FUNGAL COMMUNITY ASSESSMENT IN CANADIAN ARCTIC SOILS FROM ALEXANDRA FIORD, ELLESMERÉ ISLAND, NUNAVUT

by

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ABSTRACT

Fungal communities in arctic soils tend to be less diverse compared to the communities in temperate forest soils due to the harsher environmental conditions. Even in a single arctic site such as Alexandra Fiord, considered a terrestrial arctic oasis, fungal diversity is expected to be lower compared to soils in less extreme environments. We hypothesized that variations in environmental factors would play an important role in determining fungal community structure, as the Alexandra Fiord soils exhibits considerable environmental variation in a small geographic area. To test this hypothesis, we collected soil samples from three sites across the landscape and performed length-heterogeneity polymerase chain reaction (LH-PCR) analyses using ITS3 and NLB4 primers, which have been used successfully to characterize complex communities. Our results showed that there were large relative differences in fungal community structure between the sites. At the Alexandra Fiord Highland Dolomitic site diversity was low with genotypes relatively evenly distributed, whereas Alexandra Fiord Highland Granitic and Alexandra Fiord Lowland sites had higher diversity and a less even distribution of genotypes with a few occurring at a high frequency and many rare species. Among environmental variables, soil moisture, temperature, DOC, DON, C:N ratio and soil pH were significant influential factors in determining fungal community structure. Among these environmental factors, pH showed the strongest correlation with the fungal community data.
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<td>Alexandra Fiord</td>
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<td>AFL</td>
<td>Alexandra Fiord Lowland</td>
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<td>AFH</td>
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<td>HDS</td>
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<td>Ribosomal Ribonucleic Acid</td>
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<tr>
<td>ITS</td>
<td>Internal Transcribed Spacer</td>
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<td>LSU</td>
<td>Large Subunit</td>
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<tr>
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<td>OTU</td>
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Chapter 1 Literature Review

1.1 Composition of fungal communities across an arctic tundra landscape

Fungal community plays an important role in the function and dynamics of terrestrial ecosystems by influencing the structure of bacterial, plant and animal communities through not only symbiotic and/or parasitic interactions, but also maintaining carbon and nutrient cycles (Callaghan et al. 2004). Because of fungi’s unique biology, and their importance in nature, understanding fungal community ecology straddles the macroscopic and microscopic worlds and provides opportunities for new and unanticipated research.

The fungal community tends to be less diverse in extreme environments. Lawley et al. (2004) proposed there would be a negative correlation between fungal diversity and latitude, where fungal diversity decreases as latitude increases. For instance, in La Gorce Mountains of Antarctica, which is located close to the South Pole, the diversity of the fungal community has significantly lower diversity than Mars Oasis, which is located further from the South Pole at a lower latitude. Several investigators have examined the distribution and occurrence of fungi in arctic ecosystems. In arctic tundra of North America, over 100 species of fungi have been found, 22 species of which belong to the genera Galerina, Phaeogalera and Leptoglossum, and over sixty species belonging to the Coprinaceae, Strophariaceae, Hygrophoraceae, and Tricholomataceae (Ludley and Robinson 2008). Other soil fungi including Chrysosporium, Trichoderma, Cladosporium, Penicillium, Mortierella, Chytridiales and Saprolegniales also occur frequently in certain area of arctic tundra (Robinson and Wookey 1997; Widden 1977), though Trichoderma
spp. are known to be more common in temperate environments (Dowding and Widden 1974).

Historically, it was thought that mycorrhizal fungi were scarce in the Arctic (Muthukumar et al. 2004). This theory, however, has been disproven by many studies, which have documented that mycorrhizal fungi occur frequently across the Arctic landscape (Bledsoe et al. 1990; Dalpé and Aiken 1998; Kohn and Stasovski 1990). Mycorrhiza fungi also have been frequently reported in Arctic vegetated landscapes and some believe that mycorrhizal fungi make up a large component of fungal communities in the Arctic (Kytoviita and Ruotsalainen 2007; Pietikainen et al. 2007). The genera Laccaria, Lactarius, Russula, Cortinarius, and Hebeloma have been commonly found with Salix, Dryas, Vaccinium and Cassiope plant species in Alaska (Miller and Laursen 1974). Both ecto- and endo- mycorrhizas also have been observed in the Arctic (Dalpé and Aiken 1998; Kohn and Stasovski 1990).

1.2 Linkage between fungal community and vegetation across the arctic landscape

It is well known that many plant species interact with fungi at different levels ranging from nutrient competition to highly specific symbiotic and pathogenic relationships. Further, it has been hypothesized that plant community could affect not only the fungal communities, but also the structure and function of the ecosystem at very large scales. Thus, plant species could be a primary control over the structure and composition of fungal communities in soil across the Arctic landscape (Djukic et al.)
Many scientists believe that the production of organic substrates by plants often influences the composition of soil fungal communities by altering their abundance and composition. Zak et al. (2003) explained the relationship between fungal community and vegetation types as possibly a result of competitive interactions between microbes in soil due to plant productivity. Therefore, fungal communities in soil are largely structured by the supply of growth-liming substrates which enter soil via plant detritus and root exudation (Zak et al. 2003). Wallenstein et al. (2007) also examined fungal community structure in Arctic tundra tussock soils and shrub soils where *Eriophorum vaginatum* and *Salix* spp. were dominant, respectively. The study showed that fungal communities differed at the level of phyla, with Ascomycota dominating in tussock soils while Zygomyctae were more abundant in shrub soils (Wallenstein et al. 2007). In addition, the difference between microbial communities in tussock and shrub soil was much greater than any seasonal shifts within soils from the same vegetation type (Wallenstein et al. 2007) suggesting that plants strongly regulate microbial communities by their substrate supply to microbes and by modifying the physical environment in the active layer of soil (i.e. insulating soil by collecting drifting snow) (Schimel 1995; Tam et al. 2001). As a consequence, the diversity of fungal community may be positively correlated with plant species diversity as more plant roots release organic nutrients in soil (Zak et al. 2003). Interestingly, one study reported that there was no significant effect of plant diversity on fungal community composition (Waldrop et al. 2006). However, the study measured the composition of both active and inactive fungal species of the community. Thus, plant diversity may still influence the members the fungal community that are active.
1.3 Impacts of abiotic factors on fungal communities

1.3.1 Plant litter type/Organic carbon availability

Plant litter varies widely in chemical composition, which in turn alters organic carbon availability in soil. Litter quality is often determined by C:N ratio and lignin content in plant species. Different types of plant litter have different chemical constituents, which could influence the composition of fungal communities in soil (Hobbie 1995). Chemical composition of plant litter has been shown to lead to profound shifts in the abundance of certain members of the soil microbial community whereby some species outcompete other species, indicating that there is less competition for specific litter types (i.e. only specific species will be active with specific litter type). High lignin content of plant leaf material in black oak-white oak forest soil coincided with an abundance of basidiomycetes compared to sugar maple-basswood forest soil, where the low lignin content of plant litter corresponded with low basidiomycete abundance (Waldrop and Zak 2006). This suggests that decomposition processes in low lignin soil may be dominated by fungi other than basidiomycetes (Waldrop and Zak 2006).

The diversity of fungal communities can also be affected by plant litter quality in soil. The richness and diversity of fungi was shown to be the greatest in cellulose- and lignin- enriched soil. Cellulose and lignin are complex molecules and Hansel et al. (2008) postulate that these complex compounds are broken down to more labile compounds by a diverse assemblage of fungi; more diverse fungal communities potentially produce a more diverse suite of enzymes that breakdown the complex polymeric compounds (Trinder et al. 2008; Waldrop and Zak 2006).
1.3.2 Temperature

The temperature of the Arctic is characterized by long cold winter and short summer periods. Long cold winters in combination with high nutrient limitations often restrain decomposition rates and result in a large accumulation of organic carbon in arctic soils (Hobbie et al. 2002). Thus, the temperature oscillation in the Arctic is known to affect fungal communities which in turn alters the decomposition rate (Dang et al. 2009). Temperature increases generally affect fungal communities by enhancing their activities and increasing both functional and structural diversity, causing the fungal community structure to shift across the landscape (Allison and Treseder 2008; Schadt et al. 2003; Zak 2005). This has the potential to change the pattern of nutrient release and carbon flow. In northern Alaska, the abundance of fungi has been observed to respond to an increase of temperature, which in turn has increased CO$_2$ release, creating a positive feedback loop (Dang et al. 2009; Zak and Kling 2006). Average temperature in the Arctic is expected to increase by 4-5°C, enhancing global warming across the Arctic landscape (Dang et al. 2009).

Despite these studies supporting the hypothesis that raising temperature will cause a positive feedback on the abundance of fungi and soil carbon cycling, this is not always the case. In an Alaskan boreal forest dominated by black spruce, an increased in temperature resulted in reduced fungal abundance and soil respiration (Allison and Treseder 2008). This result, however, was more strongly constrained by declining soil moisture than by temperature. In addition, there is evidence for heterogeneity in soil responses to increasing temperature among the boreal forest sites. A black spruce
forest in Manitoba had increased soil respiration in response to soil warming, but this declined if the air above the soil was also heated (Bronson et al. 2008). In a Scots pine forest in Finland, increasing temperature also caused a positive feedback to the soil CO₂ flux (Niinistö et al. 2004), whereas a temperature increase had no significant effect in soil respiration (Verburg et al. 1999), though variation in these results could have been due to the differences in methodology.

Temperature should affect fungal communities and in turn, alter the soil carbon cycle. Many studies support this theory. However, information on the effect of temperature on fungal communities in the Arctic is still scarce. Therefore, more studies on the relationship between temperature and fungal communities along with physical soil properties should be considered.

1.3.3 Moisture level

Moisture content in soil is another important factor that has significant implications for fungal communities. In a study of fungal community in Mars Oasis in Antarctica, fungal community composition was significantly different along soil moisture gradients: Chytridiales, Mortierella, and Arrhenia/Omphalina were commonly found in wet soils while Tetracleadium, Serendipita- like, Sebacinales, and black yeast were predominant in dry soils (Bridge and Newsham 2009). Moreover, Sebacinales were exclusively found in dry soils (Bridge and Newsham 2009). It is also theorized that moisture level in soils has an impact on fungal diversity. This theory is supported by Toberman et al. (2008), who demonstrated summer drought manipulation caused a significantly decreased fungal diversity in response to low moisture content in soil. This
change in the fungal community may have resulted from reduction in the species richness of dominant fungal species (Evdokimova and Mozgova 2003; Toberman et al. 2008). In addition, the highest fungal biomass has been reported during winter and spring rather than the summer when moisture content in soil is low (Schadt et al. 2003).

1.3.4 Nitrogen availability in soil

Nitrogen is one of the most essential nutrients in soil and its availability in soil can affect not only the rate of key ecosystem processes, but also the structure and abundance of microbial communities. While many studies have been performed on the responses to simulated N enrichment by ectomycorrhizal fungi (Lilleskov et al. 2002; Peter et al. 2001; Wallenda and Kottke 1998), few studies have been carried out on the effect of nitrogen on saprotrophic fungi, that can affect the rate of key ecosystem processes.

Considering the importance of soil fungi to natural habitat function, their relationship to nitrogen in soil represents a considerable gap in research. It is difficult to draw conclusions from the few studies that have been carried out in natural ecosystems on the effect of simulated nitrogen addition on the diversity of soil fungi. However, positive effects on fungal biodiversity and abundance with nitrogen addition are commonly documented (Lagomarsino et al. 2007; Newell et al. 1996; Rühling and Tyler 1991). In contrast, Robinson et al. (1998) found a slight reduction in the diversity of saprotrophic fungi with increased N in a high Arctic ecosystem, though a greater number of colonies were observed with the treatment. Interestingly, a more recent
study showed no significant effect of nitrogen on soil fungal diversity over a two year period, suggesting microfungi in Arctic ecosystems are less sensitive to nitrogen deposition (Robinson et al. 2004). Johansson (2001) and Waldrop and Zak (2006) also showed no significant effect of nitrogen addition on fungal composition, while Sarathchandra et al. (2001) showed an increase in abundance of fungal species in response to nitrogen addition to soil. Thus, the effect of nitrogen deposition on the diversity and community structure of soil fungi in Arctic ecosystems is not clear. Furthermore, community structure of soil fungi has been elucidated in Arctic ecosystems to a lesser degree than in temperate ecosystems (Robinson et al. 2004). Therefore, it is of interest to investigate whether nitrogen availability in soil influences fungal diversity across the Arctic regions.

1.3.5 Soil pH

The pH of soil plays an important role in nutrient availability, which may indirectly control microbial activities and diversity in soil. Fierer and Jackson (2006) presented evidence that soil pH may control the biomass and composition of fungi and bacteria by selecting more adapted species. Moreover, soil pH strongly influences the fungal to bacterial ratio of the microbial community, where the fungal to bacterial ratio increased with decreasing soil pH, though substrate quality seemed to be another major controlling factor influencing the fungal to bacterial ratio (Blagdatskaya and Anderson 1998). Fungal diversity has also been found to be influenced by pH. High fungal diversity was observed with alkaline soil along with high soil organic matter content (Grishkan et al. 2008; Setälä and McLean 2004; Shah et al. 1990). This result, however,
was not supported by a recent study by Lauder et al. (2008), where the highest fungal diversity coincided with neutral soil pH to slightly acidic soil pH while lower diversity was observed in alkaline soils. This difference in result may be caused by different dominant fungal groups in a given community as community members have different pH optima. Thus, a shift in the community composition may depend more on dominant species.

Although many studies have documented the effect of soil pH on fungal communities, the majority of these reports were done in experimental settings. Experimentally manipulating the pH of a soil can result in changes in several factors (i.e. carbon availability, nutrient solubility, enzyme function) that are hard to separate and in consequence determining the proximal causes of pH effects is difficult.

1.4 Molecular approaches for fungal community analysis

For many decades, it has been difficult for mycologists to describe fungal communities due to technique limitations. Culture-dependent techniques only reveal a small fraction of natural fungal communities and do not reflect the actual diversity of fungal communities in the environment (Hofmann et al. 2003; Wintzingerode et al. 1997). Therefore, the microbial diversity in terms of species richness and abundance has been underestimated. Fortunately, many sophisticated molecular methods have been developed, offering the potential of determining the whole range of fungal taxa without culturing (Bridge et al. 2005). Such methods include, temperature gradient gel electrophoresis (TGGE), denaturing gradient gel electrophoresis (DGGE), terminal
restriction fragment length polymorphism (T-RFLP), and amplified ribosomal DNA (rDNA) restriction analysis (ARDRA) and an array of environmental sequencing techniques.

Although each of these molecular approaches is different, some of the techniques exploit the same properties of DNA. For instance, TGGE and DGGE separate DNA fragments of the same size, but of different sequences based on the DNA melting point, which varies with the G+C content, resulting in a specific profile of amplified bands. These amplicons are separated by electrophoretic mobility of partially melted DNA in polyacrylamide gels containing a linear temperature gradient or denaturation gradient (Singh et al. 2006). TGGE and DGGE analyses provide a rapid means of profiling soil fungal communities. However, these methods tend to be less sensitive to detect all the diversity within a sample, particularly, for the less abundant members of the community (Anderson and Cairney 2004). The low reproducibility of these techniques is another limitation as the amplicons are often subject to interference (Deng et al. 2008). Moreover, DGGE and TGGE are time consuming processes and are not suited for environmental isolates as databases are required (Meays et al. 2004). Nevertheless, DGGE and TGGE are recommended for pattern analysis of a community without further sequencing if a non-heterogeneous gene is used (Singh et al. 2006) and are excellent tools to investigate shifts or changes in community composition over time. In contrast, T-RFLP, through the use of an automated sequencer, has enabled significantly increased throughput compared with gel-based community profiling techniques and has been often applied to generate a fingerprint of an unknown microbial community (Marsh 1999). Along with assessing the soil fungal diversity, the identification of particular
fungal species can be achieved through T-RFLP, though the identification of fungal species requires a robust T-RFLP database (Anderson and Cairney 2004). The inability to generate sequence information from T-RFLP peaks makes the identification of unknown species in a sample very difficult. In addition, T-RFLP only detects the terminal fragment of amplified targeted DNA which potentially reduce the complexity of the community profile without reducing the diversity detected (Anderson and Cairney 2004).

Length heterogeneity PCR (LH-PCR) is another culture independent method that is commonly used to characterize fungal diversity in a community. It is an effective and reliable approach to analyze target genes with high variability in overall length (Ritchie et al. 2000). Thus, the profile peaks generated from a community by LH-PCR is a fingerprint that represents the diversity of fungi within that community. As with DGGE and TGGE, it can rapidly profile communities for comparison between sites without sequencing large clone libraries. However, the individual amplicon length can be used for phylogenetic analysis when combined with sequenced clone libraries (Venter et al. 2004). It is also known to be one of the better approaches to investigate microbial communities. One study compared LH-PCR with T-RFLP to test which method is better able to assess microbial community patterns from contaminated soils (Mills et al. 2007). It was found that LH-PCR was more reproducible than T-RFLP since T-RFLP involves post-PCR enzyme digestion which can lead to partially digested T-RFLP profiles that are not reproducible (Anderson and Cairney 2004; Mills et al. 2007; Singh et al. 2006). Moreover, LH-PCR seems to produce more unique amplicons based on length
heterogeneity as opposed to T-RFLP that produces amplicons based on restriction site sequence heterogeneity which generates a less complex profile (Ritchie et al. 2000).

Even with the efficiency of LH-PCR and its ability to amplify culturable and unculturable microbes, the sensitivity and resolution of LH-PCR do not match that of pyrosequencing (Sugiyama et al. 2010). Pyrosequencing has the potential to detect rare culturable and unculturable microbes (Acosta-Martinez et al. 2008; Ronaghi et al. 1998). With high-throughput pyrosequencing, thousands to millions of sequences from a single soil sample can be obtained, increasing the ability to detect less abundant species and allowing accurately identification of each genotype, providing a more comprehensive assessment of fungal communities in environmental samples (Acosta-Martinez et al. 2008; Sugiyama et al. 2010). Despite the effectiveness of pyrosequencing, LH-PCR is still widely used by many scientists for community ecology analysis because it is less labor-intensive, and relatively inexpensive (Anderson and Cairney 2004; Deng et al. 2008; Ritchie et al. 2000). In addition, information gained from LH-PCR results would provide insight into fungal community organization by showing the overall structure and diversity within a given community.

It is unlikely that a single approach will be universally applicable for assessing fungal community in any environmental samples. However, judicious selection of the methodology, keeping the experimental aims in mind, and the exploitation of emerging techniques will increase our understanding of not only the fungal community, but also other microbial communities in the future.
1.5 Internal Transcribed Spacer regions and primers

The ribosomal RNA (rRNA) is often used for fungal studies because the sequence of the RNA coding region is highly conserved which make an excellent tool for differentiating fungi. Within the rRNA repeat, there are non-coding regions called Internal Transcribed Spacer (ITS) region 1 and 2 (Figure 1). ITS regions are highly variable between closely related species (Egger 1995) and can be easily amplified from environmental samples (Gardes and Bruns 1996). Thus, ITS regions have been targeted to study fungal diversity in a community. Along with the ITS regions, 16S rRNA and 18S rRNA are also commonly used in fungal community analysis. Both 16S rRNA and 18S rRNA, however, are highly conserved and not sufficiently variable to compare between and within species of fungi (Dahllöf 2002; Deng et al. 2008). In addition, 18S rRNA of the Glomeromycota group has greater sequence variation between species. Therefore, 18S rRNA is used more frequently in the study of symbiotic arbuscular mycorrhizal fungi as the variation of 18S rRNA sequences is sufficient for intra-specific studies (Anderson and Cairney 2004).

**Figure 1.** Schematic representation of the internal transcribed spacer (ITS) regions in the ribosomal operon. LSU: Large subunit; SSU: Small subunit.

One of the major challenges in studying fungal diversity in soil, in which the extracted DNA pool contains DNA from a diverse range of prokaryotes and eukaryotes, is the suitability of available PCR primers. One of the earliest PCR primer sets to amplify
the ITS region are ITS1 and ITS4 which match a wide range of fungal (and other eukaryote) targets (Martin and Rygiewicz 2005). However, these primers only work well with DNA isolated from individuals and do not exclude plant sequences effectively (Martin and Rygiewicz 2005). Thus, these primers are not suitable for environmental samples. Although primers such as ITS1-F and ITS4-B, which exclude plants, have come into wide use for fungal ITS analyses, they are better matched to basidiomycetes (Gardes and Bruns 1993) and so are not a good candidate for fungal diversity estimation in environmental samples which are often dominated by ascomycetes. Ideally, primers with a broad fungal specificity would be needed to examine the diversity of fungal communities in any given set of environmental samples. The primers 2234C and 3126 T, which partially overlap with primers ITS1 and ITS4, respectively (Sequerra et al. 1997) have been used to amplify the ITS region for characterization of fungal community in soils, though these primers are specific to *Penicillium nodositatum* (Ranjard et al. 2001). Another primer that has been commonly used to estimate fungal communities in environmental samples is ITS3. The ITS3 primer (White et al. 1990) with NLB4 primer (Martin and Rygiewicz 2005) typically target ITS 2 region of RNA which is flanked by 5.8S coding sequence and large subunit sequence of the ribosomal operon (Martin and Rygiewicz 2005) (Figure 1). The ITS3 and NLB4 primers have a broad specificity to fungi, which in theory, should detect all fungal species in environmental samples. Many studies have been done using the ITS3 primer to assess fungal diversity in different environmental samples (Anderson and Cairney 2004; Dahllöf 2002; Deng et al. 2008). Therefore, the ITS3 primer with NLB4 primer would be a good candidate to investigate fungal diversity in soil samples.
1.6 Statistical applications for community ecology

Over the years, multivariate techniques have been widely used for community ecology analysis due to their accessibility and valuable information. Several applications such as nonmetric multidimensional scaling (NMS), multi response permutation procedures (MRPP) and Mantel test have been used to represent the relationship between a community and environmental variables (Clarke and Ainsworth 1993). NMS particularly accounts for all the environmental variables, even those variables that are not significant to the community (McCune and Grace 2002). Because NMS does not assume linear relationship between measured environmental variables and fungal community, it does not calculate the best solution, but gives more possibilities to explain the relationship between fungal community and environmental variables. Therefore, it gives a much wider range of structures.

Multi-response permutation procedures (MRPP) is used to explain the differences between two or more dependent variables. It also identifies whether changes in the independent variables have significant effect on the dependent variables (McCune and Grace 2002).

Mantel test evaluates the congruence between two distance (or similarity) matrices of the same dimensions. In contrast to MRPP, which compares multiple groups of various sizes, each group consisting of separate sample units, Mantel tests compare two distance matrices to the same set of sample units (McCune and Grace 2002). For example, a community can be compared against the environmental variables to
investigate how strongly environmental variables are correlated to the community.

1.7 Canonical Correspondence Analysis

Canonical Correspondence Analysis (CCA) is an ordination technique which uses environmental variables to constrain the species matrix data and is one of the most commonly used in community analysis. CCA seeks structure in the main matrix which often contains abundances of species in a set of samples in such a way as to maximize the strength of the relationship with environmental variables. Moreover, CCA allows an assessment of the importance of the measured environmental variables on the community data. Thus, it excludes community structure that is unrelated to the environmental variables and expresses pure community gradients, followed by an independent assessment of the importance of the measured environmental variables (McCune and Grace 2002).

In CCA, the environmental variables are tested as independent variables while species data within the secondary matrix are tested as dependent variables (Økland 1996). Community structure that cannot be explained by environmental variables is ignored assuming that there are no other independent variables that could affect the species matrix (McCune and Grace 2002). Consequently, CCA does not offer additional information about community structure other than demonstrating how species are related to the environmental variables measured from the same site.

One of the limitations of CCA is its vulnerability to noisy environmental data. Often, the environmental variables are moderately noisy or worse and because CCA
explicitly uses these variables in extracting the most important community gradient, the constraints on the axis become weak, resulting in less significant results (McCune 1997). Another limitation of CCA is that it tends to weigh rare species heavily which causes a distortion of data (McCune and Grace 2002).

Despite the limitations of CCA, it is possible to obtain accurate results once noise in data is cleaned. To confirm results, other techniques such as nonmetric multidimensional scaling (NMS), multi-response permutation procedure (MRPP) and Mantel test can be used.
Chapter 2 Assessment of fungal communities in the Canadian High Arctic at Alexandra Fiord

2.1 Introduction

Arctic landscapes are characterized by a diversity of ecosystems, which differ in plant species composition, litter biochemistry, and biogeochemical cycling rates. These landscapes are subjected to long cold winters followed by a few months of unfrozen conditions, making arctic environments quite variable (Wallenstein et al. 2007). It is these variations in arctic environment that makes arctic habitats, particularly soil, stressful environments, in which microbial communities are constantly subject to temperature, moisture content and nutrient level fluctuations (Lakha et al. 2005).

Although arctic landscapes accommodate many species in the ecosystem, harsh weather conditions limit the diversity of organisms including microbes in soils and therefore, biodiversity is expected to be less in Arctic than in temperate regions (Lawley et al. 2004). Across the Arctic, decreasing measures of microbial diversity in soils are correlated with increasing latitude because environments are more extreme than at lower latitude (Steven et al. 2006). Furthermore, Arctic soils vary in organic matter quality and biogeochemical flux rates which could potentially influence microbial composition in soil across Arctic landscapes (Hobbie 1996). Therefore, the range of soil environments is likely to give rise to distinct microbial communities that differ in composition across Arctic landscapes (Bossio et al. 2005).

One of the most abundant microbial taxa in soil is fungi, which are ubiquitous in the environment. Importantly, most fungal species mediate key biological processes in soil that are central to soil fertility and nutrient cycling. Thus, soil fungi are considered
as the most probable candidates for the majority of the tundra soil respiration (Callaghan et al. 2004). Despite their pivotal role in our ecosystem, our understanding of fungal communities and their relationship with their environmental factors (i.e. soil temperature, moisture content) especially in the Arctic, is scarce. Moreover, the present knowledge on microbial diversity in Arctic tundra remains the same or little better than 30-40 years ago (Callaghan et al. 2004). However, a few recent studies have shown the linkage of microbial diversity, including fungal diversity, with Arctic vegetation where microbial diversity is positively correlated with an increase in shrub abundance (Strum et al. 2001; Tape et al. 2006; Weintraub and Schimel 2005). Other studies have also documented a relationship between fungal diversity and soil moisture content and temperature (Bridge and Newsham 2009; Dang et al. 2009).

In the past years, a few studies on soil microbes in Alexandra Fiord have been conducted. Fujimura (2005) studied root-associated fungal communities in Alexandra Fiord. The results showed that warming did not affect the composition, richness and evenness in the community. Rather, the fungal community differed in genotype composition, frequency and richness according to site and soil physicochemical gradients. Thus, site and soil physical characteristics played critical, foundational role in determining community structure (Fujimura et al. 2008). This Theory was also supported by another study by Walker (2008) which documented bacterial community structure in Alexandra Fiord. The bacterial community showed overall differences between sites of Alexandra Fiord suggesting soil physical characteristics also play an important role in determining soil bacterial community structures (Walker 2008).
The Arctic forms a natural laboratory providing wide environmental gradients which may affect diversity and community structure in soils and understanding these soil microbes in the Arctic is critical for ecology analysis for our ecosystem.

The objectives of this study were to assess geospatial patterns of fungal diversity across a Canadian Arctic landscape and to investigate the relationships between fungal community and soil physical characteristics as well as to compare the diversity and structure of fungal communities between Lowland and Highland sites at Alexandra Fiord.

2.2 Research Materials and Methods

2.2.1 Study sites and sampling

Two study sites were selected for soil sample collections: Alexandra Fiord Lowland (AFL) and Highland (AFH) (Figure 2). Alexandra Fiord is a small valley on the eastern side of Ellesmere Island in Nunavut located nearly 79 degrees north latitude (78° 53′N; 75° 55′W). It is located approximately halfway up the eastern coast of Ellesmere Island near the transition from the exposed bedrock of the Canadian Shield to a younger sequence of sedimentary deposits (Freedman et al. 1994). Alexandra Fiord Lowland (Figure 3) is considered to be a terrestrial arctic oasis due to its characteristics of elevated summer temperatures and high moisture levels compared to the surrounding arctic desert. The topography of Alexandra Fiord allows a greater availability of moisture and accumulation of solar radiation within the valley basin. Consequently, it allows a greater diversity of plant species in the lowland than in
adjacent areas (Kohn and Stasovski 1990). In contrast, Alexandra Fiord Highland (Figure 4) is typically characterized by vast barren fields with mostly hard bedrock or gravel plains which severely constraints the development of vegetation (Freedman et al. 1994). Temperature fluctuates more in Alexandra Fiord Highland than Alexandra Fiord Lowland. However, the average temperature and moisture levels are much lower and microbial activities are often more limited in Highland than in Lowland (Freedman et al. 1994).

A total of 93 soil samples (per site) were collected in July 2008 by a research team led by Dr. Steven Siciliano from the University of Saskatchewan, based upon a variable-lag distance geospatial design. Three parallel transects were established along a 300m transect with 2m lateral distance between transects. A gentle slope without distinct patterns in cryoturbated soil was typically chosen for the transect. Soil cores of 5cm diameter were obtained at 31 points: 0, 0.1, 0.2, 0.5, 1, 2, 5, 10, 20, 50, 100, 100.1, 100.2, 100.5, 101, 102, 105, 110, 120, 150, 200, 200.1, 200.2, 200.5, 201, 202, 205, 210, 220, 250, 300m along each transect (Figure 5). Physical soil parameters (pH, ammonia, nitrate, phosphorus, sand, silt/clay content, % organic C, inorganic C, DOC, DON, C:N ratio), temperature, moisture, gas fluxes (CO\textsubscript{2} mmol/sec/m\textsuperscript{2}, NO\textsubscript{2} μmol/sec/m\textsuperscript{2}, CH\textsubscript{4} mmol/sec/m\textsuperscript{2}) and vegetation were also measured for all sample sites (Table 1). By using the Horiba model LA-950 Laser scattering particle size distribution analyzer (Horiba Instrument, Irvine, California, USA), soil particle size was characterized on 0.3g of air dried, sieved (<2mm) soils. Total C:N ratio was obtained by dry combustion using a Leco CNS-2000 elemental analyzer (LECO Corporation, St. Joseph, Michigan, USA). Total organic carbon and total carbon contents were also determined by combustion
using Leco CR-12 Carbon Analyzer (LECO Corporation, St. Joseph, Michigan, USA). Total inorganic carbon content was then obtained by subtracting the total organic carbon from total carbon content. To measure exchangeable NH$_4^+$ and NO$_3^-$, field-moist soil sub-samples were shaken with 0.5M K$_2$SO$_4$ for one hour and gravity filtered using Whatman 90µm filter papers (Maidstone, Kent, UK). A 3ml diluted aliquot was analyzed using the SmartChem™200 discrete chemistry analyzer (Westco Scientific Instruments Inc, Connecticut, USA). For dissolved organic carbon (DOC) and dissolved total nitrogen, TOC-VCPN analyzer (Shimadzu Scientific Instruments, Columbia, Maryland, USA) was used. Dissolved organic nitrogen (DON) was then determined by subtracting the mineral nitrogen content (sum of exchangeable NH$_4^+$ and NO$_3^-$) from dissolved total nitrogen content. Soil temperature and moisture were measured using a ProCheck digital sensor (Decagon Devices, Pullman, Washington, USA) equipped with an EC020-TE combined moisture-temperature probe. The probe was inserted into the soil and allowed to reach thermal equilibrium (~2min.). Soil pH was measured using 5g soil and deionized water mixture (1:1) with an Accumet pH meter (Accumet 925, Fisher Scientific, Massachusetts, USA).

It was discovered when examining the transect samples that the AFH transects spanned a gradient in parent materials from dolomitic to granitic, and so varied widely in pH and other soil chemical characteristics. Therefore, soil samples collected from the AFH transect ranging from 0m to 101m (45 samples) were categorized into Highland Dolomitic Site (HDS) with high pH and the samples collected within 102m-300m along the transects (48 samples) were categorized as Highland Granitic Site (HGS) with neutral to acidic pH. All of these AFL soil samples were grouped as Lowland Site (LLS).
Figure 3. A photograph of typical Alexandra Fiord Lowland with vegetation coverage.

Figure 4. A photograph of typical Alexandra Fiord Highland and the pH gradient in soil parent material. On the left side, the lighter coloured soil is dolomitic in origin and more basic and on the right side, the darker coloured soil is granitic in origin and more acidic.
Figure 5. Transects of Alexandra Fiord Highland and Lowland. Diamonds represent each soil sampling location. N=93 per site (31 per transect).
Table 1. Average and standard deviation (S.D.) of environmental variables (calculated from raw data for each sample location obtained from S. Siciliano and colleagues). HDS: Highland Dolomitic Site; HGS: Highland Granitic Site; LLS: Lowland Site.

<table>
<thead>
<tr>
<th>Variable</th>
<th>HDS Avg.</th>
<th>HDS S.D.</th>
<th>HGS Avg.</th>
<th>HGS S.D.</th>
<th>LLS Avg.</th>
<th>LLS S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>8.32</td>
<td>0.203</td>
<td>6.95</td>
<td>0.534</td>
<td>5.81</td>
<td>0.274</td>
</tr>
<tr>
<td>DOC (µg/g)</td>
<td>22.4</td>
<td>10.91</td>
<td>13.6</td>
<td>8.440</td>
<td>382.7</td>
<td>201.13</td>
</tr>
<tr>
<td>DON (µg/g)</td>
<td>1.23</td>
<td>0.394</td>
<td>1.51</td>
<td>0.528</td>
<td>19.7</td>
<td>11.66</td>
</tr>
<tr>
<td>Soil N (µg/g)</td>
<td>0.094</td>
<td>0.0334</td>
<td>0.072</td>
<td>0.0288</td>
<td>0.395</td>
<td>0.349</td>
</tr>
<tr>
<td>C:N ratio</td>
<td>61.8</td>
<td>35.82</td>
<td>10.0</td>
<td>2.827</td>
<td>11.5</td>
<td>1.772</td>
</tr>
<tr>
<td>CO₂ (mmol/sec/m²)</td>
<td>2.73</td>
<td>1.706</td>
<td>3.61</td>
<td>2.235</td>
<td>3.75</td>
<td>3.729</td>
</tr>
<tr>
<td>Soil Temperature (ºC)</td>
<td>16.8</td>
<td>3.855</td>
<td>16.4</td>
<td>2.615</td>
<td>9.81</td>
<td>2.199</td>
</tr>
<tr>
<td>Soil Moisture</td>
<td>0.083</td>
<td>0.0415</td>
<td>0.102</td>
<td>0.0506</td>
<td>0.211</td>
<td>0.0986</td>
</tr>
<tr>
<td>Ammonia (mg/kg)</td>
<td>2.930</td>
<td>1.029</td>
<td>3.612</td>
<td>1.263</td>
<td>17.06</td>
<td>16.10</td>
</tr>
<tr>
<td>Nitrate (mg/kg)</td>
<td>3.624</td>
<td>0.531</td>
<td>3.629</td>
<td>0.703</td>
<td>5.234</td>
<td>2.788</td>
</tr>
<tr>
<td>P (mg/kg)</td>
<td>0.203</td>
<td>0.116</td>
<td>0.687</td>
<td>0.394</td>
<td>2.466</td>
<td>2.719</td>
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<td>Sand (%)</td>
<td>79.24</td>
<td>4.226</td>
<td>78.05</td>
<td>9.408</td>
<td>86.21</td>
<td>8.164</td>
</tr>
<tr>
<td>Silt (%)</td>
<td>16.04</td>
<td>3.814</td>
<td>18.91</td>
<td>8.464</td>
<td>11.71</td>
<td>7.390</td>
</tr>
<tr>
<td>Clay (%)</td>
<td>4.253</td>
<td>1.501</td>
<td>2.517</td>
<td>1.672</td>
<td>0.924</td>
<td>1.088</td>
</tr>
<tr>
<td>Organic C (%)</td>
<td>0.944</td>
<td>0.250</td>
<td>0.732</td>
<td>0.301</td>
<td>4.995</td>
<td>4.849</td>
</tr>
<tr>
<td>Inorganic C (%)</td>
<td>4.366</td>
<td>2.278</td>
<td>0.061</td>
<td>0.294</td>
<td>0.194</td>
<td>0.253</td>
</tr>
<tr>
<td>CO₂ (mmol/sec/m²)</td>
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<td>1.706</td>
<td>3.636</td>
<td>2.221</td>
<td>3.751</td>
<td>3.729</td>
</tr>
<tr>
<td>NO₂ (µmol/sec/m²)</td>
<td>3.072</td>
<td>4.869</td>
<td>3.845</td>
<td>2.169</td>
<td>3.744</td>
<td>6.622</td>
</tr>
<tr>
<td>CH₄ (mmol/sec/m²)</td>
<td>0.0135</td>
<td>0.0183</td>
<td>0.0202</td>
<td>0.0181</td>
<td>0.0141</td>
<td>0.0165</td>
</tr>
</tbody>
</table>
2.2.2 DNA Extraction and LH-PCR

Soil DNA was successfully extracted from 1g of soil using Ultra Clean Soil DNA Kits (MO BIO Laboratories, Inc., Carlsbad, CA, USA) according to the manufacturer’s protocol (Alternative Protocol for maximum yields). Spectrophotometric analysis of extractions showed DNA concentrations varying from 7-176 ng/μL.

Each soil DNA sample (93 samples per site) was subjected to amplification using specific primers, with a final reaction volume of 30μL with the fluorescently labeled forward oligonucleotide primer, D4-ITS3 (5’-5D4/GCATCGATGAAGAACGCAGC-3’) and the non- labeled reverse oligonucleotide primer, NLB4 (5’-GGATTCTCACCCCTCTATGAC-3’) spanning the highly variable internal transcribed spacer region 2 (ITS2) of the ribosomal DNA. The ITS3/NLB4 primer pair was chosen for its specificity to fungi in environmental samples (Martin and Rygiewicz 2005). Each PCR reaction contained 1X PCR Buffer with 5mM MgCl₂, 80μM of each dNTPs, 0.4μM of each primer, 0.4mg/ml BSA, and 2.33U of Platinum Taq DNA polymerase and 1μl of the diluted (1:20) template DNA. Initial denaturation at 95 C for 3 minutes was followed by 35 cycles consisting of denaturation at 95 C for 90 seconds, annealing at 52 C for 90 seconds, and extension at 72 C for 90 seconds. The final extension step was performed at 72 C for 5 minutes. Any unsuccessful amplifications of samples were repeated with different dilutions of the DNA template (No dilution, 1:10, 1:50).

Bands were visualized with ethidium bromide staining in 1% agarose gel electrophoresis. The PCR products were purified using ethanol precipitation. Each precipitated DNA sample was then diluted with an appropriate amount of nuclease free
water to keep the concentrations constant for LH-PCR analysis. The diluted DNA samples were separated by length on the Beckman Coulter CEQ™ 8000 Genetic Analysis System (Beckman Coulter Inc.).

2.2.3 Fragment Analysis

All fragments were binned and analyzed using the Amplified Fragment Length Polymorphism (AFLP) program (Beckman Coulter Inc.). Analysis parameters were set for the 600bp standard size with quartic model and a bin size of 3bp. All DNA samples (both Alexandra Fiord Lowland and Highland) were analyzed together to allow comparison of the two sites. Each profile was carefully inspected to remove profiles that failed to detect targeted DNAs and/or ladder. Profiles that detected incorrect dyes were also eliminated.

The Variable Percentage Threshold (VPT) method was used to score LH-PCR profile peaks. According to Osborne et al. (2004), the VPT method accurately represents the microbial community of a sample with minimum loss of information. Data sets were standardized by determining the threshold area for the data set. Peaks with areas less than the threshold area are discarded from the profile before further analysis. The selected fragment lengths using the VPT methods ranged between 307-609bp. Any fragments that did not fall within this range were considered to be non fungal DNAs and were discarded. Eighty six profiles for Alexandra Fiord Highland and ninety profiles (out of ninety three profiles) for Alexandra Fiord Lowland were retained as well as forty-six genotypes out of 186 fragments (which included both fungal DNA profiles and
noise) for further analysis.

2.2.4 Relative Species Richness and Evenness

Relative genotypic richness was estimated by assessing the number of peaks/fragments observed in each sample using presence/absence data. Theoretically each fragment represents a different species and therefore, it potentially reflects an increase or decrease in genotypic richness. Since only the major genotypes are detected by LH-PCR, we refer to the richness comparisons as Relative Richness, because we are only assuming relative changes in diversity from sample to sample, not the total alpha diversity.

Species Evenness was determined by using the Pielou index which has equal sensitivity to minor and abundant species (Smith and Wilson 1996). The values of species evenness ranged from 0 to 1, with 0 representing minimum evenness (species are not evenly distributed) and 1 the maximum (species are evenly distributed).

2.2.5 Geospatial Analysis

Geospatial analysis was assessed using Spatial Analysis in Macroecology (SAM). SAM is a compact, but robust program which offers a comprehensive array of spatial statistical methods for Geographic Ecology, Biogeography and Macroecology. It also allows the calculations of species based on their habitat type (i.e. environmental conditions), body size or evolutionary age (Rangel et al. 2006). Presence/absence data along with environmental variables were input into SAM to examine geospatial patterns.
of fungal communities using equal number of pair setting. Moran's I coefficient was determined to ascertain whether the data were negatively or positively correlated. The results of geospatial analysis would confirm whether or not the environmental variables and the community data are significantly related to each other on the spatial scale of the transects used in this study.

2.2.6 Statistical Analysis

The following statistical ordinations were performed with PC-ORD version 5 (McCune and Mefford 2006). This program is designed to perform a variety of multivariate analyses utilizing an iterative ordination algorithm (McCune and Grace 2002).

Canonical Correspondence Analysis (CCA)

CCA was used to explore the distribution of fungal genotypes in relation to measured environmental variables. Because CCA ignores community structure that is unrelated to the environmental variables, the data that were not significantly correlated were excluded in the final analysis. Nevertheless, performing an ordination on the community data, and then secondarily relating the ordination to the environmental variables allows an expression of pure community gradients, followed by an assessment of the importance of the measured environmental variables (McCune and Grace 2002). Those significant environmental variables to the fungal genotypes include pH, dissolved organic carbon (DOC) and nitrogen (DON), C:N ratio, soil temperature, and soil
moisture. CCA Monte Carlo tests were applied with 500 randomized runs for
significance. Results were considered significant when \( p \) value is less than 0.05 while
results with \( p \) value of less than 0.1 were considered potentially significant.

**Nonmetric Multidimensional Scaling (NMS)**

This non constrained analysis can be used to confirm CCA results. It is best for
finding trends in community ecology analysis when both non constrained ordinations
such as NMS and constrained ordinations such as CCA corroborate each other (Økland
1996). NMS was run with autopilot mode in "slow and thorough" mode. The distance
measure used was Sorensen’s distance which measures the variability in the
relationship between distance in species space and environmental space.

**Multi-Response Permutation Procedure (MRPP)**

The null hypothesis that there was no difference between the two sites was
tested by means of Multi-Response Permutation Procedure (MRPP) using the Sørensen
distance measure (McCune and Grace 2002). Any statistically significant differences
between sites should be identified. MRPP provides a \( T \)-statistic value which describes
the separation among sites; the more negative \( T \) is, the stronger the separation (McCune
and Grace 2002). It also calculates a chance-corrected within group agreement \( A \)
which explains within group similarities. The \( A \) value ranges from 0 to 1, where 1 means
all items are identical within groups. \( A \)-statistical values of less than 0.1 are common for
community data (McCune and Grace 2002). As explained previously, the Alexandra
Fiord Highland transects were separated into two sites according to transect position. Each sample site positioned from 0m-101m along the transects was categorized as Highland Dolomitic Site (HDS) while samples from 102m-300m were classified as Highland Granitic Site (HGS). All samples from Alexandra Fiord Lowland were categorized as Lowland Site (LLS).

*Mantel Test*

The Mantel test was performed to confirm the significance of the relationships between fungal communities and soil characteristics by evaluating results from Mantel’s randomization test (McCune and Grace 2002). The calculated Pearson correlation ($r$), which ranges from -1 to +1 describes the strength of relationship between the fungal community data and environmental variables data. A positive value of $r$ indicates a positive association between the two.

*Indicator Species Analysis*

Indicator species analysis was used to detect and describe the species in Alexandra Fiord Highland (dolomitic and granitic sites) and Lowland that were indicative of each site. A perfect genotype/species indicator would be faithful to a particular site and would not occur at any other sites. Thus, a genotype or species with perfect indication would be present in Highland Dolomitic Site, but would not be present in Highland Granitic and Lowland Sites. Relative abundance and frequency of a species are calculated and multiplied to obtain an indicator species value (McCune and Grace 2002). Therefore, in order to obtain a high indicator value, both relative
abundance and frequency of a species need to be high. The Monte Carlo test was also applied to evaluate the statistical significance of the indicator values for each species. In addition, only those genotypes that were significant were reported in the table.

**Measurement of Species Diversity using Presence/Absence Data**

Relative species diversity was measured using three basic diversity measurements: alpha ($D_a$), beta ($\beta$), and gamma diversity ($\gamma$). Alpha diversity was typically calculated as the average richness in each site while gamma diversity was calculated as the sum of the total species richness. In contrast, beta diversity is calculated in several ways. The most frequent calculation used in community ecology studies is Whittaker’s beta diversity ($\beta_w$) which is measured as the ratio of the sum of all unique species at each site to alpha diversity (Koleff et al. 2003; Whittaker 1972). However, Wilson and Shmida’s formula for beta diversity has been used to calculate beta diversity along transects (Koleff et al. 2003; Wilson and Shmida 1984): $\beta_i = (b+c)/2a+b+c$, where $a$ is the total number of genotypes that occur in both sites, $b$ is the total number of genotype occurring only site 1 and $c$ is the total number of genotype occurring in only site 2 (Wilson and Shmida 1984). Thus, it would allow comparison of changes in fungal community structure at each site (McCune and Grace 2002). To complement the beta diversity analysis, Jaccard’s index similarity was also determined ($IS_j$) (Koleff et al. 2003). Wilson and Shmida’s beta diversity ranges from a value of 0, where there are few species differences between the sites to a value of 1, where there are many species differences between the sites. Conversely, for $IS_j$, 0 represents low
similarity and 1 represents high similarity (Koleff et al. 2003). Whittaker’s beta diversity was also calculated for comparison.

2.3 Results

2.3.1 Geospatial Analysis

Although this study was designed to assess geospatial patterns of fungal communities in Alexandra Fiord Highland and Lowland, the results were not statistically significant. There was no strong spatial scales apparent in the fungal community data. Only small-scale pattern (0m-40m) was significant and the patterns were not statistically significant as distance increased (Table 2). The results also showed a weak negative correlation between the pH of soil and the community data.

Table 2. Summary of geospatial analysis using SAM. Geographic distances between pairs of sampling sites were calculated using the geographic coordinate variables.

<table>
<thead>
<tr>
<th>Distance Class</th>
<th>Geographical distance (m)</th>
<th>Distance centre (m)</th>
<th>Moran’s I</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Class 1</td>
<td>0-5</td>
<td>2.06</td>
<td>0.05</td>
<td>0.045</td>
</tr>
<tr>
<td>Class 2</td>
<td>5-15</td>
<td>9.63</td>
<td>0.057</td>
<td>0.035</td>
</tr>
<tr>
<td>Class 3</td>
<td>15-41</td>
<td>27.9</td>
<td>0.129</td>
<td>0.005</td>
</tr>
<tr>
<td>Class 4</td>
<td>41-61</td>
<td>51.0</td>
<td>-0.003</td>
<td>0.915</td>
</tr>
<tr>
<td>Class 5</td>
<td>61-74</td>
<td>67.8</td>
<td>-0.100</td>
<td>0.015</td>
</tr>
<tr>
<td>Class 6</td>
<td>74-89</td>
<td>81.7</td>
<td>0.025</td>
<td>0.246</td>
</tr>
<tr>
<td>Class 7</td>
<td>89-110</td>
<td>99.7</td>
<td>-0.103</td>
<td>0.010</td>
</tr>
<tr>
<td>Class 8</td>
<td>110-121</td>
<td>115.6</td>
<td>-0.018</td>
<td>0.442</td>
</tr>
<tr>
<td>Class 9</td>
<td>121-142</td>
<td>131.7</td>
<td>-0.009</td>
<td>0.693</td>
</tr>
<tr>
<td>Class 10</td>
<td>142-179</td>
<td>160.6</td>
<td>-0.016</td>
<td>0.467</td>
</tr>
<tr>
<td>Class 11</td>
<td>179-194</td>
<td>186.5</td>
<td>-0.070</td>
<td>0.025</td>
</tr>
<tr>
<td>Class 12</td>
<td>194-246</td>
<td>220.0</td>
<td>-0.028</td>
<td>0.0281</td>
</tr>
<tr>
<td>Class 13</td>
<td>246-300</td>
<td>273.3</td>
<td>0.0120</td>
<td>0.548</td>
</tr>
</tbody>
</table>
2.3.2 Amplified Fragment Length Polymorphism Analysis

The AFLP program revealed genotypes ranging from 307bp to 609bp which were generated from soil samples via LH-PCR. Comparison of the LH-PCR profiles obtained from the various soil samples showed that each site is characterized by a unique pattern (Figure 6), suggesting the fungal communities are structured according to site. Each soil sample along the HDS transect yielded as little as 2 genotypes to as many as 20 genotypes (Figure 7a) while individual soil samples along the HGS and LLS transects generated genotypes ranging from 3 to 24 and 2 to 26, respectively (Figures 7b and c). When each genotype was compared between the three sites (HDS, HGS and LLS), the HDS yielded 29 different genotypes, 3 of which were exclusively found at the Highland Dolomitic Site (359bp, 362bp and 414bp) (Figure 8). In contrast, 40 different genotypes were found at the Highland Granitic Site with 2 exclusive genotypes, which include fragments of 342bp and 585bp (Figure 8). Similarly, the Lowland Site generated 38 genotypes, 5 of which were unique to only Lowland soil samples (307bp, 385bp, 467bp, 542bp and 553bp) (Figure 8). Although each site generated different numbers of genotypes, many common fragments were shared, especially between the Highland Granitic Site and the Lowland Site. In addition, the most common genotypes between the Highland Dolomitic Site and Highland Granitic Site ranged from 488bp to 523bp (Figure 8).
Figure 6. LH-PCR profiles (blue) obtained from a soil sample representing a specific fungal community structure. Red profiles represent 600bp standard ladder. Peak height indicates the abundance of that genotype.
Figure 7. Species richness at each sample site for HDS (a), HGS (b) and LLS (c). H: Highland; L: Lowland; T: transect; numbers indicate the position of sample sites.
Figure 8. Abundance of each genotype present at Highland Dolomitic Site, Highland Granitic Site and Lowland Site.
2.3.3 Species Richness and Evenness

Relative species richness and evenness were determined using the Pielou index. The Highland Dolomitic Site displayed the lowest species richness with a more even distribution of species richness ($E=0.783$, Table 3). The Highland Granitic Site and Lowland Site had lower evenness and higher relative richness, suggesting a more heterogeneous distribution of species. Moreover, the Granitic Site of Highland and Lowland Site did not exhibit large differences (only 5 species were different between the Highland Granitic and Lowland Sites). However, the Dolomitic Site and Granitic Highland Sites (28 species difference) as well as the Dolomitic Site and Lowland Site (26 species difference) exhibit high relative difference. The average species richness and evenness at each site is summarized in Table 3.

Table 3. Average evenness and richness (Pielou) at each site

<table>
<thead>
<tr>
<th>Site</th>
<th>Evenness ($E$)</th>
<th>Richness</th>
</tr>
</thead>
<tbody>
<tr>
<td>HDS</td>
<td>0.783</td>
<td>29</td>
</tr>
<tr>
<td>HGS</td>
<td>0.572</td>
<td>40</td>
</tr>
<tr>
<td>LLS</td>
<td>0.674</td>
<td>38</td>
</tr>
</tbody>
</table>
2.3.4 Indicator Species Analysis

Indicator species, as well as their abundance at each site, were obtained using fungal community data (Table 4). The Highland Granitic Site had two perfect indicator species, followed by the Highland Dolomitic Site with three indicator species and the Lowland Site with 5 (Figure 8). Although these genotypes were unique to a specific site, the frequency of each genotype at that site was relatively low (Figure 9). However, the distribution of all but one indicator species is proven to be statistically significant (Table 4). The 342bp genotype occurred only at the Highland Granitic Site, but only occurred at a frequency of 5% with $p=0.424$ (Table 4). Therefore, the 342bp genotype would not be a reliable indicator species of the Highland Granitic Site, though McCune and Grace (2002) suggested that singleton species like this (as well as infrequent species) have no possibility of being a statistically significant indicator species because the result of all of its occurrences falling in one group is quite likely.

The 542bp genotype, on the other hand, was a significant indicator of the Lowland Site, with an indicator value of 54. All of its occurrences were at the Lowland Site, and it occurred in more than a half of the site. The randomization test showed that the probability of an indicator value of 54 or higher, given this genotype’s distribution of abundances, was 0.0002 which is highly significant (Table 4). In addition, the 542bp genotype scored the highest indicator species value, while the 307bp genotype scored the lowest indicator species value of 6.7.
Table 4. Monte Carlo test of significance of observed indicator value for each genotype
$p<0.05)$. IV: Average Indicator species; Maxgrp: Site with maximum observed IV.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Maxgrp</th>
<th>IV</th>
<th>S. Dev</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>307</td>
<td>3</td>
<td>6.7</td>
<td>1.65</td>
<td>0.0540</td>
</tr>
<tr>
<td>342</td>
<td>2</td>
<td>4.7</td>
<td>1.05</td>
<td>0.4240</td>
</tr>
<tr>
<td>359</td>
<td>1</td>
<td>7.0</td>
<td>1.31</td>
<td>0.0270</td>
</tr>
<tr>
<td>362</td>
<td>1</td>
<td>7.0</td>
<td>1.31</td>
<td>0.0270</td>
</tr>
<tr>
<td>385</td>
<td>3</td>
<td>14.4</td>
<td>2.08</td>
<td>0.0036</td>
</tr>
<tr>
<td>414</td>
<td>1</td>
<td>7.0</td>
<td>2.40</td>
<td>0.0300</td>
</tr>
<tr>
<td>464</td>
<td>2</td>
<td>27.6</td>
<td>2.51</td>
<td>0.0020</td>
</tr>
<tr>
<td>467</td>
<td>3</td>
<td>23.3</td>
<td>2.37</td>
<td>0.0004</td>
</tr>
<tr>
<td>476</td>
<td>2</td>
<td>16.0</td>
<td>2.08</td>
<td>0.0016</td>
</tr>
<tr>
<td>486</td>
<td>2</td>
<td>25.0</td>
<td>3.46</td>
<td>0.0074</td>
</tr>
<tr>
<td>488</td>
<td>3</td>
<td>46.6</td>
<td>4.10</td>
<td>0.0008</td>
</tr>
<tr>
<td>501</td>
<td>2</td>
<td>59.8</td>
<td>3.47</td>
<td>0.0002</td>
</tr>
<tr>
<td>505</td>
<td>2</td>
<td>39.7</td>
<td>4.43</td>
<td>0.0020</td>
</tr>
<tr>
<td>508</td>
<td>1</td>
<td>41.6</td>
<td>4.03</td>
<td>0.0358</td>
</tr>
<tr>
<td>511</td>
<td>3</td>
<td>30.5</td>
<td>3.56</td>
<td>0.0030</td>
</tr>
<tr>
<td>512</td>
<td>1</td>
<td>26.9</td>
<td>3.18</td>
<td>0.0270</td>
</tr>
<tr>
<td>515</td>
<td>1</td>
<td>81.7</td>
<td>4.16</td>
<td>0.0002</td>
</tr>
<tr>
<td>518</td>
<td>1</td>
<td>16.1</td>
<td>2.88</td>
<td>0.0600</td>
</tr>
<tr>
<td>523</td>
<td>1</td>
<td>32.9</td>
<td>3.31</td>
<td>0.0014</td>
</tr>
<tr>
<td>542</td>
<td>3</td>
<td>54.4</td>
<td>3.24</td>
<td>0.0002</td>
</tr>
<tr>
<td>553</td>
<td>3</td>
<td>21.1</td>
<td>2.55</td>
<td>0.0012</td>
</tr>
<tr>
<td>562</td>
<td>2</td>
<td>15.0</td>
<td>2.94</td>
<td>0.0300</td>
</tr>
<tr>
<td>564</td>
<td>3</td>
<td>20.8</td>
<td>2.92</td>
<td>0.0020</td>
</tr>
<tr>
<td>570</td>
<td>2</td>
<td>29.5</td>
<td>3.59</td>
<td>0.0010</td>
</tr>
<tr>
<td>573</td>
<td>1</td>
<td>20.7</td>
<td>3.23</td>
<td>0.0248</td>
</tr>
<tr>
<td>579</td>
<td>3</td>
<td>25.7</td>
<td>3.67</td>
<td>0.0102</td>
</tr>
<tr>
<td>580</td>
<td>3</td>
<td>13.0</td>
<td>2.56</td>
<td>0.0524</td>
</tr>
<tr>
<td>585</td>
<td>2</td>
<td>9.3</td>
<td>1.45</td>
<td>0.0074</td>
</tr>
</tbody>
</table>
Figure 9. Relative frequency (%) of genotypes in each site: HDS, HGS, and IDS.

Relative Frequency (%)
2.3.5 Canonical Correspondence Analysis

CCA showed that fungal community composition is different between the three sites. Figure 10 shows the separation of Alexandra Fiord Highland and Lowland into three distinctive sites. The fungal community composition variability at the Lowland site was more strongly related to DOC, DON, and soil moisture, while the composition at the Highland Dolomitic Site and the Highland Granitic Site was better explained by C:N ratio and pH, respectively. Overall, pH seemed to be the environmental factor most strongly affecting fungal communities in all three groups. Each vector indicates the direction and relative strength of environmental variables.

Most of the variation (5.2%) explained is in the first axis as shown in Table 5. Axes 2 and 3 explain little of the variance; \( p \) values from the Monte Carlo test for axes 2 and 3 were not calculated because they explain little variance (Table 6; Figures 11 & 12). As seen in Figures 11 and 12, ordinations explained by Axis 1 and 3 or axis 2 and 3 show weak correlations with many overlapping sample points between the sites. Conversely, the ordination explained by Axis 1 and 2 (Figure 10) showed the strongest correlations with environmental variables and the fungal community data. The eigenvalue for the first axis was also relatively higher than the range expected by chance with the \( p \) value of 0.0020. This axis is strongly related to the environmental variables (Table 6 and Figure 10).
Table 5. Summary statistical reports on each axis. Pearson correlation is a standard correlation coefficient between environmental variables and the fungal community while Kendall correlation is a rank correlation coefficient.

<table>
<thead>
<tr>
<th></th>
<th>Axis 1</th>
<th>Axis 2</th>
<th>Axis 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eigenvalue</td>
<td>0.325</td>
<td>0.172</td>
<td>0.107</td>
</tr>
<tr>
<td>Variance in species data</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% of variance explained</td>
<td>5.2</td>
<td>2.8</td>
<td>1.7</td>
</tr>
<tr>
<td>Cumulative % explained</td>
<td>5.2</td>
<td>8.0</td>
<td>9.7</td>
</tr>
<tr>
<td>Pearson Correlation</td>
<td>0.857</td>
<td>0.733</td>
<td>0.581</td>
</tr>
<tr>
<td>Kendall (Rank) Correlation</td>
<td>0.631</td>
<td>0.501</td>
<td>0.257</td>
</tr>
</tbody>
</table>

Table 6. Monte Carlo test results for eigenvalues and species environmental correlations (Spp-Envt Corr.) based on 499 runs with randomized data.

<table>
<thead>
<tr>
<th>Axis</th>
<th>Real data</th>
<th>Randomized data</th>
<th>Mean</th>
<th>Min</th>
<th>Max</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Eigenvalue</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>0.325</td>
<td></td>
<td>0.091</td>
<td>0.053</td>
<td>0.171</td>
<td>0.0020</td>
</tr>
<tr>
<td>2</td>
<td>0.172</td>
<td></td>
<td>0.066</td>
<td>0.36</td>
<td>0.101</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>0.107</td>
<td></td>
<td>0.051</td>
<td>0.030</td>
<td>0.079</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>0.857</td>
<td></td>
<td>0.531</td>
<td>0.390</td>
<td>0.672</td>
<td>0.0020</td>
</tr>
<tr>
<td>2</td>
<td>0.733</td>
<td></td>
<td>0.487</td>
<td>0.374</td>
<td>0.607</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>0.581</td>
<td></td>
<td>0.451</td>
<td>0.359</td>
<td>0.604</td>
<td></td>
</tr>
</tbody>
</table>
Figure 10. CCA ordination of sites (triangles) in environmental space explained by Axis 1 and Axis 2. The significance of each environmental variable explained by vectors. Each cross (blue) represents each genotype. Axis 1: $r^2=0.098$; Axis 2: $r^2=0.105$
**Figure 11.** CCA ordination of sites (triangles) in environmental space explained by Axis 1 and 3. The significance of each environmental variable is explained by vectors. Each cross (blue) represents each genotype. Axis 1: $r^2=0.096$; Axis 3: $r^2=0.083$. 
Figure 12. CCA ordination of sites (triangles) in environmental space explained by Axis 2 and 3. The significance of each environmental variable is explained by vectors. Each cross (blue) represents each genotype. Axis 2: $r^2=0.105$; Axis 3: $r^2=0.083$. 
2.3.6 Nonmetric Multidimensional Scaling (NMS)

Because trends in community structure are best explained by both constrained (e.g., CCA) and non-constrained ordinations (Økland 1996), NMS was applied as a non-constrained ordination method. NMS revealed almost identical trends as CCA, confirming the CCA results. However, NMS showed in a low correlation between the key environmental variables and the ordination scores, but provided a better representation of the overall community structure (Table 7, Figure 10 and Figure 13). Ordinations of NMS explained by all axes showed less distinct separation between the Highland Dolomitic Site and Granitic Site (Figures 13, 14, 15). The vectors, which indicate the direction and strength of the correlations between the grouping of sample sites and the environmental variables revealed the same pattern as CCA (Figure 10 & Figure 13). The distribution of fungal genotypes across the sample sites was also very similar. The final stress for 3-dimensional solution was 18.05 with a final instability of 0.0005.

<table>
<thead>
<tr>
<th>Variance represented (%)</th>
<th>CCA</th>
<th>NMS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Axis 1</td>
<td>5.2</td>
<td>24.5</td>
</tr>
<tr>
<td>Axis 2</td>
<td>2.8</td>
<td>22.9</td>
</tr>
<tr>
<td>Axis 3</td>
<td>1.7</td>
<td>24.8</td>
</tr>
</tbody>
</table>
Figure 13. NMS ordination of sites (triangles) in environmental space explained by Axis 1 and 2. The significance of each environmental variable is explained by vectors. Each cross (blue) represents each genotype.
Figure 14. NMS ordination of sites (triangles) in environmental space explained by Axis 1 and 3. The significance of each environmental variable is explained by vectors. Each cross (blue) represents each genotype.
Figure 15. NMS ordination of sites (triangles) in environmental space explained by Axis 2 and 3. The significance of each environmental variable is explained by vectors. Each cross (blue) represents each genotype.
2.3.7 Measurement of Species Diversity

Species diversity was compared at each site using $\beta_1$ and $IS_J$ (Table 8). According to these values, the Highland Dolomitic Site and the Lowland Site had the highest $\beta_1$ value ($\beta_1 = 0.573$) with the lowest $IS_J$ (0.271) suggesting that there are differences in community composition with some overlapping species between the two sites. The Highland Dolomitic and Granitic Sites also showed similar trends with a relatively high $\beta_1$ and a low $IS_J$. The Highland Granitic Site and the Lowland Site on the other hand had very similar fungal community compositions. This was not expected as the Highland Granitic Site and the Lowland have very different altitude and moisture regions and have very different soil properties. Table 8 summarized the measurements of species diversity at each site.

Table 8. Summary of species diversity measurements between the sample sites.

<table>
<thead>
<tr>
<th>Site</th>
<th>Alpha ($\alpha$)</th>
<th>Beta ($\beta$)</th>
<th>Gamma ($\gamma$)</th>
<th>$IS_J$</th>
</tr>
</thead>
<tbody>
<tr>
<td>HDS and HGS</td>
<td>29</td>
<td>0.433</td>
<td>3</td>
<td>0.395</td>
</tr>
<tr>
<td>HGS and LLS</td>
<td>40</td>
<td>0.103</td>
<td>3</td>
<td>0.814</td>
</tr>
<tr>
<td>HDS and LLS</td>
<td>38</td>
<td>0.573</td>
<td>5</td>
<td>0.271</td>
</tr>
</tbody>
</table>

2.3.8 Mantel Test

The fungal community was compared to each of the environmental variables. All of the environmental variables showed positive associations with fungal community structure, confirming the significance of the correlation between the two matrices. As with CCA and NMS results, pH appeared to have the strongest correlation with the community data ($r=0.285$, $p<0.05$). The moisture content also showed a very strong correlation ($r=0.278$). All the associations with the fungal community data were
statistically significant ($p<0.05$) based on the randomization test. The results are summarized in Table 9.

**Table 9.** Standardized Mantel statistic ($r$) of each environmental variable using the asymptotic approximation of the Mantel test.

<table>
<thead>
<tr>
<th>Environmental variables</th>
<th>$r$</th>
<th>$p$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>0.285</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>C:N ratio</td>
<td>0.179</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>DOC</td>
<td>0.155</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>DON</td>
<td>0.175</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Temperature</td>
<td>0.203</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Moisture content</td>
<td>0.278</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

2.3.9 MRPP

Based on the MRPP results, we can reject the null hypothesis that no differences exist among these three sites. The three sites occupy different regions of species space, as shown by the strong chance-corrected within-group (site) agreement ($A$) and test statistic ($T$) (Table 10). The comparisons between the sites yielded statistics comparable to the overall comparisons. The $T$ values indicated that the separations between the sites are distinct, therefore, the species composition between the sites is different.

**Table 10.** Summary statistics for MRPP comparing across all groups, as well as for multiple pairwise comparisons for the Sørensen distance.

<table>
<thead>
<tr>
<th>Sørensen distances</th>
<th>$T$</th>
<th>$p$</th>
<th>$A$</th>
</tr>
</thead>
<tbody>
<tr>
<td>HDS vs HGS</td>
<td>-16.001</td>
<td>&lt;0.05</td>
<td>0.1001</td>
</tr>
<tr>
<td>HDS vs LLS</td>
<td>-32.664</td>
<td>&lt;0.05</td>
<td>0.1037</td>
</tr>
<tr>
<td>HGS vs LLS</td>
<td>-16.092</td>
<td>&lt;0.05</td>
<td>0.0681</td>
</tr>
</tbody>
</table>
2.4 Discussion

2.4.1 Geospatial Analysis

Initially, the intention of this study was to examine geospatial patterns of fungal communities in Alexandra Fiord Highland and Lowland. The three transects that were 300m long covered a substantial portion of the landscape and the sampling was designed to allow geospatial analysis. However, the spatial analysis showed little significant spatial structure, which made it difficult to interpret the data. This may be due to the design of transects (S. Siciliano and collaborators) with the aim to assess small, medium and large scale patterns. Furthermore, the soil samples were collected in as much as a homogeneous landscape patch as possible, thus the environmental variables at each sample point along the transects within a particular site was expected to be similar. The exception was the transect on the Alexandra Fiord Highland, which inadvertently was sampled across a gradient in soil parent materials (dolomitic versus granitic) and so differed greatly in pH and other soil factors. The lack of significant statistical spatial structure (after subsampling the Highland transect) suggests that the diversity patterns of the fungal community may be random or neutral (i.e. diversity patterns were driven primarily by stochastic processes). However, environmental variables may be spatially structured at a smaller scale resulting in slightly significant small scale pattern across the Alexandra Fiord transects. According to the literature, different sampling designs could result in different statistical values which would change the interpretation of data (Legendre et al. 2002). The response of an organism to the environment is particular to a specific scale and may respond differently at a larger or smaller scale (Legendre et al. 2002). Therefore, choosing a scale that is
appropriate to the ecological process in question is hard and is important. One method that may be suited for geospatial analysis along the environmental gradient is to collect soil samples in aggregates of 5 sampling units in a systematic pattern (Legendre et al. 2002). This design would allow a better estimation of autocorrelation between the community and environmental data (Dutilleul 1993). Thus, the geospatial analysis results in this study may have been different (i.e. resulting in strong geospatial patterns) if the study had a different sampling method.

2.4.2 Diversity of fungal communities in Alexandra Fiord Highland and Lowland

The diversity assessment was developed by measuring the relative abundance, relative frequency and relative genotype richness of samples. As expected, the Dolomitic Site of Alexandra Fiord Highland yielded fewer genotypes as the Dolomitic Site has much harsher environmental conditions with limited soil nutrients. In contrast, the Highland Granitic Site and the Lowland Site (Figure 7) yielded higher diversity. Moreover, many genotypes present at the Highland Dolomitic Site occurred at a very low frequency with low abundance (Figures 8 & 9). For instance, the genotype with a fragment size of 512bp occurred at the Highland dolomitic site at a frequency of 9% while it occurred at a frequency of 86% and 59% at the Highland Granitic Site and Lowland Site, respectively. This suggests that the occurrence of this genotype at the Highland Dolomitic Site could be a chance event rather than representing adaptation to this niche. Furthermore, soil chemical components of Alexandra Fiord depend on parental materials and may play an important role in regulating a fungal community. For example, the Highland Dolomitic soil originated from dolomitic parental material.
which possesses excess calcium (Ca) and carbonate. Ca reacts with P, to create calcium phosphate which only a few fungi can solubilize (Fujimura et al. 2008; Golubić and Schneider 1979) and excess carbonate increases the pH. Therefore, fungi that can solubilize calcium phosphate at high pH would have a competitive advantage over fungi that cannot. Although the Highland Dolomitic Site has a high C:N ratio, this is not conducive to the breakdown of organic matter and may be a limiting factor at the Highland Dolomitic Site. Thus, the Highland Dolomitic Site may be too harsh for many fungal species to survive, resulting in low fungal diversity. This suggests that the Highland Dolomitic Site may be characterized by species that can tolerate high pH and solubilize materials that are relatively insoluble. Moreover, the low diversity at the Highland Dolomitic Site may also reflect the nutrient poor status of the arctic soil samples used in this study. However, it is difficult to conclude that the low diversity is a function of increasing latitude or is simply representative of a different Alexandra Fiord Highland (AFH) biogeographical zone, though the community data showed some significant relationship with environmental variables. In contrast, the Highland Granitic Site generated the greatest number of genotypes indicating the most diversified fungal community. The lowland site also yielded a large number of genotypes. In addition, the Pielou index evenness values (Table 3) associated with each site indicated that fungal species are not evenly distributed in the soil samples.

Despite the harsh soil conditions (i.e. low soil nutrients and moisture) in the Highland Sites, the Granitic Site yielded high diversity. Soils of Alexandra Fiord Highland (AFH) have very low soil nutrients (i.e. low DOC and DON levels) and low moisture level with relatively high temperature fluctuation compared to Alexandra
Fiord Lowland (AFL). Therefore, the high diversity of fungal communities at the Highland Granitic Site, was surprising as it was expected to be lower than the lowland site. According to the literatures, soil nutrients such as nitrogen do not have much effect on fungal community composition (Johansson 2001; Robinson et al. 2004; Waldrop and Zak 2006), despite reports that soil nutrients influence fungal communities (Drenovsky et al. 2004; Trinder et al. 2008; Wallenstein et al. 2007). This result is consistent with the high diversity at the Granitic Site where soil nutrients were low suggesting fungal communities in Arctic ecosystem are not responding strongly to soil nutrients.

However, when pH levels at the Dolomitic Site and Granitic Site of AFH were compared, it was distinctly different; the Dolomitic Site being basic while the Granitic Site had neutral pH. According to the literature, bacterial functional diversity is strongly influenced by the soil pH (Hansel et al. 2008). Because both bacteria and fungi respond quickly to environmental changes and have different ranges of pH optimum level, soil pH may have the same effect on fungi as it has on bacterial community. In fact, a study shows that arbuscular fungal diversity is significantly affected by the soil pH (Dumbrell et al. 2010). Thus, we expected that our high diversity results at the Highland sites was influenced by pH difference between the Dolomitic and the Granitic Sites, with the neutral pH of the soils at the Granitic Site, resulting in higher diversity. The Lowland Site also yielded high fungal diversity with slightly lower pH. This slightly acidic condition would still support a healthy community of neutrophiles similar to the Highland Granitic Site. Moreover, the moisture condition and temperature fluctuations are less severe with much higher organic nutrients. Hence, it is not surprising that the Lowland Site had high fungal diversity. In addition, the lowland site had much more
vegetation than the Highland Dolomitic and Granitic Sites. An increase in plant diversity seem to increase soil nutrients for fungal community as more plant roots release variety of organic nutrients in soil (Waldrop and Zak 2006; Zak et al. 2003) which is consistent with our observations where the lowland site had higher soil organic nutrients with abundant plant species. Because soil organic substrates often influence the composition of fungal community, it is possible that differences in community structure at each sample site may be affected by organic substrates produced by different plant species across the Alexandra Fiord landscape. Plants also modify the physical environment in the active layer of soil by collecting drifting snow, insulating soil which would help explain our observation of low soil temperature fluctuation at the lowland site.

In spite of the differences in soil characteristics at each site, many genotypes were shared by all three sites. Thus, the community composition was largely differentiated by changes in the relative abundance and frequency of each genotype. Because the most frequent genotypes were present across all sites, some differences between sites are due to small changes in the frequency distribution of common genotypes. In addition, many of the genotypes occurred in greater abundances with high frequencies in particular sites, and some genotypes occurred at only one or two sites, giving rise to the unique fungal communities at each site. The Highland Dolomitic Site had three exclusive genotypes while the Highland Granitic Site and Lowland Site had two and five unique genotypes, respectively. However, the relative abundance of some of these exclusive genotypes was low, suggesting that they represent rare fungal species at that site. Furthermore, because the relative abundance measurements are confounded by the presence of rare species, it may create noise in the data (McCune and
Grace 2002). Nevertheless, the relative abundance and frequency of each genotype show how each site is structured by both dominant and unique fragments creating a distinct community at each site.

Although fungal species richness seemed to be relatively high across the Alexandra Fiord landscape, the diversity may have been underestimated since the same length fragment could reflect more than one species. To investigate conclusively whether diversity was underestimated DNA sequencing would be required to identify the operational taxonomic units (OTUs). However, there is no reason to believe that estimates would be biased towards results for particular sites or samples, so even if the diversity was underestimated, relative comparison of fungal communities between the sites should remain valid.

In addition, this study of fungal community assessment at AF did not consider vertical distribution of fungal species and the results only reflect the upper organic layer of soil (which is most active). However, we cannot eliminate the possibility of differentiation of fungal community structure along the soil profile. A study by Dickie et al. (2002) documented vertical distribution of fungal species of forest floor and showed distinctive community patterns among the four soil layers. According to the study, certain ectomycorrhizal fungi were present only in deeper layers of soil (Dickie et al. 2002). Because our soil samples were collected from the upper layer, these mycorrhizal fungi that habitat in deeper soil layer may not be detected in our study. Furthermore, any plant roots that may be associated with mycorrhizal fungi and mycelia were removed during sampling. As a consequence, our results of root-associated fungal
diversity may have been underestimated. With vertical niche differentiation of fungal species in soil, the patterns of fungal community structure between the sites may have been more distinctive if samples had been collected through the soil profiles. Thus, the fungal community structure between the sites (HGS, HGS, LLS) may be less significant than we initially thought as collecting soils from the same soil layer may have over-emphasized fungal species that were shared between all three sites. Nevertheless, the study results still provided important information on the composition of the fungal community and the significance of environmental factors across the sample sites.

2.4.3 Fungal community comparison between Alexandra Fiord Highland and Lowland

Alexandra Fiord Highland and Lowland were expected to be very different in terms of fungal communities. According to the beta diversity measurement, the Highland Dolomitic Site and Lowland Site had the highest beta diversity value (\(\beta=0.0.573\) (Table 8), which indicates that the amount of compositional variation between the Dolomitic Site of AFH and the Lowland Site is great, resulting in distinctive fungal community compositions. These results were expected as the Highland Dolomitic Site and the Lowland Site are separated by a very distinctive landscape and soil characteristics. The Highland Dolomitic Site is rocky with almost no vegetation and has low DOC and DON and moisture content, and high pH (caused by excess Ca and Mg from the parental material, as well as an abundance of carbonate). Conversely, the Lowland Site was characterized by diverse vegetation, high soil moisture content, high nutrients,
less temperature fluctuation, and neutral to low pH. There is no doubt that fungal communities are strongly influenced by such environmental factors (Dumbrell et al. 2010; Lawley et al. 2004) and changes in these variables would select populations that are most competitive in terms of growth rates and ability to absorb nutrients. Furthermore, disadvantaged genotypes would either be extirpated or go dormant (Persiani et al. 1998), which may be the case for the species at HDS as it has harsher environmental conditions. In addition, the average relative abundance of fungal species at the Lowland site was not significantly different from the other two sites. Furthermore, some genotypes from the LLS had relatively low abundance (Figure 8). Some studies have suggested that bacteria control fungal growth by outcompeting fungal species (De Boer et al. 2003; Romani et al. 2006). Because the LLS has less harsh environment conditions with high organic nutrients, it is also an ideal environment for bacteria to survive in the Arctic. However, this study did not look at bacterial community structure and therefore, we can only speculate that bacterial species may be thriving at Lowland site and suppress fungal growth, and ultimately decrease the frequency in the community.

When Highland Dolomitic and Granitic Sites were compared, the results were astonishing as the composition of fungal community between the two sites was very different despite the similar physical soil characteristics other than pH. Rather, the fungal community of the Highland Granitic Site was more similar to the Lowland Site ($\beta=0.103$). Regardless of the large differences in physical soil characteristics and landscape differences between Highland and Lowland, the Highland Granitic Site and the Lowland Site registered a very low beta diversity value, sharing many of the same
genotypes between the sites and have fewer exclusive genotypes at each site. This may be explained by the fact that the site pair (Highland Granitic Site and Lowland Site) contains many common fungal species. Conversely, the high beta diversity value of Highland Dolomitic Site may be influenced by the presence of rare and infrequent species. However, the results in community comparison between the sites were not surprising when soil physical characteristics along transects, especially pH were considered.

Niche theory suggests that changes in species composition can be related to changes in environmental variables. Thus, the structure of communities can be explained by the coexistence of species and maintenance of biodiversity due to adaptation to specific soil niches (Dumbrell et al. 2010). Niche theory is widely accepted and it is believed by most ecologists that niche plays the major role in regulating community structure. This is consistent with our observations, as our results revealed the importance of soil environmental variables in community structure. However, spatial structure may also play an important role in determining a community structure. Neutral theory (which suggests that community composition is related to geographic distance between samples as a result of dispersal limitation) played some role in structuring an arbuscular mycorrhizal (AM) fungi community, but niche theory, particularly in relation to pH, played a more important role in both our study and the study by Dumbrell et al. (2010). The dissimilarity between the sites of Alexandra Fiord could have occurred from dispersal limitation, as suggested by neutral theory, but there was more evidence that the dissimilarities were driven by adaptation to soil conditions (i.e. niche theory).
Another study by Fujimura et al. (2008), who studied the effect of warming on the root associated fungal community in Alexandra Fiord documented that site and soil physicochemical gradients are the most important factors determining fungal community structure. This result was consistent with our study observations, where the number of different genotypes, richness, and evenness between the sites were different according to site and soil physicochemical gradients.

Evenness values explained the distribution of genotypes at each site. The Highland Dolomitic Site had a high evenness value, indicating that species across the Dolomitic Site are more evenly distributed (i.e. the community is not dominated by a few species). This may be due to the fact that the distance of soil samples collected at each position across the Highland Dolomitic Site were relatively in close proximity and that standard deviation of environmental variables (particularly soil pH) was low. For example, the soil pH at the Highland Dolomitic Site varied less (i.e. measured soil pH at each sample site of HDS was close to the mean), giving more even pH level across the Highland Dolomitic Site which may affected the dominance of each genotype to be more evenly distributed at HDS. The Highland Granitic and Lowland Sites on the other hand had relatively low evenness values, indicating that these two sites had more typical patterns of fewer abundant species with many rare species. According to Tam et al. (2000) the shift in dominant species is affected by variations of substrates in soils, as differential use of the substrates depends on different microorganisms in soils. Thus, the distribution of soil nutrients and other soil organic materials may play a role in the community evenness. This could be suggesting that fungal species may have a restricted geographic distribution because of environmental sensitivity. Therefore, the structure
of fungal communities at each site may still be distinctive.

2.4.4 Environmental factors influencing fungal communities in Alexandra Fiord Highland and Lowland

It is well known that environmental factors play a large role in determining fungal communities in soil. This study showed that soil pH, moisture, temperature and DOC and DON content are the most influential factors affecting fungal communities in Alexandra Fiord Highland and Lowland. The Dolomitic Site of AFH had high soil temperature with low moisture content which coincided with lowest fungal diversity and abundance while both the Granitic Site and Lowland Site showed high fungal diversity and abundance. Soil water content influences communities both directly and indirectly through impacts on oxygen concentrations and nutrient availability. Flooding reduces soil oxygen levels and selects for facultative and anaerobic microbial organisms (Drenovsky et al. 2004).

Northern latitude soils tend to contain large quantities of soil organic matter which may include DOC and DON that accumulate and persist due to low temperatures (Neff and Hooper 2002). This dissolved organic matter in soils may play an important role in the nutrition of soil and arctic vegetation, which could potentially affect fungal communities (Drenovsky et al. 2003). Myers et al. (2001) studied fungal community shifts during seasonal change and concluded that the availability and types of organic substrate which may alter chemical constituents in soil fosters fungal growth and their community structure. Although our samples were collected only during the summer, it
is expected that seasonal change in the Arctic would influence fungal community structure by changing chemical constituents in soil. To confirm this hypothesis, soil samples should be collected periodically.

Although organic nutrients have an important role in determining fungal community structure, their role, especially on the arctic fungal community is poorly understood. Only limited information is available on the effect of nitrogen on saprotrophic basidiomycetes in temperate ecosystems, but these are from surveys of their hyphae and fruiting bodies and often are contradictory (Lagomarsino et al. 2007; Peter et al. 2001). Thus, it is difficult to draw conclusions about how C:N ratio affect fungal communities in polar desert. Our results suggested that even though dissolved organic carbon and nitrogen correlated with the fungal community data, the diversity at each site did not seem to be strongly affected. The Highland Dolomitic and Granitic Sites, which both maintained very low DOC and DON had very different results in terms of diversity and abundance. It is likely that most of fungal genotypes we sampled were saprotrophic fungi as the sampling tended to exclude roots from the soil samples. From our results and scant information available on the effect of nitrogen availability on saprotrophic fungi in the Arctic, we can only speculate that the effect of nitrogen in polar desert ecosystems is not as marked as with mycorrhizal fungi. Thus, nitrogen availability may not play such an important role in shifting fungal communities in the Arctic. However, nitrogen may affect more of a role at the functional level rather than at the structural level or simply temporal changes in nitrogen availability in soil over long periods of time may have an effect on fungal communities. In addition, saprotrophic
fungi in temperate soil appear to be less consistent regarding the effect of nitrogen, perhaps reflecting the fewer studies overall.

Among all the environmental variables, our results certainly show that pH has the strongest correlation with the community data. Hence, it is the most influential factor in determining fungal community structure, which corresponds with other studies (Blagodatskaya et al. 1998; Fernández-Calvino and Bååth 2010; Rousk et al. 2010). The soil pH regulates dissolved organic matter availability, solubility of metals, gross N fluxes and availability of other micronutrients, which may indirectly control microbial activity and diversity in soil (Bååth et al. 1995; Robinson et al. 2004). In the literature, the highest fungal diversity was detected at neutral to acidic pH in temperate forest soils (Lauder et al. 2008) and because most fungal species absorb nutrients better and show optimal growth at neutral pH, it is not coincidental that the granitic site, even with low DOC and DON showed high fungal diversity. The Lowland site, with slightly acidic soil, also had high diversity. Blagodatskya and Anderson (1998) reported high fungal and bacterial biomass ratio at pH of 6 suggesting that many fungal species prefer acidic soils. However, the pH they tested ranged only from 3 to 6 and therefore, the effect of pH on fungal communities from acidic soils to neutral soils to basic soils cannot be compared. A very recent study by Rousk et al. (2010) strengthened the previous theory who demonstrated the impact of pH (ranging from 4 to 8) on fungal communities in vitro which resulted in high growth rate of fungal species at slightly acidic to neutral pH and low fungal growth rate in high pH soils. These results also reflect our findings where the highest fungal diversity was seen in soils ranging from 5 to 7. Conversely, the lowest diversity was detected in basic soils (pH~8.4). This low
diversity at high pH may be due to competition with bacteria in soil. According to Fernández-Calvino and Bååth (2010), the bacterial growth rate was much higher in higher pH soils. This suggests that the competitive pressure exerted by bacteria in soil may have inhibited fungal activity and growth at high pH, causing fungal community shifts which help explain our observations of low diversity in higher pH soil at the Highland Dolomitic Site.

2.4.5 Methodological limitations

LH-PCR was used to detect fungal DNA to identify community differences and to determine changes in community structure between sites. Although LH-PCR has been widely used to study fungal communities, several potential limitations have been reported. Fungi are difficult to sample and keep separate due to their range of delicate and diffuse structures (Avis et al. 2006). Moreover, obtaining fungi in their natural environment can result in the inadvertent collection of complex mixtures of different structures and species even if steps are taken to target only specific types of species (Avis et al. 2006). Thus, collecting different species from a heterogeneous mixture (i.e. soil) could contribute to conflicting interpretations of the distributions of fungal communities.

In the LH-PCR fragment analysis, it was assumed that each fragment represents a different fungal genotype. Although some fragments may represent a single taxon, a fragment may represent more than one taxon since more than one taxonomic group can have LH-PCR products that are similar enough in length to be placed in the same bin (Ritchie et al. 2000). Since the software relies upon an algorithm for binning the
fragments into unique genotypes, it is also possible that profiles have contiguous amplicon distributions that are difficult to resolve (i.e. a single genotype may be placed in two different bins if it is sized between bins). Therefore, the diversity of fungal communities is likely to be underestimated.

Another methodological limitation is selectivity of LH-PCR. Although LH-PCR is a widely used molecular approach for community ecology analysis, it most likely detects active fungal species. Dormant cells often have thick cell wall-like structures that are less likely to fracture during DNA extraction. Because dormant cells are less likely to be detected by LH-PCR, diversity of this component may have been underestimated. Nevertheless, the LH-PCR approach is an effective and sensitive method for detecting differences in the active microbial community at various spatial scales (between and within-site variability).

The underestimation of diversity could also be facilitated by the ethanol DNA precipitation procedure (Fregel et al. 2010). The concentration of DNA tends to be reduced during the procedure and the method is not ideal for samples with low DNA concentrations or small DNA fragments. Despite the limitations, we believe that our DNA samples were successfully precipitated using ethanol precipitation, though some of the peaks were low in abundance and richness.

The ITS regions have been widely used to study fungal communities using ITS primers. However, the specificity of primers could lead to biased results. The selected primers have broad specificity to fungi and do not amplify bacteria (Martin and Rygiewicz 2005). However, the primers are homologous to some ribosomal sequences
from eukaryotic species that are unrelated to fungi (Ranjard et al. 2001). Although most of these DNAs are unlikely to be encountered in soil, we cannot totally exclude the possibility that some closely related organisms could harbor amplified sequences. To date, the primers are still widely used and have successfully amplified fungal DNAs in many studies to characterize complex communities (Mills et al. 2007).

Fungi exist in nature as complex, community-oriented entities responsible for processes that define and shape their surrounding environments. For a better understanding of the overwhelming hidden diversity that is present within fungal communities and their associated ecosystems, it would be necessary to have concerted interdisciplinary efforts, greater dissemination of information, and perhaps even higher resolution methods to understand these complexities. Nevertheless, many culture-independent approaches such as LH-PCR will still continue to help narrow our knowledge gap in understanding of fungal community’s role within the global ecosystem.
Conclusions

Although the fungal communities in Alexandra Fiord did not show strong geospatial patterns, the communities showed differences in terms of diversity and abundance at each site. Our results provided strong evidence that environmental factors are more important than geographic distance in determining fungal community structure. Moreover, it was the pH gradient in soil that was the most influential factor affecting fungal communities in Alexandra Fiord. Because the pH gradient may alter other soil properties such as carbon availability, it is difficult to identify specific factors affecting fungal communities. Therefore, it is necessary to study interactive effects (i.e. the effect of combination of carbon quality and soil pH) in both natural and experimental settings. In addition, further investigation such as the identification of each genotype via sequencing would bring more accurate results to determine the structure of fungal communities and give an insight to whether individual species in the Arctic are indeed pH tolerant.

This study compared fungal communities in Alexandra Fiord and studied how the communities are affected by environmental factors. However, this study only considered the spatial distribution of genotypes across the arctic landscape. With samples that are collected more frequently, examining the temporal change of fungal communities in the Canadian High Arctic would be possible. Fungal diversity is expected to increase with increasing temperature in the Arctic due to climate warming. Because fungal communities are heavily involved in respiration and decomposition, monitoring fungal communities in the Arctic could give an insight to how fungal species
could contribute to global warming in the Arctic. The outcome of these uncertainties need further research and could be critically important to global carbon cycle and in predicting positive and negative feedbacks to atmospheric CO₂ levels and climate change in the Canadian Arctic regions. In addition, a thorough integration of microbial ecology into the field of biogeography (i.e. studying the distribution of species spatially and temporally) may provide a more comprehensive understanding of the factors controlling biodiversity and biogeochemistry.
Literature cited


