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Wood-inhabiting, polyporoid fungi in aspen-dominated forests managed for biomass in the U.S. Lake States

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ABSTRACT

To better understand the potential long-term effects of biomass harvesting on biodiversity, the polyporoid fungi community was characterized from 120 plots in four aspen-dominated forests in Minnesota. Four deadwood variables (substratum species, substratum type, decay class and diameter class) were recorded for each polyporoid species occurrence. A total of 2 358 polyporoid occurrences, representing 86 species, were recorded on 16 tree species. Eight species (*Trichaptum biforme*, *Bjerkandera adusta*, *Trametes hirsuta*, *Phellinus tremulae*, *Fomes fomentarius*, *Irpex lacteus*, *Fomitopsis ochracea* and *Antrodia serialis*) made up 67 % of occurrences. Four polyporoid species (*Funalia trogii*, *Pycnoporellus fulgens*, *Rigidoporus crocatus* and *Skeletocutis chrysella*) are potentially rare and/or threatened in the Lake States. Non-metric multidimensional scaling and rarefaction curves demonstrated that small diameter substrata (especially those <5 cm) most strongly influenced polyporoid species occurrences. Aspen-dominated systems show great potential for biomass production, but these forests also support a species-rich community of polyporoid fungi, including potentially rare species.

Introduction

Aspen forests in the U.S. Lake States and elsewhere are increasingly being viewed as a source of renewable energy in the form of woody biomass feedstocks (henceforth, biomass) that are burned to produce electricity (Kauter *et al.* 2003). A primary concern regarding repeated biomass harvesting, particularly when conducted on short rotations, is the potential negative impact on ecosystem services, including biodiversity, soil nutrient availability, carbon cycling, and overall ecosystem productivity (Mariani *et al.* 2006; Haeussler *et al.* 2007; Mundell *et al.* 2008; Lattimore *et al.* 2009). These services are influenced by the abundance of fine and coarse woody debris present on the forest floor, which will likely be extensively reduced following biomass harvests. While clear-cutting in aspen-dominated forests is meant to mimic natural disturbances (e.g., a stand-replacing wildfire), research from mixed aspen-conifer forests has shown that coarse woody debris (CWD) volumes in burned stands can be significantly higher compared to clear-cut sites (Pedlar *et al.* 2002). Biomass harvests, as they are currently conducted in the U.S. Lake States, are especially extractive because they remove existing

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sound woody debris, as well as a wide range of living material (shrubs, boles, tops, and branches) that would otherwise die and add to the woody-debris pool. Thus, when conducted over repeated entries and on short rotations, these harvests are expected to dramatically reduce woody-debris volume and preclude the accrual of large diameter, well-decayed logs.

As a consequence, biodiversity threats from biomass harvesting are likely greatest to those organisms that require various forms of coarse and fine woody debris as a substratum, such as wood-decaying polyporoid fungi. Research from Fennoscandia has convincingly established that reductions in woody debris quality and quantity have adverse effects on the richness and abundance of polyporoid fungi (e.g., Bader *et al.* 1995; Rydin *et al.* 1997; Kruys & Jonsson 1999; Nordén *et al.* 2004; Toivanen *et al.* 2012). The risk of biomass harvesting to these organisms is exacerbated by the fact that certain fungal species require large-diameter, well-decayed woody debris, a substratum unlikely to be found on biomass harvesting sites. Similarly, small-diameter woody debris is also known to be an important substratum for wood-inhabiting fungi (Küffer & Senn-Irlet 2005; Juutilainen *et al.* 2011).

Because fungal enzymatic activity is the primary mechanism by which deadwood is mineralized in forested systems, significant changes to the composition and function of these communities could have long-term, negative impacts on patterns of nutrient and carbon cycling, along with site productivity. In particular, wood-decay fungi can be considered 'ecosystem engineers' because they directly alter wood structure and resource availability for many other groups of organisms (Lonsdale *et al.* 2008). Moreover, the diversity and species composition of these fungal communities has been linked to nutrient turnover rates (Torres & González 2005; Lindner *et al.* 2011). Due to the central role these organisms play in carbon and nutrient cycling, understanding how biomass harvesting affects wood-decay fungal communities is critical for assessing the environmental sustainability of such harvests.

To address the ecological impact of biomass harvesting, a large-scale, manipulative experiment in mature aspen stands (>50 yr without active management) has been established in northern Minnesota, USA. The study includes four sites with replicated silvicultural treatments representing various levels of woody debris retention and various configurations of living-tree retention. As part of that long-term study, we are testing the impacts of these harvest treatments on wood-inhabiting fungal communities. Thus, the objective of the current, initial study is to characterize pre-treatment (prior to harvest) polyporoid species composition, including factors that influence fungal diversity, in mature aspen-dominated sites. Although the intent of these baseline data is to allow the tracking of changes in the wood-inhabiting polyporoid community over time in response to the harvesting treatments, they also provide detailed insights into the polyporoid community structure in these systems. We are focusing our efforts on the polyporoid fungal because this group directly relies on woody substrata for resources and habitat, and have been shown to be sensitive to harvesting pressures in Fennoscandia. Further, polyporoid fungi are useful for assessing biodiversity because the large number of species present in northern temperate forests facilitates robust species richness and diversity analyses (Lindner *et al.* 2006; Halme *et al.* 2009).

This information is especially meaningful in North America where this important group of organisms is rarely considered in biodiversity studies (but see Crites & Dale 1998; Lindner *et al.* 2006), and much remains unknown regarding community composition. Also, the aspen forest type, dominated primarily by quaking aspen (*Populus tremuloides*), is the most widely distributed forest type in North America (Eyre 1980), representing the most common forest type in portions of the upper Great Lakes region, including Minnesota, where it covers over two million hectares. Considering the predominance of aspen forests in North America, the current lack of information on wood-inhabiting polyporoid fungi represents a significant gap in our knowledge and has hampered efforts to develop biomass harvesting guidelines that ensure the sustainability of these critical ecosystem components.

The specific objectives of this study were to: (1) characterize the overall polyporoid species community within aspen-dominated forest systems in Minnesota; (2) establish if rare and/or threatened polyporoid species are present in managed aspen forests, so they may be utilized as ecosystem indicators once biomass harvesting is implemented; and (3) determine if specific deadwood characteristics could be recognized as important for polyporoid species, so as to inform management recommendations for conserving these species.

Materials and methods

Site selection and sampling

Four sites selected for our long-term study of biomass harvesting (Independence, Lost River, Melrude, and Pelican Lake) in northern Minnesota were used for this assessment of the polyporoid species community. Sites were dominated by *P. tremuloides*, having naturally regenerated following clear-cut harvesting, and ranged in age from 55 to 68 yr (in 2009). Site characteristics are listed in Table S1.

The sampling scheme consisted of thirty 400 m^2 circular plots (radius = ~11.3 m) distributed across each of the four sites, resulting in 120 total plots sampled. All fine and coarse woody debris (FWD and CWD, respectively), along with standing trees (living or dead, to a height of 2 m) within each 400 m^2 plots were non-destructively inventoried for polyporoid fruit bodies during late Sep. and Oct. of 2009 or 2010. All substrata with a diameter ≥1 cm were sampled regardless of length. When a fruit body was encountered, the following characteristics were recorded: fungal species (when known; see below for unknowns), substratum type (branch, log, suspended log, snag, stump, and living-tree), substratum species, diameter class (1 to <5, 5 to <10, 10 to <15, 15 to <20, 20 to <25, and >25 cm), and decay class [following the five-class system of Maser *et al.* (1979)]. Dead fruit bodies were also inventoried, unless their state of degradation precluded identification. Inventories were conducted during early autumn to ensure detection of those species producing annual fruit bodies. This sampling detail and intensity is comparable to similar studies in Scandinavia (see Junninen & Komonen 2011). Sampling was

carried out by three people, together working until all polyporoid fungi occurring on substrata with a diameter ≥1 cm were sampled per plot, which on average took 30 min.

Polyporoid species identification

For fungal species not easily identified in the field, a sample of the fruit body was collected and dried before being returned to the laboratory. Samples were identified by either microscopic analysis of morphological features or DNA sequencing of the internal transcribed spacer (ITS) or nuclear large subunit (nLSU) regions. In addition, voucher specimens of common polyporoid species were also collected to generate reference sequence data. All DNA extractions, PCR and sequencing protocols were carried out as described in Lindner & Banik (2009). The Basic Local Alignment Search Tool (BLAST) was used to search for similar sequences in GenBank (Altschul *et al.* 1997) to identify unknown isolates, using a 97 % similarity threshold for species-level identifications. For isolates that could not be matched to known species in GenBank, molecular operational taxonomic units (MOTUs) were designated using the program jMOTU (https://nematodes.org/bioinformatics/jMOTU/). The jMOTU program uses the Needleman–Wunsch megablast search algorithm to align sequences, then user-defined parameters to designate MOTUs. Within jMOTU, the minimum sequence length used for analysis was set at 300 base pairs (bp) to eliminate short sequences, and MOTUs were designated at 3 % sequence divergence. Because of intraspecific variation and the large number of indels present within the ITS region, a low BLAST identity filter was set at 90 %, and the default sequence alignment overlap (60 % of the minimum sequence length) was used.

Once unknown isolates were grouped into MOTUs, phylogenetic analyses were performed using sequences representing the most closely related species for comparison, as determined from the original BLAST search. Sequences were aligned with MAFFT v. 6 using the FFT-INS-i option (Katoh *et al.* 2005), and phylogenetic reconstruction of ITS and partial nLSU sequences was performed in MEGA v. 5 (Tamura *et al.* 2011) using methods described in Brazee *et al.* (2011). The use of molecular diagnostics greatly increased our accuracy in identifying both rare species and common species exhibiting high phenotypic plasticity. The use of morphological features alone would likely have resulted in a reduced number of uncommon and rare species identified. Polyporoid nomenclature was based on Index Fungorum (www.indexfungorum.org) with minor exceptions, and collected specimens have been preserved in the USDA Forest Service, Center for Forest Mycology Research (CFMR) herbarium.

Statistical analyses

One of our primary objectives was to characterize the polyporoid species community, including the sources of variation, found within aspen-dominated forest systems. Therefore, we conducted a non-metric multidimensional scaling (NMS) ordination in PC-ORD v. 6.0 (McCune & Mefford 2011), using the Sørensen distance measure and random starting coordinates. Two frequency matrices were used for analysis, with the primary matrix composed of polyporoid species (columns; $N = 57$) by plots (rows; $N = 120$) while the secondary matrix was composed of deadwood variables (columns; $N = 27$) by plots. To down-weight the influence of very abundant polyporoid species, a general relativization by column totals was used to transform the polyporoid species matrix. In addition, polyporoid species with less than three occurrences (29/86 species) were excluded from the analysis. The percent variance explained in the distance matrix was calculated using the Sørensen measure, and the two axes with the highest increment R^2 were selected to best describe the data. To determine the significance of the deadwood variables and polyporoid species that were structuring the NMS axes, bivariate correlations using Kendall's tau-b were performed in SPSS 16.0 (SPSS Inc., Chicago, IL), with a sequential Bonferroni correction set at $P = 0.05$ (Holm 1979).

To further assess the relatedness of the polyporoid communities by plot, we carried out an analysis of nestedness using the equations developed by Brualdi & Sanderson (1999) and Jonsson (2001). A determination of nestedness would indicate that uncommon polyporoid species are more likely to be found in species-rich plots, an important consideration for conservation efforts. In PC-ORD, the Nestedness6 application was run using all 86 species from 120 plots with 1 000 randomizations. If the observed nestedness (N_a) equals zero, then nestedness equals the random expectations from a null population. A negative value indicates the population is more strongly nested, while a positive value indicates the population is more weakly nested compared to a random sample.

To determine how polyporoid species richness changed by each of the four deadwood variables (substratum, substratum type, diameter class and decay class), we generated species accumulation curves (SACs) using rarefaction equations developed by Sanders (1968) and modified by Hurlbert (1971). SACs were generated in R (www.R-project.org) and details regarding the equations used have been described previously in Lindner *et al.* (2006). For substratum species, we generated curves with and without *P. tremuloides*, so that we could better interpret the curves of the less abundant, non-aspen hosts.

To determine how similar the polyporoid communities were by each of the four deadwood variables, a presence/absence matrix of polyporoid species (rows; $N = 86$) by deadwood variables (columns; $N = 26$) was created. The Sørensen similarity index, for which joint absences are excluded from consideration and matches are double weighted, was used to create the distance matrices. The Sørensen measure was also performed in SPSS.

Results

Polyporoid species identification and occurrence

From 120 plots, 2 358 occurrences of polyporoid fungi were recorded, representing 86 unique species from 16 host tree species (Table 1). Most fruit bodies could be identified to species in the field (2 088/2 358; 89 %), while the remainder (270/2 358; 11 %) required collection for morphological and molecular analyses. From the 270 collections, 230 ITS and 11 nLSU sequences were generated, with GenBank accession

Table 1 – Polyporoid species, total observations, substratum characteristics, and GenBank accession numbers of ITS and nLSU sequences

No.	Polyporoid species[a]	Abbrev.[b]	Total Obs.	Substratum species[c]									Substratum diameter (cm)	GenBank accession Nos.
				POTR	BEPA	UNKH	Acer	ACSP	ABBA	Alnus	Other	BEAL		
1	*Trichaptum biforme*	TriBif	489	430	51	1	3	1	1	0	2	0	11.2	–
2	*Bjerkandera adusta* **(A)**	BjeAdu	311	300	9	1	0	0	0	0	1	0	17.5	–
3	*Trametes hirsuta* **(A)**	TraHir	201	199	0	0	1	0	0	1	0	0	9.6	–
4	*Phellinus tremulae* **(A)**	PheTre	173	172	0	1	0	0	0	0	0	0	23.6	–
5	*Fomes fomentarius*	FomFom	150	40	107	0	0	0	0	0	2	1	18.7	–
6	*Irpex lacteus*	IrpLac	106	34	1	7	24	29	0	11	0	0	3.7	–
7	*Fomitopsis ochracea* **(A)**	FomOch	84	84	0	0	0	0	0	0	0	0	21.6	JQ673051-JQ673054
8	*Antrodia serialis* **(A)**	AntSer	59	49	0	9	0	0	1	0	0	0	10.6	JQ673032-JQ673047
9	*Trametes pubescens*	TraPub	47	5	25	3	0	0	0	14	0	0	7.5	JQ673025-JQ673026
10	*Tyromyces chioneus*	TyrChi	47	36	2	5	0	1	0	2	1	0	8.0	–
11	*Junghuhnia nitida* **(A)**	JunNit	42	31	0	10	0	1	0	0	0	0	5.8	JQ673148-JQ673150
12	*Piptoporus betulinus*	PipBet	40	0	38	0	0	0	0	0	2	0	10.6	–
13	*Stromatoscypha fimbriata*	StrFim	38	29	2	7	0	0	0	0	0	0	9.6	–
14	*Ceriporiopsis aneirina* **(A)**	CeiAne	37	35	0	2	0	0	0	0	0	0	6.0	JQ673087-JQ673099
15	*Trechispora mollusca*	TreMol	33	25	0	4	0	0	3	1	0	0	6.1	JQ673209
16	*Antrodiella* sp. 1	AntSp1	32	27	0	3	1	0	0	0	0	1	6.4	JQ673134-JQ673142
17	*Trichaptum abietinum*	TriAbi	31	0	0	0	0	0	30	0	1	0	12.1	–
18	*Phellinus igniarius* s.l.	PheIgn	30	1	21	1	0	0	0	0	3	4	13.5	JQ673181
19	*Schizopora* c.f. *radula*	SchRad	25	21	1	2	1	0	0	0	0	0	8.4	JQ673187-JQ673189
20	*Cerrena unicolor*	CerUni	23	3	4	0	13	2	0	0	1	0	15.3	–
21	*Fuscoporia* sp. 1	FusSp1	22	11	0	7	1	0	3	0	0	0	11.1	JQ673155-JQ673173
22	*Fomitiporia punctata*	FomPun	20	2	0	2	1	15	0	0	0	0	5.1	JQ673197-JQ673204
23	*Postia caesia* **(A)**	PosCae	20	13	0	7	0	0	0	0	0	0	5.6	–
24	*Datronia scutellata*	DatScu	19	12	0	3	0	0	0	4	0	0	2.1	JQ673031
25	*Postia* sp. 2	PosSp2	18	13	0	1	0	0	3	0	1	0	16.1	JQ673056-JQ673064
26	*Fuscoporia ferrea*	FusFer	16	6	0	3	3	3	0	1	0	0	4.1	JQ673174-JQ673178
27	*Skeletocutis nivea*	SkeNiv	15	10	1	1	0	1	0	1	1	0	4.4	JQ673114-JQ673123; JQ673211
28	*Fomitopsis pinicola*	FomPin	14	0	8	1	1	0	2	0	2	0	17.1	JQ673055
29	*Polyporus brumalis*	PolBru	14	5	2	2	0	0	0	5	0	0	3.4	JQ673029-JQ673030
30	*Antrodia xantha* **(A)**	AntXan	11	10	0	1	0	0	0	0	0	0	14.2	JQ673048-JQ673049
31	*Postia subcaesia* **(A)**	PosSub	11	10	0	1	0	0	0	0	0	0	3.7	JQ673070-JQ673078
32	*Ceriporiopsis pannocincta*	CeiPan	10	8	0	0	1	0	0	0	1	0	18.5	JQ673100-JQ673106
33	*Gloeoporus dichrous*	GloDic	10	8	1	0	1	0	0	0	0	0	13.6	JQ673109
34	*Skeletocutis chrysella* **(RL) (A)**	SkeChr	10	10	0	0	0	0	0	0	0	0	10.7	JQ673126-JQ673132
35	*Daedaleopsis confragosa*	DaeCon	9	0	3	3	0	0	0	0	2	1	8.8	–
36	*Fuscoporia ferruginosa* **(A)**	FusFeu	9	7	0	2	0	0	0	0	0	0	4.4	JQ673212-JQ673220
37	*Ischnoderma resinosa*	IscRes	8	0	5	2	0	0	0	0	0	1	17.8	–
38	*Antrodiella semisupina* **(A)**	AntSem	7	5	0	2	0	0	0	0	0	0	8.1	–
39	*Lenzites betulina*	LenBet	6	0	6	0	0	0	0	0	0	0	7.5	–
40	*Polyporus arcularius* **(A)**	PolArc	6	5	0	1	0	0	0	0	0	0	3.3	–
41	*Trametes versicolor*	TraVer	6	4	0	0	1	1	0	0	0	0	10.2	JQ673021-JQ673022
42	*Ceriporiopsis* sp. 2 **(A)**	CeiSp2	5	4	0	1	0	0	0	0	0	0	9.2	JQ673081-JQ673085
43	*Ganoderma applanatum*	GanApp	5	4	1	0	0	0	0	0	0	0	26.2	–
44	*Oxyporus populinus*	OxyPop	5	0	0	0	3	0	0	0	2	0	24.2	–
45	*Trametes ochracea* **(A)**	TraOch	5	5	0	0	0	0	0	0	0	0	11.6	JQ673023-JQ673024; JQ673027-JQ673028

(*continued on next page*)

Table 1 – (*continued*)

No.	Polyporoid species[a]	Abbrev.[b]	Total Obs.	Substratum species[c]									Substratum diameter (cm)	GenBank accession Nos.
				POTR	BEPA	UNKH	Acer	ACSP	ABBA	Alnus	Other	BEAL		
46	*Antrodiella romellii*	AntRom	4	1	0	1	0	0	2	0	0	0	8.5	JQ673144-JQ673145
47	*Inonotus obliquus*	InoObl	4	0	2	0	0	0	0	0	0	2	10.8	–
48	*Oxyporus corticola*	OxyCor	4	2	0	1	1	0	0	0	0	0	10.3	JQ673194-JQ673196
49	*Funalia trogii* **(RL) (A)**	FunTro	3	3	0	0	0	0	0	0	0	0	17.7	JQ673018-JQ673020
50	*Mensularia radiata*	MenRad	3	0	0	1	0	2	0	0	0	0	3.3	JQ673179-JQ673180
51	*Perenniporia subacida*	PerSub	3	1	1	0	0	0	1	0	0	0	17.7	JQ673014-JQ673016
52	*Phellinus laevigatus*	PheLae	3	0	2	0	0	0	0	0	0	1	13.3	JQ673182-JQ673183; JQ673210
53	*Polyporus alveolaris*	PolAlv	3	2	0	0	0	1	0	0	0	0	2.7	–
54	*Polyporus varius*	PolVar	3	1	2	0	0	0	0	0	0	0	4.7	–
55	*Postia sericeomollis*	PosSer	3	0	0	0	0	0	0	0	3	0	40.0	JQ673065-JQ673067
56	*Rigidoporus crocatus*	RigCro	3	0	1	0	0	0	1	0	1	0	33.3	JQ673152-JQ673154
57	*Skeletocutis* sp. 1 **(A)**	SkeSp1	3	2	0	1	0	0	0	0	0	0	2.2	JQ673124-JQ673125
58	*Antrodiella* sp. 2 **(A)**		2	2	0	0	0	0	0	0	0	0	6.5	JQ673143; JQ673146
59	*Fomitopsis cajanderi*		2	1	0	0	0	0	0	0	1	0	22.5	JQ673050
60	*Gloeophyllum sepiarium*		2	0	0	1	0	0	1	0	0	0	13.5	JQ673111-JQ673112
61	*Gloeoporus* sp. 1		2	0	0	2	0	0	0	0	0	0	4.0	JQ673110
62	*Perenniporia medulla-panis*		2	1	0	1	0	0	0	0	0	0	8.0	JQ673013
63	*Perenniporia* sp. 1 **(A)**		2	1	0	0	0	0	0	1	0	0	5.5	JQ673017
64	*Postia lactea* **(A)**		2	1	0	1	0	0	0	0	0	0	8.0	JQ673079-JQ673080
65	*Postia* sp. 1 **(A)**		2	2	0	0	0	0	0	0	0	0	4.0	JQ673068-JQ673069
66	*Schizopora* sp. 1		2	1	0	0	1	0	0	0	0	0	20.0	JQ673191-JQ673192
67	*Trechispora* sp. 1		2	0	0	0	0	0	2	0	0	0	9.0	JQ673207-JQ673208
68	*Trechispora* sp. 2 **(A)**		2	2	0	0	0	0	0	0	0	0	3.5	JQ673205-JQ673206
69	*Antrodiella* sp. 3 **(A)**		1	1	0	0	0	0	0	0	0	0	14.0	JQ673147
70	*Ceriporia purpurea* **(A)**		1	1	0	0	0	0	0	0	0	0	5.0	JQ673108
71	*Ceriporia* sp. 1 **(A)**		1	1	0	0	0	0	0	0	0	0	18.0	–
72	*Ceriporiopsis* sp. 1		1	0	0	0	0	0	1	0	0	0	14.0	JQ673107
73	*Ceriporiopsis subvermispora* **(A)**		1	1	0	0	0	0	0	0	0	0	20.0	JQ673086
74	*Elmerina caryae*		1	1	0	0	0	0	0	0	0	0	25.0	JQ673151
75	*Fuscoporia gilva*		1	0	0	0	0	0	0	1	0	0	4.0	–
76	*Gloeophyllum carbonarium*		1	0	0	0	0	0	0	0	1	0	45.0	JQ673113
77	*Oxyporus* sp. 1 **(A)**		1	1	0	0	0	0	0	0	0	0	6.0	–
78	*Phaeolus schweinitzii*		1	0	0	0	0	0	1	0	0	0	35.0	–
79	*Phellinus conchatus*		1	0	0	0	1	0	0	0	0	0	4.0	JQ673185
80	*Phellinus* sp. 1 **(A)**		1	1	0	0	0	0	0	0	0	0	11.0	JQ673184
81	*Porodaedalea* sp. 1		1	0	0	0	0	0	0	0	1	0	22.0	JQ673186
82	*Pycnoporellus fulgens* **(RL)**		1	0	0	0	0	0	0	0	0	1	5.0	JQ673193
83	*Schizopora* sp. 2 **(A)**		1	1	0	0	0	0	0	0	0	0	20.0	JQ673190
84	*Schizopora* sp. 3		1	0	0	0	0	0	1	0	0	0	25.0	–
85	*Skeletocutis amorpha*		1	0	0	0	0	0	1	0	0	0	10.0	–
86	*Skeletocutis* sp. 2 **(A)**		1	1	0	0	0	0	0	0	0	0	4.0	JQ673133
Total			2358	1705	296	105	63	57	54	42	24	12	12.7	

a Polyporoid species present on aspen ≥95 % of the time (excluding "unknown hardwood") are denoted with (A), while species with informal red list status in the U.S. Lake States are denoted with (RL).
b Abbreviations (Abbrev): species without abbreviations had less than three occurrences and were excluded from the NMS analysis.
c Host substrata (ordered by number of observations): POTR = *Populus tremuloides*; BEPA = *Betula papyrifera*; UNKH = unknown hardwood; Acer = *Acer* spp.; ACSP = *Acer spicatum*; ABBA = *Abies balsamea*; Alnus = *Alnus* spp; Other (*Betula* spp., *Fraxinus nigra*, *Picea glauca*, *Pinus strobus*, *Salix* spp., unknown, and unknown conifer); and BEAL = *Betula alleghaniensis*.

numbers listed in Table 1. Of the 230 ITS sequences, 224 met the minimum sequence length threshold of 300 bp set in jMOTU. The mean sequence length was 634 bp with a range of 394–771 bp, and a 60 % minimum sequence overlap value of 238 bp. Although 86 unique taxa were identified, 22 could not be accurately assigned a species epithet based on microscopic characters and ITS/nLSU sequences (Table 1).

Populus tremuloides supported 62 species of polyporoid fungi from 1 705 observations (Table 1). The next most abundant host was *Betula papyrifera*, which supported 24 species from 296 observations (Table 1). Eight polyporoid species (*Trichaptum biforme*, *Bjerkandera adusta*, *Trametes hirsuta*, *Phellinus tremulae*, *Fomes fomentarius*, *Irpex lacteus*, *Fomitopsis ochracea* and *Antrodia serialis*; listed in decreasing abundance) made up 1 573/2 358 (67 %) of all observations. While, 45 species were encountered five times or less, and 18 species were encountered only once (Table 1). When only the polyporoid fungi that occurred on aspen ≥95 % of the time are considered, 31 species were present (Table 1). Of those, 24 species (77 %) occurred on aspen deadwood pieces with mean diameters in the three smallest diameter classes (1 to <15 cm), and no species were recorded on aspen deadwood with a mean diameter in the largest class (>25 cm; Table 1). The mean diameter of aspen substrata supporting these 24 polyporoid species was 7.2 cm ($SD = 3.4$; $N = 441$), while the remaining seven species found primarily on aspen were on substrata with a mean diameter of 19.8 cm ($SD = 2.2$; $N = 574$). Using the polyporoid species list generated in this study along with the results from a previous study in nearby Wisconsin and Michigan (Lindner *et al.* 2006), we generated an informal red list of rare and/or threatened polyporoid species for the U.S. Lake States (Table 1).

Polyporoid community analysis

A three-axis NMS solution (final stress = 20.01; final instability < 0.00001; $P = 0.004$) best described the data set (cumulative $R^2 = 0.71$). Fourteen quantitative deadwood variables were significantly correlated with NMS axes 1 and 2, which explained 30 and 22 % of the variation, respectively. For axis 1, small diameter deadwood was determined to be the variable with the strongest tau-b coefficient (diameter class I: $\tau = 0.427$; $P < 0.001$; Fig 1 and Table 2). The correlations with axis 1 indicate that plots in the positive segment of this axis contained more diameter class I deadwood, and a greater abundance of *A. serialis*, *Antrodiella* sp. 1, *Datronia scutellata*, *F. ochracea*, *Junghuhnia nitida*, *Polyporus brumalis* and *Stromatoscypha fimbriata* (Fig 1 and Table 2). Meanwhile, plots in the negative section of this axis had more *B. adusta* and *T. biforme* (Fig 1 and Table 2). While several deadwood variables were significantly correlated to axis 2, they explained less of the overall variation and are not presented in the NMS diagram (Fig 1 and Table 2). In addition, 17 polyporoid species were significantly correlated to both axes, with tau-b correlation coefficients ranging from −0.400 to 0.431 (Table 2). Yet overall, there were no tau-b coefficients that exceeded ±0.5 for any of the deadwood variables or polyporoid fungi. Results of the nestedness analysis ($N_a = -1.14$) were not significantly different than expected under the null hypothesis ($P = 0.13$ from a *t*-distribution and $P = 0.14$ from null matrices).

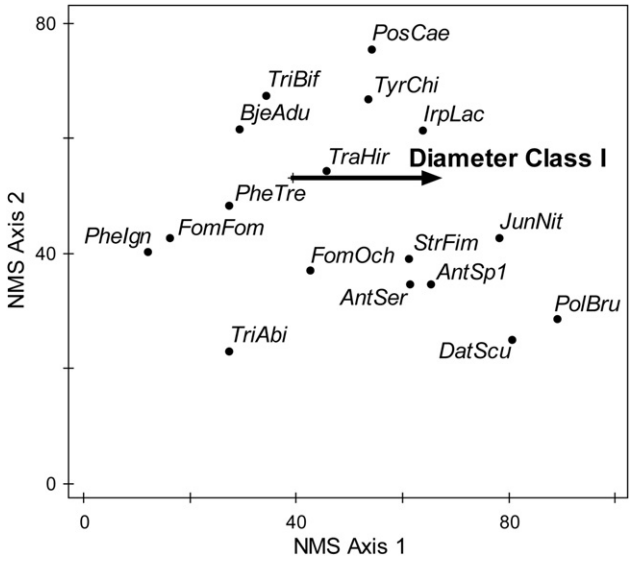

Fig 1 – Non-metric multidimensional scaling (NMS) summarizing the variation explained for those polyporoid fungal species significantly correlated to the axes. Polyporoid species not significantly related to the axes are not shown. Polyporoid species abbreviations are listed in Table 1. The length of the arrow reflects the importance of the explanatory variable. The first and second axes summarized 30 and 22% of the variation explained, respectively.

Species accumulation curves of polyporoid species occurrence by each of the deadwood characteristics are presented in Fig 2. The SAC using substratum species illustrated that presence of non-aspen hosts are important for polyporoid species richness, especially *Abies balsamea*, *Acer* sp., and *Betula alleghaniensis* (Fig 2; the SAC using all substratum species is not shown). SACs using additional deadwood variables confirmed that small diameter substrata were important for polyporoid species richness compared to larger diameter classes, and well-decayed substrata supported a higher diversity of polyporoid species compared to less decayed wood (Fig 2).

Similarity of polyporoid communities

The Sørensen similarity index showed that the composition of polyporoid species varied for each deadwood variable (diameter class, decay class, substratum type, and substratum species), but differences by substratum species were most pronounced (Table S2). The mean similarity (percentage of shared polyporoid species using presence/absence) by substratum species was only 28 %, and the substratum species with the highest similarity to *P. tremuloides* was *B. papyrifera*, at 37 % (Table S2). Diameter classes I (1 to <5 cm) and II (6 to <10 cm) supported nearly identical numbers of polyporoid species, 53 and 51 respectively, but were only 70 % similar to one another (Table S2).

Table 2 – Significant Kendall's tau-b correlation coefficients from the first and second NMS axes and quantitative variables (polyporoid species and deadwood characteristics)

Variables	NMS 1[a]	NMS 2
Polyporoid species		
Antrodia serialis	0.329**	ns
Antrodiella sp. 1	0.264**	ns
Bjerkandera adusta	−0.255**	ns
Datronia scutellata	0.269**	ns
Fomes fomentarius	ns	−0.400**
Fomitopsis ochracea	0.220*	ns
Irpex lacteus	ns	0.431**
Junghuhnia nitida	0.310**	0.324**
Phellinus igniarius	ns	−0.334**
Phellinus tremulae	ns	−0.255**
Polyporus brumalis	0.317**	ns
Postia caesia	ns	0.252*
Stromatoscypha fimbriata	0.249*	ns
Trametes hirsuta	ns	0.211*
Trichaptum abietinum	ns	−0.271**
Trichaptum biforme	−0.424**	0.255**
Tyromyces chioneus	ns	0.284**
Deadwood Variables		
Diameter class I (1to <5 cm)	0.242**	0.427**
Diameter class II (5 to <10 cm)	ns	0.295**
Diameter class V (20 to <25 cm)	ns	−0.217*
Diameter class VI (>25 cm)	ns	−0.285**
Decay class II	ns	0.200**
Decay class III	0.217*	ns
Substratum type (branch)	ns	0.411**
Substratum type (log)	ns	0.200*
Substratum type (tree)	ns	−0.212**
Host (*Abies balsamea*)	ns	−0.315**
Host (*Acer spicatum*)	ns	0.279**
Host (*Betula papyrifera*)	ns	−0.316**
Host (*Populus tremuloides*)	ns	0.302**
Host (Unknown hardwood)	ns	0.327**

a Significance was determined using a sequential Bonferroni correction at $P = 0.05$ and are coded as: $^{*}P < 0.01$, $^{**}P < 0.001$, ns = non-significant.

Discussion

Our principal objective in this study was to characterize the species composition and richness of polyporoid fungi in mature, aspen-dominated forests typical of the U.S. Lake States. While polyporoid fungi have been used extensively as ecosystem indicators in Fennoscandia (Bader *et al.* 1995; Berglund *et al.* 2005; Junninen *et al.* 2007; Halme *et al.* 2009; Stokland & Larsson 2011), researchers in North America have yet to utilize this diverse group to assess the impacts of forest management (e.g., biomass harvesting) on biodiversity. Thus, our results serve as an important baseline data set, and when used to further investigate the effects of biomass harvesting on polyporoid fungi, will help to facilitate the application of these organisms as ecosystem indicators in North America.

Our results show that aspen-dominated forests in the U.S. Lake States region support a rich assemblage of polyporoid species. Despite the dominance by a single tree species, the limited presence of additional sub-dominant tree species and maximum tree ages less than 70 yr, this forest type supported 86 polyporoid species, with 62 species present on aspen alone. However, the polyporoid community was dominated by several very abundant species: eight species accounted for 67 % of all observations. In comparison, Junninen *et al.* (2007) identified 46 polyporoid species, from 499 total observations, in aspen-dominated forests in eastern Finland with 11 present on that country's red list of threatened species. Recently, Lõhmus (2011) documented 36 species of polyporoid fungi, from 518 total observations, in Estonian aspen forests with two species included on that country's red list.

Currently, no red list exists for North American fungi, and studies documenting the community structure of polyporoid fungi are nearly nonexistent. However, our informal red list based on the results of the present study and a previous study in nearby Wisconsin and Michigan (Lindner *et al.* 2006), shows that four polyporoid species encountered in Minnesota aspen forests (*Funalia trogii*, *Pycnoporellus fulgens*, *Rigidoporus crocatus*, and *Skeletocutis chrysella*) warrant consideration as rare and/or threatened in the Lake States region. Two of these three species were found at multiple sites, suggesting that while rare, they are potentially widespread across the aspen forest type in the Lake States.

As depicted in the NMS ordination and species accumulation curves, small diameter deadwood was the variable most strongly correlated with the patterns in polyporoid community composition. Overall, 48 polyporoid species were found on branches, and while no species relied solely on this substratum, species that utilize branches could be adversely affected by biomass harvesting, which removes the small diameter woody debris that would otherwise be abundant in natural forests. Small diameter substrata (branches and twigs 1 to <5 cm in diameter) supported 58 of 133 (44 %) polyporoid species identified from several forest types in central Finland (Juutilainen *et al.* 2011). The authors concluded such substrata could be vital for the survival of certain fungi in intensively managed systems where larger substrata are not available. Also, Heilmann-Clausen & Christensen (2004) found that small trees and branches in mature beech forests in Denmark supported a higher number of polyporoid species per unit volume compared to large diameter substrata. Recent studies have also shown that small diameter woody substrata are important in maintaining overall species diversity for saproxylic beetle communities (Brin *et al.* 2011). These results indicate that management guides in aspen-dominated forests of the U.S. Lake States should seek to maintain or promote small diameter woody substrata to help preserve biodiversity.

Across all sites, there were very few sampled substrata classified as well-decayed (0.5 % of all substrata grouped into decay classes IV and V) or large diameter (1.6 % of all sampled substrata had a diameter ≥40 cm, and 0.6 % of all CWD surveyed independent of fungal occurrence had a diameter ≥40 cm). Previous research has shown that certain polyporoid species require large diameter, well-decayed CWD. In northern Sweden, Kruys *et al.* (1999) found that eight species of red-listed fungi showed a significant preference for large diameter, well-decayed CWD. In North America, Lindner *et al.* (2006) found a higher mean species richness on larger diameter wood in northern hardwood forests. However, because

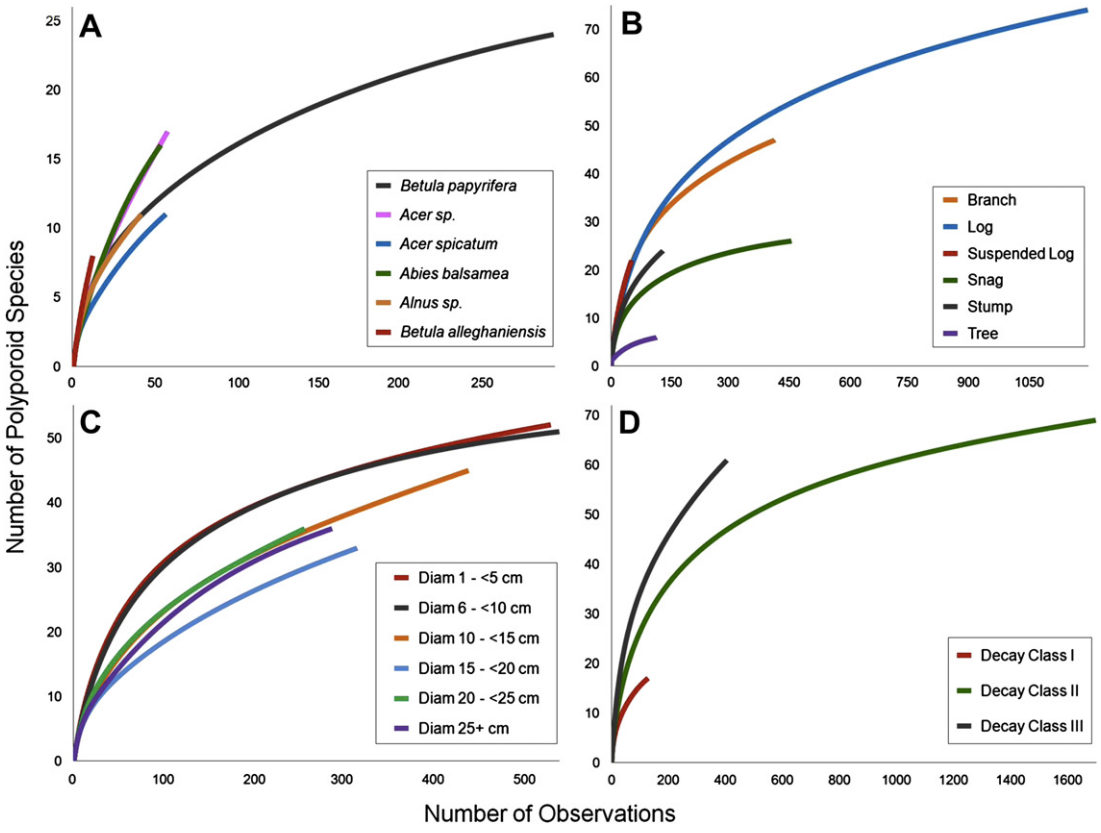

Fig 2 – Species accumulation curves of polyporoid species abundance by each deadwood variable: (A) substratum species (for substratum species with >20 observations, and excluding *Populus tremuloides* and "unknown hardwood"); (B) substratum type; (C) diameter class; and (D) decay class.

aspen is a short-lived tree species that is highly susceptible to decay (Burns & Honkala 1990), polyporoid species that prefer aspen may not exclusively rely on large diameter substrata since relatively few would have existed in pre-settlement forests (Frelich 2003). Targeted sampling of large diameter, well-decayed aspen logs is needed in the future to determine whether any polyporoid species rely on these rare substratum classes.

Substratum species also proved to be an important factor contributing to polyporoid species diversity. The two most common substrata in the present study, *P. tremuloides* and *B. papyrifera*, shared only 40 % of the polyporoid fungi that occurred on each. In addition, several uncommon polyporoid species were collected only from non-aspen substrata (see Table 1). Heilmann-Clausen *et al.* (2005) also determined that substratum species diversity was important for polyporoid species diversity in mixed hardwood forests in southern Denmark. Patterns in polyporoid communities revealed by the NMS ordination also suggest that additional site and/or substratum variables not addressed in the present study influence the composition and structure of polyporoid communities in aspen forests. Approximately 30 % of the total variance remained unexplained, and the observed tau-b coefficients failed to exceed ±0.50. Additional site variables influencing polyporoid species composition may be related to site quality.

The typical rotation for managed aspen forests in the Lake States is relatively short (40–60 yr), creating a challenge for forest managers to maintain both large-diameter trees and well-decayed CWD while still achieving management objectives aimed at maximizing economic returns or product yields. Post-settlement high-grading, wildfires and short-rotation management in natural forests containing aspen in north-central North America (see Whitney 1987) may have already extirpated those uncommon or sensitive polyporoid species that require large diameter aspens. Research in managed forests of Fennoscandia has shown that intensive harvesting can reduce polyporoid species abundance and diversity (Sippola *et al.* 2001; Stokland & Larsson 2011; Toivanen *et al.* 2012).

Conclusions

The primary conclusions can be summarized as follows: (1) the aspen forest type in Minnesota supports a diverse assemblage of polyporoid species, including potentially rare and/or threatened species; (2) small diameter substrata, especially those <5 cm, were the most significant driver of polyporoid species composition, and management plans

aimed at maintaining or increasing polyporoid species richness should seek to promote or retain these substrata; and (3) substratum species diversity was also an important contributor to polyporoid species richness, and future management efforts should focus on retaining or promoting advanced regeneration of non-aspen species to increase levels of substratum diversity. Given these findings, management of these ecosystems for biomass procurement should include provisions for the retention of a diversity of fine and coarse woody debris substratum sizes and species to ensure the maintenance of these critical components of biodiversity within managed landscapes.

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Supplementary material

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.funeco.2012.03.002.

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