



An assemblage of macrofungi associated with a boreal forest community in northern Manitoba, Canada

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General Note

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ABSTRACT

In an initial effort to characterize the assemblage of macrofungi associated with a boreal forest community in the Leaf Rapid Region (N 56° 28' 0" N; W 99° 44' 59" W, elevation 320 m) of northern Manitoba, Canada, specimens were collected during the period of mid-June to early August 2018. The forest community from which the specimens were collected is dominated by conifers, with an admixture of broadleaf trees. Prior to the present study, our null hypothesis was that there was no significant difference between macrofungi species' frequency in boreal forests. A total of 47 specimens of macrofungi were collected, but DNA suitable for sequencing could not be obtained from 14 of these. For the other specimens, only those specimens which yielded sequences that passed a quality score set by GeneWiz were blasted against the NCBI database with a blast option to identify the taxon involved, usually to the level of species. Ultimately, a total of 27 different taxa were identified. Ectomycorrhizal fungi and wood-decay fungi were the two dominant groups present. Jmp statistical software revealed that null hypothesis holds. That is, there was no statistically significant difference in macrofungi species' frequency in boreal forests of Manitoba Canada. Prob >F = 0.0833.

Key words: Biodiversity, DNA sequences, Ecology, Basidiomycota, Boreal forest

1. INTRODUCTION

Diverse assemblages of macrofungi are associated with forest communities throughout the world. The macrofungi making up these assemblages have a number of important ecological roles. These include those macrofungi which establish mycorrhizal relationships with trees as well as other macrofungi responsible for the decomposition of coarse woody debris and other plant-derived organic material. Many of the studies that have been directed towards determining the composition of the assemblage associated with a particular forest community have been limited to certain taxonomic groups (e.g., Huhtinen, S. 1993, Högberg, N., and J. Stenlid 1999, Rolstad et al. 2004) or is contained in field guides (e.g., Bossenmaier 1997, Laursen and Seppelt 2009). Relatively comprehensive studies (e.g., Salo, K. 1993) of the assemblage of fungi present at a particular locality are much less common.

The overall objective of the study reported herein was to characterize the macrofungi associated with a boreal forest community in the Leaf Rapid Region (N 56° 28' 0" N; W 99° 44' 59" W, elevation 320 m) of northern Manitoba, Canada (Fig. 1A). The forest community from which specimens were collected is dominated by conifers, with an admixture of a few broadleaf trees. The primary species of conifers are balsam fir (*Abies balsamea* [L.] Mill), black spruce (*Picea mariana* [Mill.] B.S.P., white spruce (*Picea glauca* [Moench] Voss), Jack pine (*Pinus banksiana* Lamb.), tamarack (*Larix laricina* [Du Roi] K. Koch), and red pine (*Pinus resinosa* Aiton), whereas the two most common species of broadleaf trees are paper birch (*Betula papyrifera* Marsh.) and quaking aspen (*Populus tremuloides* Michx.). We hypothesized that there were no statistically significant differences between macrofungi species' frequency in boreal forests. All specimens of fungi were collected during the period of mid-June to early August 2018.

2. METHODS

Fruiting bodies were located in the general study area using an opportunistic search method as described by Cannon & Sutton (2004). When fruiting bodies were observed, they were photographed in the field and then removed from the substrate upon which they occurred with the aid of a knife or a small hatchet. The fruiting bodies were loosely wrapped in aluminum foil, placed in a compartmentalized plastic collection box and taken to the laboratory. A food dehydrator was used to dry the specimens at a temperature of 42-55°C. The dried specimens were placed in plastic bags, the bags were labelled with unique collection numbers and placed in the herbarium at the University of Arkansas.

Samples and morphological observations

Morphological aspects of the specimens collected in the field were determined with the use of a standard stereomicroscope. The initial species identification was based on phenotypic characteristics. The morphological features such as the color, size, and shape of the fruiting body, the presence or absence of such structures as a distinct cap or stipe, and the nature of the hymenium were useful. The use of such sources of information as Binion et al. (2008) and Elliott & Stephenson (2018) also helped in identifications.

DNA extraction, PCR and sequencing

DNA was extracted from one or more representative specimens for each different taxon tentatively identified on the basis of morphological features of the fruiting body. This extraction was carried out using a Wizard® genomic purification kit (Promega Corporation, Madison, Wisconsin). The amplification of genomic deoxyribonucleic acids (gDNA) was done using the fungal-specific primers ITS4 (5'-TCCTCCGCTTATTGATATGC-3') and ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') as reported by Toju et al. 2012. PCR amplifications were performed in a thermocycler programmed for an initial denaturation at 95°C for 5 minutes, followed by 35 cycles of denaturation at 95°C for 45 seconds, annealing at 50°C for 45 seconds, and extension at 72°C for 1.5 minutes. The final extension was performed for 10 minutes at a temperature of 72°C. The length of amplified products was confirmed by 1% agarose gel electrophoresis gel using 0.5 × TAE buffer, SYBR safe staining dye, and 1 kb DNA ladder (New England Biolabs, Ipswich, Massachusetts). The amplicons were sent for Sanger sequencing to GeneWiz (South Plainfield, New Jersey). The sequences obtained from the GeneWiz Company were formatted (cleaned up), edited using SeqMan-program version 7.1.0 (44.1) software and then manually corrected before alignment was done to obtain a consensus sequence. All sequences were then compared in-silico with the results of a nucleotide search, using the Basic Local Alignment Search Tool (BLAST) available at The National Center for Biotechnology Information (www.ncbi.nlm.nih.gov), for DNA-based identification. At 98% identity, sequences were considered identified to species, at less than 98% identity, sequences were considered identified only to genus. We followed Index Fungorum (www.indexfungorum) for taxonomic names.

3. RESULTS

A total of 47 specimens of macrofungi were collected. DNA suitable for sequencing could not be obtained from 14 of these. For the other specimens, only those specimens which yielded sequences that passed a quality score set by GeneWiz were blasted against the NCBI database with a blast option to identify the taxon involved, usually to the level of species. Ultimately, a total of 27 different species were identified (Table 1).

These 27 species belong to 18 different genera and 14 different families. The families represented by two or more taxa were the Cortinariaceae (6 species), Polyporaceae (4 species), Russulaceae (3 species), Fomitopsidaceae (3 species) and Hymenochaetaceae (2 species, albeit one identified only to genus). The nine other families were each represented by a single species. Twelve of the 27 species are ectomycorrhizal fungi, another 12 are wood-decay fungi, and only three are saprophytes typically associated with the litter layer on the forest floor.

Table 1. Taxa of macrofungi recorded during the study described herein. Note: %ID = percent sequence identity and SGB = sequence in GenBank.

Taxon	Family	% ID	SGB
<i>Agaricus nivescens</i> F.H. Møller	Agaricaceae	98	AY484670.1
<i>Collybia hariolorum</i> (Bull.) Quél.	Tricholomataceae	98	MH856329.1
<i>Cortinarius caesioarmeniacus</i> Kytöv	Cortinariaceae	99	KP137504.1
<i>Cortinarius caperatus</i> (Pers.) Fr.	Cortinariaceae	99	KP889889.1
<i>Cortinarius collinitus</i> (Sowerby) Gray	Cortinariaceae	99	AF325573.1
<i>Cortinarius distans</i> Peck	Cortinariaceae	98	MH979323.1
<i>Cortinarius infractiflavus</i> Kytöv., Niskanen	Cortinariaceae,	99	FJ039612.1
<i>Cortinarius muscigenus</i> Peck	Cortinariaceae	100	FJ717533.1
<i>Fomitopsis pinicola</i> (Sw.) P. Karst	Fomitopsidaceae	99	MH561766.1
<i>Gymnopus confluens</i> (Pers.) Antonín	Marasmiaceae	99	MF908467.1
<i>Hebeloma velutipes</i> Bruchet	Strophariaceae	99	MF954943.1
<i>Inocybe praetervisa</i> Quél.	Inocybaceae	99	HQ604492.1
<i>Limacella</i> sp. Earle	Amanitaceae	99	KY263616.1
<i>Phellinus</i> sp. Quél.	Hymenochaetaceae	92	KU668962.1
<i>Piptoporus betulinus</i> (Bull.) P. Karst	Fomitopsidaceae	99	KC585371.1
<i>Pleurotus abieticola</i> R.H. Petersen	Pleurotaceae,	99	AY450348.1
<i>Pluteus leucoborealis</i> E.F. Justo	Pluteaceae	99	KJ009739.1
<i>Polyporus varius</i> (Pers.) Fr.	Polyporaceae	99	MG748571.1
<i>Pseudochaete tabacina</i> (Sowerby) T	Hymenochaetaceae	99	JQ279611.1
<i>Rhodofomes cajanderi</i> (P. Karst.)	Fomitopsidaceae	99	MG735350.1
<i>Russula aurantioflammans</i> Ruots., Sarnari	Russulaceae	99	KU928167.1
<i>Russula delica</i> Fr.	Russulaceae	98	KX812842.1
<i>Russula versicolor</i> Jul. Schäff	Russulaceae	98	JX425372.1
<i>Schizopora</i> sp. Jülich	Schizoporaceae	100	MF161257.1
<i>Trichaptum bifforme</i> (Fr.) Ryvarden	Polyporaceae	99	MF161260.1
<i>Trichaptum fuscoviolaceum</i> (Ehrenb.)	Polyporaceae	99	AM269816.1
<i>Trichaptum laricinum</i> (P. Karst.)	Polyporaceae	99	U63471.1



Fig 1. Representative of the boreal habitat and specimens. **A–I:** A. Forest community where the macrofungi reported herein were collected. B. *Piptoporus betulius*. C. *Russula aurantiiflammas*. D. *Schizopora* sp. E. *Cortinarius collinitus*. F. *Polyporus varius*. G. *Collybia harioloum*. H. *Inocybe praetervisa*. I. *Russula versicolor*.

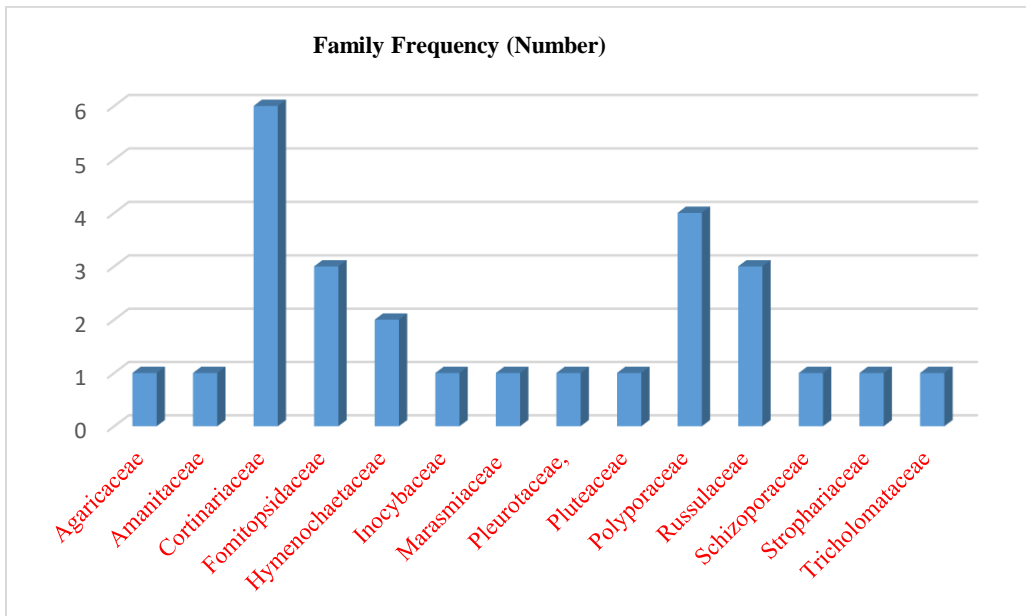


Fig. 2- Frequency of macrofungi families in boreal forest of northern Manitoba

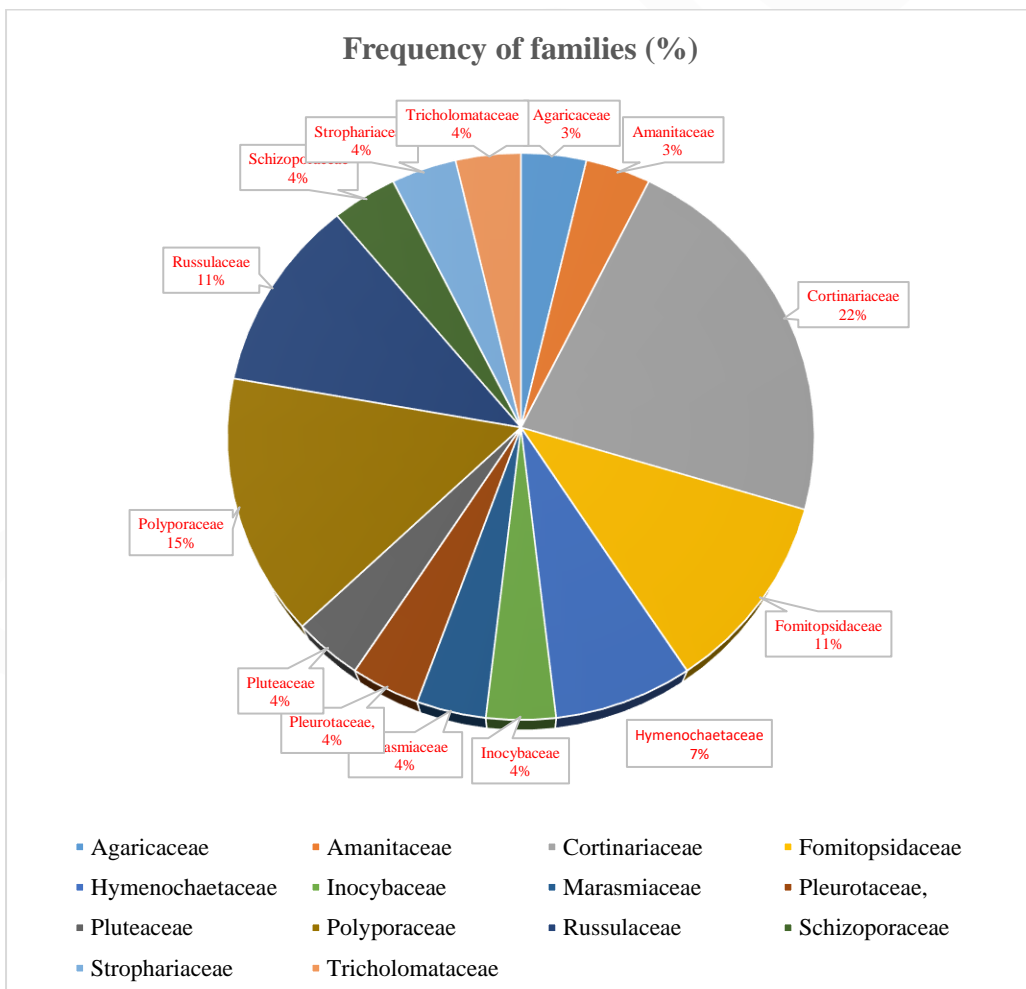


Fig. 3- The abundance of the various macrofungi families found in the study area.

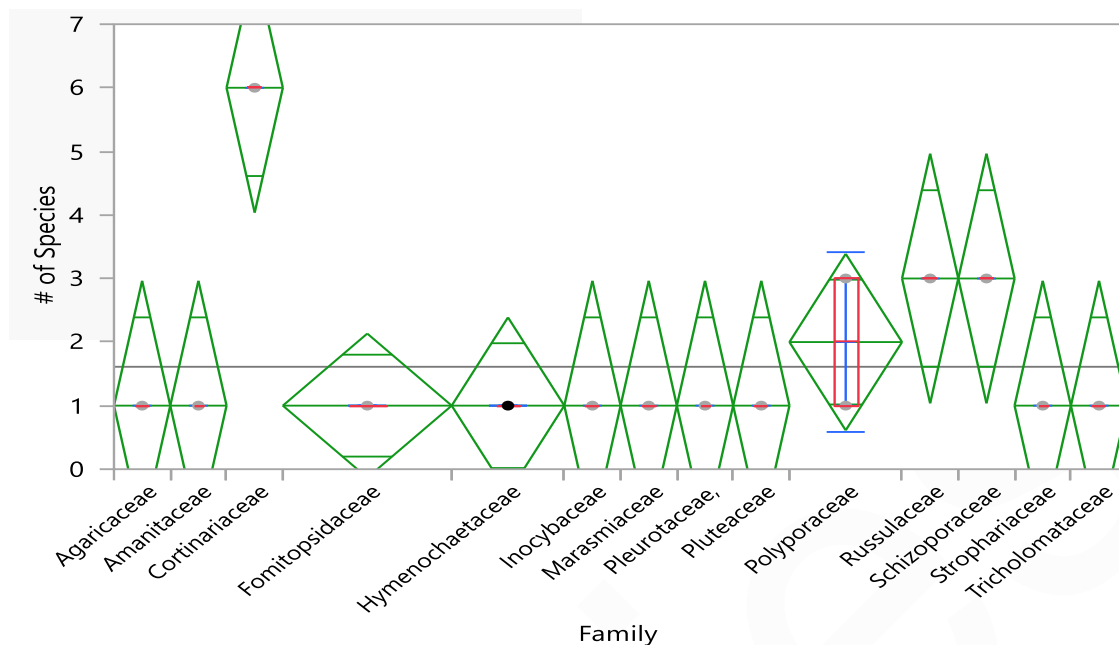


Fig. 4. Oneway Analysis of # of Species by Family

Oneway Anova Summary of Fit

R-square	0.933945
Adj R-square	0.719266
Observations (or Sum Wgts)	18

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Family	13	28.277778	2.17521	4.3504	0.0833
Error	4	2.000000	0.50000		
C. Total	17	30.277778			

Fig.5. Analysis of Variance

Using jmp statistical software, the analysis of variance (Oneway Anova) reveals that there is no statistical difference on species' frequency as seen on fig.5 (Prob.>F = 0.0833). The software also gives a R^2 -value of 0.93. This is a very strong indication that the relationships between dependent and independent variables are perfectly explained by the model (Fig.4).

4. DISCUSSION

Many of the wood-decay fungi such as *Fomitopsis pinicola* (Sw.) P.Karst and *Phellinus hetulinus* (Bull.: Fr.) P.Karst are well known heart-rot taxa whose ecological and economic impact on forest ecosystems is considerable (Figs 1.). As a general observation, the assemblage of macrofungi present at the locality investigated in the present study would seem to be what might be expected for a boreal forest, where the coniferous trees present provide an abundant supply of coarse woody debris to serve as substrates for wood-decay fungi. Moreover, since the majority of trees at this locality are species known to form ectomycorrhizal associations, their fungal associates would be expected to occur. Because the study reported herein was carried out over a relatively brief period of time, the data obtained represent what might be considered as a "snapshot" of the total taxonomic assemblage of macrofungi of macrofungi present in the forest of northern Manitoba. However, these data do represent a starting point for additional future studies. The frequency data gives the picture of species abundance in this ecosystem.

5. CONCLUSION

The results of this study indicated that boreal forest of norther Manitoba has diverse macrofungi families and there is no statistical significant differences between macrofungi species' frequency. Therefore, the null hypothesis of not having significant differences between macrofungi species' frequency in boreal forests is accepted.

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Conflict of interest

The author has no conflict of interest to declare that are relevant to the content of this article.

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Data and materials availability:

All data associated with this study are present in the paper.

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