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Mycological diversity on Jack Pine and Black Spruce bark by Payuk Lake, Manitoba

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Abstract

Fungi provide an essential role to the ecosystem they inhabit by decomposing dead organic material; however, they have been studied little in Northern Manitoba. This is seen in Payuk Lake; located within a chain of lakes that make up the headwaters of the Grass River. This region is currently under consideration for conservation. The objectives of this study were to investigate the mycological diversity of Jack Pine (*Pinus banksiana*) and Black Spruce (*Picea mariana*) at Payuk Lake, MB, and to examine the differences in mycological diversity between heights of tree trunks and between Jack Pine (*Pinus banksiana*) and Black Spruce *(Picea mariana*). Bark samples were collected from three heights on Jack Pine and Black Spruce trees along the ridges of Payuk Lake. The bark samples were dried and placed in moist chambers for three weeks and their characteristics were described via the use of the dissecting and compound light microscopes. A total of 18 organisms were cultured from the bark samples, nine of which were classified as myxomycetes and nine as zygomycetes. Two-way cluster analysis showed that the most common organisms according to abundance were zygomycetes, and the uncommon were myxomycetes. Moreover, cluster analysis showed distinct clustering between tree species and heights. Shannon's diversity index was not significant between Jack Pine and Black Spruce, but trends were evident. The diversity of fungi, and other organisms, could be large and should be further investigated to better understand the importance of the Payuk Lake region, which would help conserve the headwaters of the Grass River.

Keywords: Conservation, fungal diversity, Grass River, Payuk Lake, myxomycetes

Introduction

Fungi are heterotrophic organisms that have a critical role in all ecosystems. Fungi decompose organic debris to be used in the nutrient cycles of the ecosystem and are important food sources for many animals, including humans (Kendrick, 2000). The "macroscopic" groups of fungi, the Basidiomycota (club fungi) and Ascomycota (sac fungi) are most well-known; however, the "microscopic" groups of fungi are equally important (Kendrick, 2000). Zygomycetes, the bread molds, colonise substrates rich in carbon and are important decomposers of rich organic materials (Kendrick, 2000). Zygomycetes also form symbiotic relationships with the roots of economically important trees, such as pine and spruce, and are found worldwide. Myxomycetes, or the slime molds, are found on moist substrata, such as wood and litter (Schnittler et al. 2006), which is filled with bacteria. They can be found in all types of habitats including alpine regions (Roniker & Roniker, 2009), dry deserts (Wrigley de Basanta et al., 2008), various forest types, and aquatic ecosystems (Lindley et al., 2007).

Both groups of microscopic fungi have been known to grow on trees (Kendrick, 2000). Bark provides a good microhabitat for many fungi. Bark characteristics, such as texture and pH, vary between tree species. Different bark textures allow for the retention of water and other nutrients that may be essential for the survival of many fungi. In addition to, bark pH and moisture influences the distribution of fungi between tree species as seen along different heights of tree trunks (Everhart et al., 2008), since different species exhibit different tolerances to pH and water content. While these organisms have been greatly studied, the diversity of Zygomycetes and Myxomycetes of Northern Manitoba has been studied little (Bisby et al., 1938), and little is currently known about the bark inhabiting fungi of this region.

Payuk Lake is a post-glacial lake nestled within the boreal forest of Northern Manitoba. It is a part of a chain of lakes in the Mystik Creek system, and is one of the many important water bodies in the headwaters of the Grass River (T. Booth, personal communication, 23 April 2012). Payuk Lake is the last lake in the chain, before the start of the Grass River. The Grass River is protected within the Grass River Provincial Park, and is an important river system ecologically. The Grass River Provincial Park protects the river system, as well as preserves the geological transition between the Churchill River Uplands and the Manitoba Lowlands within the boreal forest (Manitoba Conservation, 2012). The park also serves to protect the natural habitat of the endangered woodland caribou. The park prides itself for the pristine, clear waters of the Grass River which is protected by the Clean Environment Commission (Manitoba Conservation, 2012). The Mystik Creek system, however, has been decreasing in water quality over the years (T. Booth, personal communication, 23 April 2012), due to mining and misuse of the water through recreation. The continued pollution of Mystik Creek and its interconnected lakes could eventually harm the Grass River region, affecting not just the caribou, but plants and other animals as well. Locals around the Flin Flon area are currently trying to extend the conservation boundaries of the Grass River Provincial Park to include the Mystik Creek system (T. Booth, personal communication, 23 April 2012). Investigation of the plants, fungi, lichens, and animals within this region will be used to justify the extension of the park boundaries.

The objectives of this study were 1) to observe the diversity of Zygomycetes and Myxomycetes on Jack Pine (*Pinus banksiana*) and Black Spruce (*Picea mariana*) at Payuk Lake, MB, 2) to examine the difference in mycological diversity between Jack Pine and Black Spruce , and 3) to examine the change in diversity with respect to height within Jack Pine and Black Spruce populations.

Results

There were 18 cultured organisms present after the three week culturing period (Table 1). Six organisms had a plasmodium and the rest had hyphae present. Nine of the organisms had fruiting structures and spores, while the rest were sterile (Table 1).

Shannon’s Diversity Index was not significantly different between Jack Pine and Black Spruce (Figure 2 A), or within either Jack Pine or Black Spruce. However there were opposite trends within the two tree species. Within Jack Pine, Shannon’s Diversity Index showed a decrease in diversity from the bottom height to the top height while the opposite was true within Black Spruce (Figure 2 B-C).

Two-way cluster analysis between organisms present and bark samples of Jack Pine and Black Spruce revealed two distinct clusters within the organisms and four distinct clusters between bark samples (Figure 3). The organisms clustered by commonness, with organisms 1, 5, 7, and 13 being most common (Figure 3 cluster B), and the rest being uncommon (Figure 3 cluster A). All myxomycetes present except organism 7 clustered within the uncommon group, with organism 7 clustering in the common group.

Cluster I of the bark samples contained only Black Spruce samples, with all heights present, and Cluster II contained only Jack Pine samples, with all heights present (Figure 3). Cluster III had two distinct sub-clusters, one containing only Black Spruce top samples (Figure 3 cluster III.1), and one containing only Jack Pine samples with all heights present (Figure 3 cluster III.2). Sub-cluster IV.1 had only Black Spruce bottom samples and sub-cluster IV.2 clustered a mix of both Jack Pine and Black Spruce, with all heights present (Figure 3).

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Table 1. Morphological characteristics of cultured organisms found on Jack Pine and Black Spruce bark from Payuk Lake, Manitoba.

Organism	Overall Colour	Main Strcture	Main Structure Colour	Septation	Branching	Main Structure Tips	Cell Walls	Fertility	Spore Size (units)	Spore Shape	Spore Colour	Number of Cells per Spore	Spore Texture	Spore Walls
1	White	Hyphae	Hyaline	Septate	Yes	Blunt	Single	Sterile or Immature	N/A	N/A	N/A	N/A	N/A	N/A
2	Orange	Plasmodium	Hyaline to Orange	Aseptate	No	Blunt	Single	Fertile	5	Circular	Clear	1	Smooth	Single
3	Bright Red	Plasmodium	Red	Aseptate	No	Blunt	Single	Fertile	6	Circular	Clear	1	Smooth	Single
4	Yellow	Plasmodium	Yellow	Aseptate	No	Blunt	Single	Sterile or Immature	N/A	N/A	N/A	N/A	N/A	N/A
5	Green and White	Hyphae	Light Green	Septate	No	Blunt	Single	Fertile	2	Circular	Clear	1	Smooth	Single
6	White	Hyphae	Hyaline	Septate	Yes	Blunt	Single	Sterile or Immature	N/A	N/A	N/A	N/A	N/A	N/A
7	Black	Plasmodium	Hyaline	Septate	No	Blunt	Single	Fertile	5	Circular	Brown	1	Ridged	Single
8	White	Hyphae	Hyaline	Septate	Yes	Blunt	Single	Sterile or Immature	N/A	N/A	N/A	N/A	N/A	N/A
9	White	Hyphae	Hyaline	Septate	Yes	Blunt	Single	Sterile or Immature	N/A	N/A	N/A	N/A	N/A	N/A
10	White	Hyphae	Hyaline	Septate	Yes	Tapering	Single	Sterile or Immature	N/A	N/A	N/A	N/A	N/A	N/A
11	White	Hyphae	Hyaline	Septate	Yes	Blunt	Single	Sterile or Immature	N/A	N/A	N/A	N/A	N/A	N/A
12	Light Pink-Orange	Hyphae	Hyaline	Septate	Yes	Blunt	Single	Sterile or Immature	N/A	N/A	N/A	N/A	N/A	N/A
13	Grey-Clear	Hyphae	Light Brown	Septate	Yes	Tapering	Single	Sterile or Immature	N/A	N/A	N/A	N/A	N/A	N/A
14	Light Green	Hyphae	Hyaline	Septate	Yes	Blunt	Single	Sterile or Immature	N/A	N/A	N/A	N/A	N/A	N/A
15	White	Hyphae	Hyaline	Septate	Yes	Blunt	Single	Fertile	1	Circular	Clear	1	Smooth	Single
16	White and Brown	Hyphae	Hyaline to Light Brown	Septate	No	Blunt	Single	Fertile	6 long, 2 wide	Oval/ Ellipsoid	Clear	1 to 2	Smooth	Single
17	Brown	Plasmodium	Brown	Aseptate	No	Blunt	Single	Fertile	6	Circular	Light Brown	1	Rough	Single
18	Yellow-Grey	Plasmodium	Yellow-Grey	Aseptate	No	Blunt	Single	Fertile	5	Circular	Clear	1	Smooth	Single

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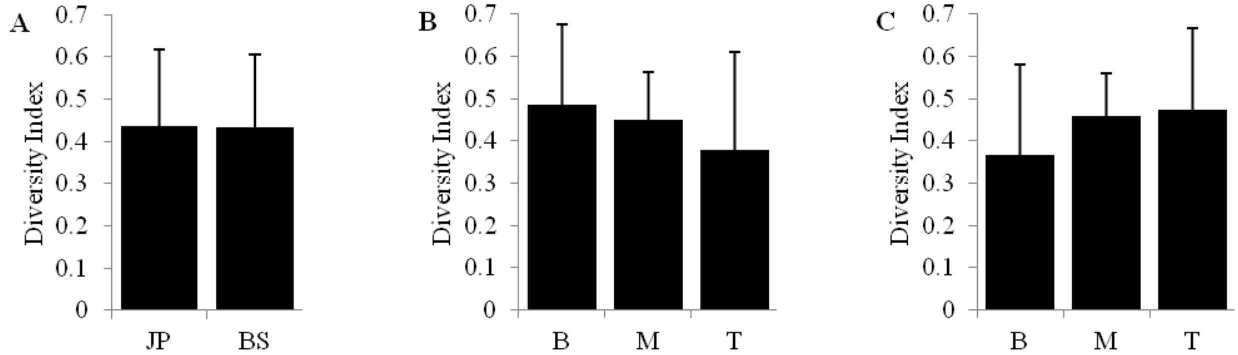

Figure 1. A comparison of Shannon's Diversity Index on Jack Pine (JP) and Black Spruce (BS), showing standard deviations. A: Differences between JP and BS, n = 18. B: Differences in height above ground (B = 0 m, M = 0.65 m, T = 1.3 m) of JP, n = 6. C: Differences in height above ground (B = 0 m, M = 0.65 m, T = 1.3 m) of BS, n = 6 (A).

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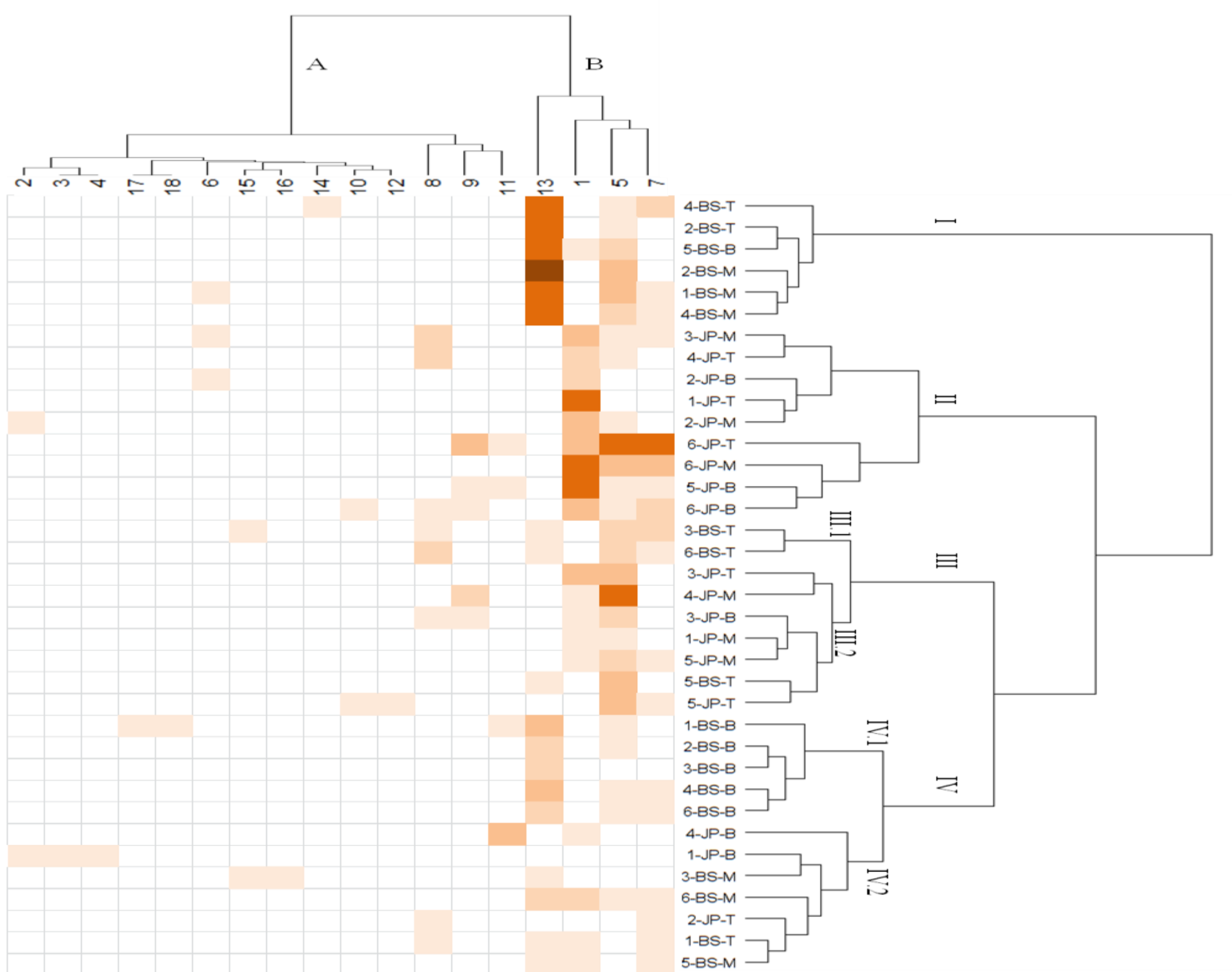

Figure 2. Two-way clustering of cultured organisms (1-18) and bark samples (transect #, tree species, height) of Jack Pine (JP) and Black Spruce (BS) from three heights (B = 0 m, M = 0.65 m, T = 1.3 m above ground) (A).

Discussion and Conclusion

Historically, little has been known about the mycological diversity of northern Manitoba (Bisby et al., 1938), and little is currently known about the bark inhabiting fungi of this region. Organisms 2, 3, 4, 7, 17, and 18 had plasmodia and were considered to be myxomycetes (Table 1). Organism 16 may have been an ascomycete due to the presence of well-developed asci and cup-like appearance. The other organisms were either zygomycetes or ascomycete anamorphs, though due their immature or sterile form cannot be completely determined (Table 1).

The relationship with tree species and height indicating the presence and absence of the organisms is quite interesting. The most common organisms present were either zygomycetes or anamorphs (Figure 3 cluster B). Organism 13 was found only on Black Spruce, with the highest frequencies in the top and middle heights (Figure 3 cluster I) and some occurrences in the bottom height (Figure 3 cluster IV.1), which could be a result of higher pH in Black Spruce bark compared to Jack Pine (Dix and Webster, 1995). Organism 1 was found almost exclusively within the lower height (Figure 3 cluster II, IV.2). Dix and Webster (1995) suggest that fungal species tend to colonise substrata with either a low or high pH. Organisms 5 and 7 were found to occur in both Jack Pine and Black Spruce samples, with no preference to height (Figure 3 cluster I, II, III), which could suggest these organisms tolerated a range of pH conditions or other environmental conditions.

The effect of pH on growth occurs alongside multiple factors and cannot be the sole cause of fungal substrate preference (Dix and Webster, 1995). Another factor that could have resulted in organism preference of one tree species is moisture content, as determined by the texture of the bark (Dix and Webster, 1995). Black Spruce bark had more scaly bark than Jack Pine, which had very flaky, layered bark. The different texture of the barks could result in varying moisture content as water is trapped within the layers. Rougher, scalier bark would be able to hold more moisture than smoother, more appressed bark (Dix and Webster, 1995). Since organism 13 only occurred on Black Spruce bark, it could be suggested that it has a higher tolerance for lower moisture content than the other common organisms and therefore could grow on this particular bark. It can be further suggested that organism 1 has a higher tolerance to higher moisture content than the other organisms and is able to grow on Jack Pine bark. Organisms 5 and 7 could have a moderate tolerance for either moisture content and could grow on either Jack Pine or Black Spruce bark.

The exact clustering of the bark samples was also caused by the presence of uncommon organisms. Within cluster A, the uncommon organisms showed two clustering patterns. The uncommon anamorphs and zygomycetes (Figure 3 cluster A) showed no distinct preference for either tree species or height category, except for organism 6 which only occurred in the middle heights of both Jack Pine and Black Spruce. This preference may be a result of a balance of many factors such as pH, moisture, and other factors. The myxomycetes (organisms 2, 3, 4, 17, and 18) clustered only in bottom samples, with organisms 2, 3, and 4 occurring on Jack Pine and organisms 17 and 18 present on Black Spruce. The preference to the bottom height may be due to different pH and moisture content as compared to the other heights in both Jack Pine and Black Spruce. Also, myxomycetes feed on bacteria, which are abundant in soil (Kendrick, 2000). Being closer to the ground would ensure a plentiful food supply to survive. The myxomycete, organism 7, occurred on all the heights in both tree species, suggesting that this organism may have a different feeding strategy or is more adapted to varying environmental conditions.

Shannon's diversity index showed opposite height trends in Jack Pine and Black Spruce. Diversity was higher on the bottom of Jack Pine than the top, and lower on the bottom of Black Spruce than the top (Figure 2). This trend is confirmed by the cluster analysis (Figure 3) where all the Black Spruce bottoms formed a cluster characterised by low organism frequencies (Figure 3 cluster IV.I). Although, Jack Pine did not form as distinct a cluster as the Black Spruce, the Jack Pine bottoms generally displayed higher organism frequencies than the other Jack Pine heights in all clusters (Figure 3). Jack Pine bottoms may have had higher frequencies of organisms due to a more suitable environment, higher nutrient levels or interactions of environmental factors. Diversity differences between heights on the Black Spruce may be due to differences in bark texture. At the bottom, the bark was less flaky and more tightly compressed. While, higher up the bark, it was found to be flakier, allowing for water and nutrients to be trapped. This is similar to the flaky bark found on all heights of the Jack Pine trees.

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In summation, the mycological diversity on Jack Pine and Black Spruce at Payuk Lake Manitoba has been investigated and 18 morphological organisms have been described, though not identified. There appears to be some preference between Jack Pine and Black Spruce bark, as well as with height above ground within Jack Pine and Black Spruce. Further investigation of the mycological diversity should be undertaken with other tree species, such as White Spruce, Tamarack, and Aspen, and among the lakes within the Mystic Creek/Grass River headwaters.Also, molecular identification and phylogenetic characterisation of the fungi at Payuk Lake would complement the research into the diversity and provide a complete collection of the fungi in this area. Finally, continued research and understanding of the diversity within this area could help to extend the conservation area of the Grass River Provincial Park. By understanding the diversity in this area, we can infer and further investigate the environmental changes in this ecosystem and how it may be affected by the tourism and recreational development, which can be used to protect the headwaters of this important river.

Materials and Methods

Site Description

Payuk Lake is a lake in Northern Manitoba nestled within the Canadian Shield. The study sites around Payuk Lake were characterised by boreal forest dominated by Jack Pine (*Pinus banksiana*), Black Spruce (*Picea mariana*), and White Spruce (*Picea glauca*). Other trees present included White Birch (*Betula papyrifera*), Balsam Fir (*Abies balsamea*) and Trembling Aspen (*Populus tremuloides*). The dominant shrubs present were Alder (*Alnus* spp.) and Willow (*Salix* spp.). In addition to this, common herbs included Common Juniper (*Juniperus communis*), Blueberry (*Vaccinium myrtilloides*), Bearberry (*Arctostaphylos uva-ursi*), Red Osier Dogwood (*Cornus sericea*), and Twinflower (*Linnaea borealis*). Grass and moss species were also present. Finally, common ground-dwelling lichens present were *Cladonia* spp., *Cladonia stellaris*, *Cladonia rangiferina*, *Cladonia arbuscula*, *Stereocaulon* spp., *Xanthoparmelia* spp., and *Umbilicaria* species.

GIS

Initial study sites were selected in the open source software Quantum GIS v. 1.7.0 Wroclaw (Quantum GIS Development Team, 2012), using forest inventory maps (Manitoba Land Initiative 2004). Transect locations were randomly selected in appropriate forest types (Jack Pine (JP) ≥ 10%, Black Spruce (BS) ≥ 10%) along the shores of Payuk Lake and the coordinates were imported onto the GPS (Garmin GPSmap 76C x, datum WGS 84). Twelve sites were randomly selected, though only 6 suitable sites visited were to be sampled. This allowed for some error in the accuracy of the 8-year-old forest inventory.

Field Methods

Study sites were reached by canoe and the GIS forest compositions confirmed. The GPS coordinates were recorded (Figure 1). The overall site vegetation was recorded. A 50 m transect was laid down following the ridge to minimise the change in slope. The closest Jack Pine and Black Spruce to the transect were sampled every 10 m. Each tree was sampled at three heights (B = 0 m, M = 0.65 m, T = 1.3m) on the north side of the tree. Bark samples at each height were collected by scraping off the outer bark with a pocket knife and placed into paper bags for transport back to the lab. A 10 cm x 10 cm area of bark was collected for each sample.

Culturing

Bark samples were air dried in the lab for two days (Lindley et al., 2007). Deep dish petri plates were labelled and filled with 10 tsp of fine-grain vermiculite. The vermiculite was soaked overnight with sterile distilled water. The bark samples were placed in their respective dishes to minimise overlapping and to cover the surface of the vermiculite (Lindley et al., 2007). The petri dishes were covered to act as moist chambers, and placed in a temperature controlled room at 15°C with the lights remaining on. The samples were cultured for three weeks and observed at the end of the three weeks under the dissecting microscope for mycological growth. If growth was present, the organism was described using the features observed under the dissecting scope and compound light microscope.

Data Analysis

Shannon's Diversity Index (Shannon, 1948), measure of α diversity including the evenness of the samples, was calculated by summing the numbers of organisms present for each height category together for each transect so that a single diversity index was calculated for each height of each tree species in each transect. Shannon's Diversity was compared between tree species and among heights with a Student's t-test and ANOVA in Minitab 14 Student Edition (Minitab 14 Statistical Software, 2010), with α = 0.05. Two way cluster analysis using Euclidean distances and Ward's method of clustering was performed between the organisms present and the bark samples using R (R Development Core Team, 2009).

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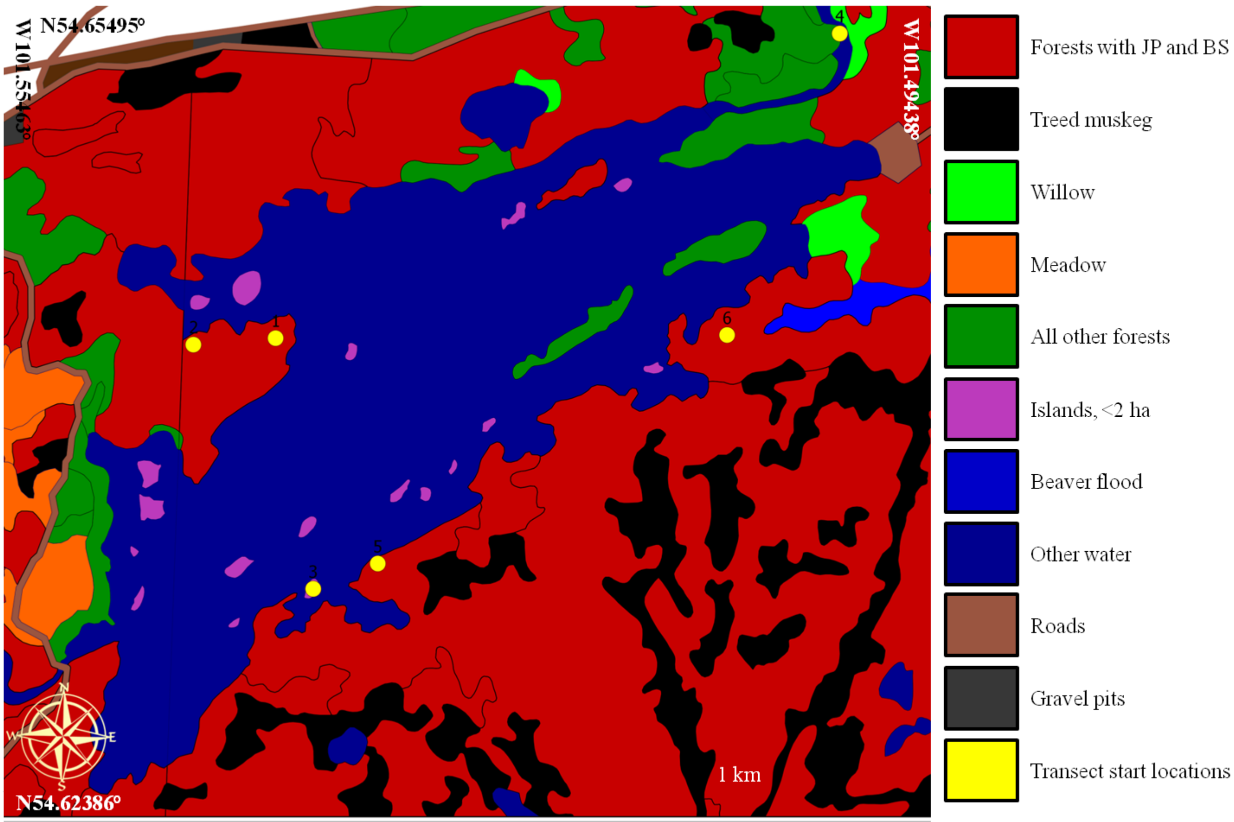

Figure 3. Forests around Payuk Lake, Manitoba, showing sampled transects and in forests with ≥ 10% Jack Pine (JP) and ≥ 10% Black Spruce (BS).

References

1. Bisby, G.R., Buller, A.H.R., Dearness, J., Fraser, W.P., Russel, R.C., and Gussow, H.T. (1938). *The fungi of Manitoba and Saskatchewan*. Ottawa, Ontario: National Research Council of Canada.
2. Dix, N.J., and Webster, J. (1995). *Fungal ecology*. New York, NY: Chapman & Hall
3. Everhart, S.E., Keller, H.W., and Ely, J.S. (2008). Influence of bark pH on the occurrence and distribution of tree canopy myxomycetes species. *Mycologia* 100, 191-204.
4. Kendrick, B. (2000). *The fifth kingdom.* (3rd ed.). Newburyport, MA: Focus Publishing, R. Pullins Company.
5. Lindley, L.A., Stephenson, S.L., and Spiegel, F.W. (2007). Protostelids and Myxomycetes isolated from aquatic habitats. *Mycologia* 99, 504-509.
6. Manitoba Conservation. (2012). Grass River Provincial Park. Retrieved 28 June 2012, from http://www.gov.mb.ca/conservation/parks/popular_parks/northwest/grass_info.html.
7. Manitoba Land Initiative. (2004). Forest inventory maps. Retrieved 28 June 2012, from http://mli2.gov.mb.ca.
8. Minitab 14 Statistical Software. (2010). [Computer software]. Minitab, Inc. URL: http://www.minitab.com.
9. Quantum GIS Development Team. (2012). Quantum GIS Geographic Information System [Computer software]. Open Source Geospatial Foundation Project. Retrieved 28 June 2012, from http://qgis.osgeo.org.