech is the most productive timber species in Caspian forests occupying 17.6% of the total land area and representing 30% of the standing tree volume. Beech in this area can exceed 40 m in height and reach 1.5 m dbh (Resaneh *et al.,* 2001). The Caspian forests of Iran are largely natural temperate broad leaved with minimal active management. Those sections that are managed for timber production have been chiefly kept in a two-aged shelterwood system.

Dead tree selection and description

Two hundred and five dead beech and hornbeam trees (standing, leaning, or down) were randomly selected from two forest compartments (Patom and Namkhaneh) within the University of Tehran's Experimental Forest (Table 1) where all dead wood had been surveyed (Sefidi *et al.,* 2008). Different type of dead wood: (a) the logs as downed dead bole, (b) snags as standing dead tree, and (c) stumps as standing dead tree up to 2 m were recorded. The logs and snags extended outside the forest compartment boundaries were excluded from measurement. All dead wood pieces were measured for diameter, length and stage of decay. Diameters of logs, snags, and stumps were measured using calipers. For tall snags, diameter of the top end was visually estimated and calibrated with a snag top that was within manual reach and was measured (Harmon & Sexton, 1996; Sefidi *et al.*,2014). Diameter at breast height was measured on dead trees in the early stages of decay. However, in the latter stages of decay the diameter of the original (living) tree was estimated, due to collapse of bole structures. The lengths of down logs were measured with a tape and snag heights were measured with a meter stick to calculate volume. For snags taller than 4 m, a clinometer was used to measure height. The pieces of dead wood were divided into three size classes based upon their maximum diameter as follows: Class 1 included pieces with a maximum diameter < 25 cm; Class 2 was 25 –50 cm; Class 3 was 50 < cm. Decay was classified into four stages based on outer physical features of dead trees (Table 2). Many dead wood pieces had a combination of different decay stages along their entire length and therefore the dominant decay stage was assigned to each piece. The slope and topographic position at which each inventoried dead wood was located was also recorded.

Table 1. Stand structural and climatic features of the two selected study sites

District Name, Number	Area (ha)	Age (yr)	Dominant Tree Species	Regeneration	Elevation (m)	Location
Patom, 112	59	100+	*Fagus orientalis* *Acer capadocicum, Carpinus betulus*	Natural	480-630	36°36'13"N, 51°33'47"E
Namkhaneh, 214	39	120+	*Fagus orientalis* *Acer velutinum,* *Carpinus betulus*	Natural	950-1110	36°36'17"N, 51°33'51"E

Table 2. The classification system for identifying dead wood decay stages (Albrecht, 1990)

Stage	Morphological characteristics
1	Dead wood freshly fallen, bark all wood solid, current year twigs still attached
2	Dead wood still supports own weight, sapwood decayed but some still present, bark and heartwood mainly solid, twigs absent
3	Dead wood does not support its own weight, bark sloughs, sapwood mainly absent, heartwood not structurally solid ,branch stubs can be removed
4	Heartwood mainly fragmented, forming ill-defined, elongate mounds on the forest floor sometimes invisible from surface

Inventory of fungi

The presence of sporocarps species was recorded on each individual dead tree, but quantified separately for three different types of dead wood. Dead trees were inventoried for fungal sporocarps. Fungi were either determined on site or were collected for determination in the laboratory. All sporocarps were photographed and determined according to Phillips (1981). When we encountered difficulties in determination, photographs were sent to Dr. Siranoush Nanagulian from Yerevan State University in Armenia for assistance with identification. Non-stromatic Pyrenomycetes and inoperculate Discomycetes with sporocarps regularly smaller than 10 mm were excluded from the sampling. The excluded groups are typically not considered important for wood decay in European beech forests, though they constitute an important component of species diversity (Ódor & van Hees, 2004). We could not measure fungal cover because many wood inhabiting fungi develop fruiting bodies, while other species produce infrequent and/or short-lived fruiting bodies which may be easily missed during sampling (Boddy, 2001; Berglund *et al.,* 2005). All sampling took place between June and July of 2006 and therefore the fungal species should be viewed as a "snap-shot" of fungal diversity and not a true representation of all the fungi present on the dead wood.

Data analysis

Tree species (three levels: beech vs. hornbeam and minor species), dead wood size (3 classes: < 10-25, 25-50, >50 cm diameter), log position (downed, small standing pieces and standing logs taller than 2 m), decay stage (4 stages, Table 2) were used as predictor factors for the number of sporocarps species (NSS) as a fungal species richness and diversity in each dead log using analysis of variance (ANOVA). Less than 10 % of dead wood was standing dead wood, so log position was excluded from the analysis. The effect of dead wood variables (size class, species and decay stage) and macrofungi diversity after normalization were analyzed by one-way ANOVA.

Furthermore, the analysis a general linear model was used with the following criteria: (1) dependent variable: NSS; (2) explanatory variables: decay stage (factor), DBH (interval), Length or Height (factor). The model building was based on backward elimination from the full model (including all interactions). During the selection the effects of eliminations were tested by deviance analysis (Crawley, 1993). This analysis use to find the best model fitness in stepwise regression. In this analysis, the variance of variables compare and some of them eliminate from the model to provide the best model.

The data had to be log transformed because the assumption of homogeneity of variance was not met. All statistical tests were considered significant at the $P < 0.05$ level.

The type of diversity used here is α- diversity which is the diversity of species on the dead woods as micro habitat. Some diversity index was used in order to study the fungi species diversity in different groups including :

The Shannon-Wiene's diversity index (H´) (Pitkanen, 1998) used to evaluate diversity.

$$H' = \sum_{i=1}^{S} (p_i)(\log_2 p_i)$$

Where H' is the index of diversity, p_i is a proportion of total samples belonging to the *i* species. In this index the larger the value of H', the greater the uncertainty. Aslo Simpson`s (E1/D) (Krebs, 1999),

$$\frac{1}{D} = \frac{1}{\sum p_i^2}$$

Where 1/D is the Simpson reciprocal index and p_i is a proportion of species *i* in the community. The reciprocal of Simpson's formulation (1/D) varies from 1 to s, the number of species in the sample. In this form Simpson's diversity can be more easily interpreted as the number of equally common species required to generate the observed heterogeneity of the sample.

Pielou`s evenness index (Peet, 1974) also used in this study:

$$J' = H' / H'_{max}$$

Where J' is the Pielou`s evenness index and H' is the Shannon – Wiener diversity index, H'_{max} equal to InS which S is the total number of species in the sample. J' is constrained between 0 and 1. The less variation in the communities between the species, the higher J' is.

A Margalef `richness index used as a simple measure of species richness (Margalef, 1958).

$$M = (S - 1) / \ln N$$

Where M is the Margalef `species richness index and S is the total number of species, N total number of individuals in the sample and ln is the natural logarithm.

Results

A total of 40 species of macro-fungi inhabited the dead beech and hornbeam wood in the Caspian forests of the study site (Table 3). The number of sporocarps species (NSS) varied in different dead wood size and decay classes (Figure 3). The different stages of decay ($F = 20.0$, $P = 0.000$) and the different size classes ($F = 5.40$, $P = 0.009$) of dead wood had significantly different species richness of macrofungi (Figure 3, Table 4). Deadwood in decay stage 3 and the dead wood in size Class 4 (> 75 cm diameter) contained the highest diversity of fungi.

More downed logs had macrofungi present on them than standing snags. Most of fungi identified on both logs and snags belonged to Basidiomycetes and Ascomycetes (Table 3). The highest value for richness and evenness indices calculated in large diameter dead wood in decay class III (Table 5). Steps of multiple regression model building of species richness of fungi during backward selection were shown in Table 6. The results indicated the size ($df = 60$, F-value 5.210, $p < 0.01$) and decay class ($df = 3$, F-value 19.928, $p < 0.001$) of dead wood describe the greatest variance of the model that means the highest number of sporocarps species inhabited on the large dead wood in advanced stage of decaying.

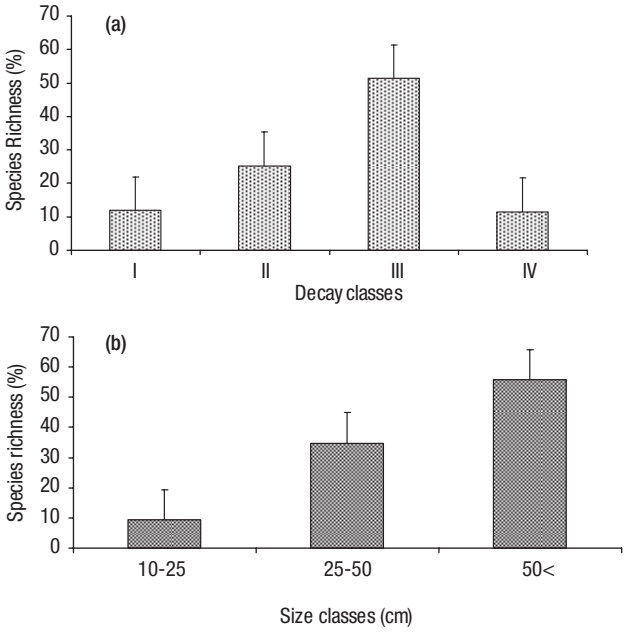

Figure 3. Species richness of fungi species (the number of sporocarps species) by decay class ($F = 20.0$, $P = 0.000$) (a) and size class ($F = 5.40$, $P = 0.009$) (b), Decay class I defined as freshly fallen dead wood, in the decay class II sapwood decayed but some still present, in the decay class III dead wood does not support its own weight, bark sloughs, sapwood mainly absent, in the Class IV Heartwood mainly fragmented and elongate mounds on the forest floor sometimes invisible from surface.

Table 3. Species of dead-wood inhabiting macrofungi growing in the beech forests of northern Iran

Aleuria aurantia (Fr.) Fuckel	*Lentinellus ursinus* (Fr.) Kühner
Amanita phalloides (Fr.) Link	*Meripilus giganteus* (Fr.) Karst.
Auricularia auricula-judea (Fr.) Schröt	*Mycena galericulata* (Fr.) Gray
Calocera viscosa (Persoon ex Fries) Fr.	*Nectria cinnabarina* (Tode) Fr.
Calvatia excipuliformis (Pers.) Perdek	*Oligoporus caesius* (Schrad.) Gilb. & Ryv.
Clavulinopsis corniculata (Schaeff.) Corner	*Onnia tomentosa* (Fr.) Karst.
Coprinus micaceus (Bulliard ex Fries) Fr.	*Phellinus pomaceus* (Pers.) Maire.
Coriolus hirsutus (Wulf. ex Fr.) Quél.	*Phyllotopsis nidulans* (Pers.) Singer
Coriolus versicolor (L. ex Fr.) Quél.	*Piptoporus betulinus* (Bull. ex Fr.) Karst.
Craterellus cornucopioides Persoon	*Pleurotus ostreatus* (Jacq. ex Fr.) Kumm.
Crepidotus variabilis (Pers. ex Fr.) Kumm.	*Pluteus romelii* (Fr.) Bres.
Fistulina hepatica Shaeff. ex Fr.	*Polyporus squamosus* (Huds. ex Fr.) Schu.
Flammulina velutipes (Fr.) Karst.	*Psathyrella candolleana* (Fr.) Maire.
Fomes fomentarius (L. ex Fr.) Kickx.	*Ramaria apiculata* (Fr.) Donk
Fomitopsis pinicola (Sw. ex Fr.) Karst.	*Ramaria formosa* (Fr.) Quél.
Ganoderma adspersum (Schulz.) Donk.	*Ramaria stricta* (Fr.) Quél.
Ganoderma applanatum (Pers.) Pat.	*Schizophyllum commune* Fr.
Gloeophyllum abietinum (Fr.) Karst.	*Sparassis crispa* Wulf. ex Fr.
Gloeophyllum sepiarium (Fr.) Karst.	*Thelephora terrestris* (Ehrh.) Fr.
Inocybe geophylla (Pers.) Kumm	*Xylaria polymorpha* (Pers.) Grev.

Table 4. Results of one-way ANOVA's of tree level fungi species richness in different types, decay and size classes of dead wood, deciduous broad-leaved forest of Northern Iran

Characteristics of dead wood	index	df	ANOVA		
			Mean Square	F value	P value
Tree species	Margalef (M)	2	1.78	28.97	< 0.001
	Shannon-Wiener (H`)	2	0.07	2.11	0.165
	Simpson (1/D)	2	14.75	9.04	0.004
	Pielou (J)	2	0.01	5.51	0.020
Size class	Margalef (M)	2	15.23	199.94	< 0.001
	Shannon-Wiener (H`)	2	3.31	111.62	< 0.001
	Simpson (1/D)	2	227.09	128.74	< 0.001
	Pielou (J)	2	0.01	3.09	0.082
Decay class	Margalef (M)	3	7.61	28.01	< 0.001
	Shannon-Wiener (H`)	3	1.82	10.71	< 0.001
	Simpson (1/D)	3	236.14	57.44	< 0.001
	Pielou (J)	3	0.02	1.03	0.404

Table 5. Diversity indexes (±SE) in different types, decay and size classes of dead wood, deciduous broad-leaved forest of Northern Iran

Characteristics of dead wood	Diversity index					
	Sample size	Species richness (%)	Shannon-Wiene *(H`)*	Simpson *(1/D)*	Margalef *(M)*	Pielou *(J)*
Tree species						
F.orientalis	102	48.45	2.181 ± 0.341	7.651± 2.141	2.519± 0.542	0.909± 0.234
C. betulus	98	45.12	2.647 ± 0.567	12.425± 1.453	3.467± 0.841	0.934± 0.352
Size class						
10-25 cm	35	9.3	1.806 ± 0.441	5.236± 0.941	1.888± 0.343	0.928± 0.333
25-50 cm	83	34.8	2.561± 0.731	11.351± 2.365	3.253± 0.841	0.945± 0.124
50 cm <	87	55.9	3.195± 0.844	21.402± 2.748	5.418± 1.311	0.959± 0.376
Decay class						
I	48	11.9	2.272 ± 0.875	8.627± 0.941	2.823 ± 0.271	0.948± 0.123
II	74	25.28	3.097 ± 0.654	20.46± 4.344	4.926 ± 1.311	0.962± 0.278
III	49	51.30	3.334 ± 0.732	26.37± 3.343	6.50 ± 0.874	0.965± 0.345
IV	34	11.52	2.861 ± 0.876	13.126± 1.321	4.049 ± 0.441	0.946± 0.435

Table 6. Multiple regression, model building of species richness of fungi

Steps	SS	DF	MS	F	P
Full Model	3538.053a	194	18.237	6.437	**
Diameter	885.651	60	14.761	5.210	**
Decay class	169.390	3	56.463	19.928	***
Length	250.913	33	7.603	2.684	n.s.
D × L	10.017	7	1.431	.505	n.s.
D× DC	1.583	2	.792	.279	n.s.

The error estimation was normal. SS, DF, MS: sum of squares, degrees of freedom and mean square of the regression. Explanatory variables are the midpoint diameter of log or snags decay stage and the length or height of dead tree, ''p'' is the significance level of deviance analyses using F statistics, n.s.: not significant,*p < 0.05, **p < 0.01, *** p < 0.001. R^2 value of the regression was 0.854.

Discussion

Dead trees in Iranian forests were first identified as an important component of forest ecosystems in the scientific literature a decade ago (Marvie-Mohadjer, 2001). Nowadays managing forest ecosystem based on ecological properties is a new challenge in the near to nature forestry in the mixed beech stands. In the new forestry maintaining dead trees and inhibiting organism as biological legacy is a crucial challenge for forest managers. This current study contributes to a growing body of knowledge by providing a quantitative assessment of the diversity of macrofungi inhabiting different types of dead wood in this forest type. Macro fungi are closely linked in cycling organic material in forest ecosystems back to the forest soil; therefore the close link between diversity and decomposition stage is likely an indication of the activity of the dead wood and the soil transition zone. Larger dead wood provides more surfaces for connections between the soil and thus is likely the cause for higher fungi diversity on larger pieces of dead woods (Christensen *et al.,* 2005). Larger pieces of dead wood have greater structural diversity which provides more niches for macrofungi establishment and the pieces have a slower decomposition rates which provides a longer time for colonization (Ódor & van Hees, 2004). Another characteristic of large trees that favors higher macrofungi diversity is that large trees likely have older heartwood that has already had a long infection history prior to the tree's death (Heilmann-Clausen & Christensen, 2004).

The species richness of macrofungi from this study (Table 3) may seem small in comparison to other descriptions of macrofungi from this region, e.g., Hallenberg (1981) recorded 275 species of wood fungus from across a wide range of substrates in the Caspian forests of northern Iran. However, our species list must be viewed within the context that the substrate was limited to dead beech and hornbeam trees and the sampling occurred only in the months of June and July. From this perspective our species richness closely matches smaller-scale studies from this region that have found 25 wood decaying fungi on conifers and 51 on broad-leaved trees (Afyon *et al.,* 2005). Most of the species identified in this study have previously been described as growing in the beech forests of this region. Fallahyan (1973) first described *Amanita phalloides* (as cited in Bahram *et al.,* 2006); Soleimani (1976) described *Coriolus hirsutus, Ganoderma adspersum, Ganoderma applanatum, Gloeophyllum sepiarium, Polyporus squamosus, Schizophyllum commune,* and *Xylaria polymorpha* present on deadwood from broad-leaved trees in the Caspian Forest in Iran; and Ghobad-Nejhad *et al.,* (2009) identified *Fomes fomentarius*, *Fomitopsis pinicola,* and *Onnia tomentosa* as present in the Caucasus region of Iran. Although the sampling for this study was limited to dead wood of beech and hornbeam, several of the fungal species are also capable of, and sometimes more commonly found, growing on live trees [e.g., *Auricularia auricular-judae* (Le Goc, 1914)], dead conifers [e.g., *Gloeophyllum sepiarium* (Alli *et al.,* 2007)], or soil [e.g., *Onnia tomentosa* (Ghobad-Nejhad *et al*., 2009)].

Maximizing the number of large dead trees in different stages of decay seems to be a good management strategy for increasing the structural complexity and biological diversity of forests in northern Iran and in hardwood forests around the world (Kruys & Jonsson, 1999; Ódor & Standovár, 2001; Nordén *et al.,* 2004). Decay stage and size class appear to be key variables relating significantly to both species richness and rare species incidence (Heilmann-Clausen & Christensen, 2004). Diversity indexes significantly was higher in the large and advancly decayed dead woods. Impact of dead wood size and decay stage on presence of dead wood dwelling fungi had reported in the same studies (Nordén *et al*., 2004). Fungi are the most important agents of wood decay in forest ecosystems and hence they open up the wood resource for most other dead wood dwelling organisms (Boddy, 2001).

In most situations, the only active management that would be required to maintain intact large pieces of dead wood in these forests would be to provide the opportunity for trees to grow to full maturity and if timber harvesting occurs within these forests, ideally it would be appropriate to eliminate or reduce the crushing of existing dead wood when machinery transports harvested trees out of the site. Maintenance of large dead trees will sustain the ecological diversity and integrity of these forest ecosystems.

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