# THE INFLUENCE OF WARMING, SITE CHARACTERESTICS, AND HOST PLANT ON ROOT-ASSOCIATED FUNGAL COMMUNITIES FROM ALEXANDRA FIORD IN THE CANADIAN HIGH ARCTIC

by

Kei E. Fujimura

B.S., University of Wisconsin, 1991

M.S., Oregon State University, 1999

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Abstract

Arctic systems are expected to be impacted earlier and more severely by global warming than temperate ecosystems. However, much of the research on the impact of warming on arctic ecosystems has centered on plant communities. One objective of this thesis was to examine how passive warming would impact the root-associated fungal community at Alexandra Fiord, Nunavut. The root-associated fungal community consists mostly of mycorrhizal, dark-septate and hyaline-septate fungi, which are considered important mutualists in arctic ecosystems. The objective was to compare the fungal community from plots warmed by open-top chambers to ambient plots, using two methodologies: 1) fungal DNA extracted directly from root tips with terminal restriction fragment length polymorphisms (T-RFLPs) used to estimate variation, and 2) fungal cultures isolated from root tips to which PCR-RFLP techniques were applied to assess variation.

T-RFLPs were used to examine the root-associated fungal community on *Salix arctica*. Differences between the communities were analyzed using canonical correspondence analysis (CCA). Genotype diversity was tested using a 2-way, 2-stage, nested ANOVA. Warming did not significantly change genotype cumulative frequency or diversity of the root-associated fungal community, but cumulative frequency tended to increase on the warmed plots. Genotype richness was significantly different according to site, which was correlated with differences in soil chemistry.

Again site, not warming, was the main factor that distinguished the root-associated fungal community of *Salix arctica, Saxifraga oppositifolia, Cassiope tetragona*, and *Dryas integrifolia* based on fungal cultures. Warming did not have a detectable impact on cumulative frequency and diversity, based on CCA and a nested, 3-way ANOVA. Fungal cultures were identified based on sequence analysis and morphology. *Phialocephala fortinii*

was the most frequently identified taxon, but almost half of the fungal isolates remained unknown.

The root-associated fungal community was examined along a glacier forefront characterized by a directional, non-replacement primary plant succession pattern. CCA was used to examine genotype frequency; linear regressions were used to test for changes of cumulative frequency and diversity as succession advanced. The fungal community on only one of the host plants increased in frequency and richness as succession advanced. The dark- and hyaline-septate endophyte communities were distinct on different host plants, providing evidence for host specificity and higher diversity than previously reported.

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# I. Introduction

## A. *Rationale*

The root-associated fungal community includes mycorrhizal fungi, dark and hyaline septate fungi, and possibly parasitic or pathogenic fungi. Of these, the mycorrhizal and dark- and hyaline-septate fungi are the most abundant members of this community. Mycorrhizal fungi are known to have mutualistic relationships with vascular plants, and are important components of most ecosystems. Dark and hyaline septate fungi have been reported to be both pathogenic and mutualistic. Their role as mutualists is hypothesized to be greater in arctic ecosystems with the absence of arbuscular mycorrhizae (Bledsoe et al. 1990).

Global warming is an important source of disturbance of arctic ecosystems. The primary effect of global warming is the increase in mean temperature. Global warming is also significant because of its secondary effects; it has been linked to the increase of sea levels, hurricane occurrence, fire and insect outbreak in coniferous forests, and species extinction. These problems are compounded by the release of greenhouse gases.

Arctic environments are opportune ecosystems to examine ecological questions about the root-associated fungal community. These environments have low plant species diversity, which simplifies the complexity of examining the fungal community found on roots. In addition, arctic ecosystems are expected to be impacted more severely by global warming, and in advance of other ecosystems. By simulating warming in arctic environments using open-top chambers, insights can be gained into how warming may impact the root-associated fungal community in other environments.

Warming indirectly affects arctic ecosystems by causing glaciers to recede. This provides an opportunity to examine changes in the belowground community in response to a

unique form of primary plant succession that occurs in the high arctic, directional non-replacement succession, where host plants are not replaced as succession proceeds so diversity increases along the chronosequence.

Alexandra Fiord, Nunavut provides an excellent opportunity to study the effects of climate change on the root-associated fungal community. The biology and autecology of plants in this area have been studied extensively, and passive warming experiments are part of the International Tundra Experiment (ITEX), which was created to monitor the effects of warming in arctic regions. Alexandra Fiord also hosts a unique type of primary plant succession, which provides a natural experimental design for studying how the root-associated fungal community on a common suite of host plants responds to an increase in plant diversity along a chronosequence.

## B. *Literature review*

### 1. Concepts in community ecology

#### a) Definition of community

There has been considerable debate among ecologists regarding the conceptual definition of "community". Wilson (1991), for example, questions whether plant communities are really integrated, discrete entities. He argues that plant communities do not exist unless the definition is delimited by a list of criteria including assembly rules, niche limitation, discreteness, discontinuity, and integratedness (Wilson 1991, Palmer and White 1994).

Looijen and van Andel (1999) asserted that the problem with the definition of community is that the term is too ambiguous: 1) it can be applied to different levels of taxa,

2) no objective boundaries can be made, and 3) communities are heterogeneous with respect to species composition. They suggest the following definition: "community may be defined as a set of individuals of two or more species that occur in the intersection of areas occupied by populations of these species" (Looijen and van Andel 1999). This limits the definition of communities to be used only for coexisting species belonging to a single taxonomic group, such as phyla or class, that has a static boundary (Looijen and van Andel 1999).

Parker (2001) refutes Looijen and van Andel's definition because of scale limitations and unidentified assumptions. He argues that the scale limitation leads to ambiguity or conflict with respect to what organisms belong to a given community (Parker 2001). He identifies three assumptions from Looijen and van Andel's model which are often violated in community ecological studies: 1) there must be a unique underlying process, 2) there must be consistency of processes among replicates and, 3) there must be independence from other communities. Instead, Parker's definition combines concepts from Brand and Parker (1995) and Pickett et al. (1992): 'communities are continuous in time and space, and processes underlie composition and dynamics'. His definition includes a conceptual model where the community focuses on a single individual and its interactions with other members of the community (i.e. consumers, symbionts, pathogens, mutualists, and competitors); each individual of the community has its own set of interactions (Parker 2001).

Some argue to forgo the conceptual definition (McCune and Grace 2002, Palmer and White 1994) and use an operational definition (Palmer and White 1994), such as a "collection of organisms found at a specific place and time" (McCune and Grace 2002). This operational definition is similar to Parker's definition (2001) but does not include his conceptual model, which implies that interactions are necessary in a community. The

operational model assumes that variation in species composition is random spatially and temporally (McCune and Grace 2002). With the operational definition, conceptual or theoretical implications are circumvented (McCune and Grace 2002, Palmer and White 1994).

To avoid the ambiguous use of the term community, Fauth et al. (1996) proposed a more restricted set of definitions. 'Taxa' is reserved for phylogenetically-related species regardless of where they occur; 'communities' include all species co-occurring in one place (i.e. corresponds to the operational definition Palmer and White (1994)); and 'guilds' are sets of species exploiting the same resources (Fauth et al. 1996). Other concepts are used to describe overlapping combinations of these three terms. 'Assemblages' are phylogenetically- restricted groups that occur in a community (i.e. overlap between taxa and communities). When guilds and taxa overlap, then a group of related species exploit the same resource. 'Local guilds' are formed from the intersection of communities and guilds and are groups of species that share a common resource and occur in the same community (Fauth et al. 1996). When all three overlap, then the term 'ensemble' is used; this is a taxonomically restricted group of species that exploit the same resources, and are located in one place (Fauth et al. 1996).

The term that perhaps best applies to this study is the 'ensemble' according to Fauth (1996), because this study incorporates phylogenetic, community and guild perspectives. However, operationally, I will use community in the sense of Palmer and White (1994), which corresponds to Fauth's definition.

2. Biodiversity

a) Definitions

***Biodiversity*** is a loosely applied term, and has been used to describe diversity from the genetic level to the biome level (Hooper et al. 2005). Biodiversity can be described as the number of different genotypes, species, ecosystem types, etc. and includes the evenness of their distribution (Hooper et al. 2005). Species richness is only part of this definition and refers to the number of taxonomic units (usually species or genotypes).

***Ecosystem function*** is the effect of the activities of organisms on the physical and chemical processes of an environment. A functioning ecosystem is characterized by these processes (Naeem et al. 1999). According to Naeem et al. (1999), ecosystem functioning can be measured by quantifying rates of movement, such as nutrient transportation, or by measuring growth or production, such as plant stem growth or seed production.

***Disturbance*** used to be limited to events that were massively destructive and rare (Rykiel 1985). This definition is no longer acceptable because disturbances are not always catastrophic and can be recurring events in ecosystem. Rykiel (1985) attempted to formulate a general definition by defining disturbance as a physical force, agent, or process that causes a perturbation in an ecological component or system. Disturbance can be abiotic or biotic and: 1) can cause destruction, where biomass is quantitatively reduced; 2) can cause discomposition, where certain populations are eliminated, reduced, added, or expanded; 3) can cause interference, where matter, energy and/or processes are hindered; or 4) can be caused by suppression, where natural disturbances are prevented (Rykiel 1985). The outcome of a disturbance is perturbation, which is a deviation of values that are used to describe the properties of the ecological component or system (Rykiel 1985). Rykiel's

definition assumes that reference conditions must be known in order to understand disturbance (Pickett et al. 1989).

White and Jentsch (2001) argued that one general definition is unachievable for disturbance. They argued that disturbance should incorporate four different areas: 1) variation in disturbance events, which would include the timing and intensity of the disturbance; 2) variation in the disturbance effects within an ecosystem, which would cover spatial and temporal variation; 3) variation in ecosystem response by including differences in biota and physical environments; this would include the rates of response and species adaptation would be included; and 4) inferences of scale of observations and measurements, which are affected by observations, sampling, and analysis by the researcher. These four topics that they argue should be included are covered in the definition proposed by Pickett et al. (1989).

The definition by Pickett et al (1989) consists of identifying the object disturbed, distinguishing what is and is not disturbed, and recognizing the minimal level of hierarchical organization. The following levels are their recommended hierarchical organization: individual, population, community, ecosystem, and landscape. Disturbance, which is defined as an external force of a given level, would affect the structure, function, and attributes of that level. For example, at the community level, structural disturbance would include effects on vertical and horizontal patterns, species composition, or functional groups; functional disturbance would include effects on resource levels, competition, or mutualistic interactions; and attribute disturbance would include effects on coexistence, evenness, or dominance. At the ecosystem level, structural disturbance would include effects on functional groups, functional disturbance would include effects on fluxes in the ecosystem, and attribute

disturbance would include effects on resistance and resilience of the ecosystem. In this hierarchical organization, temporal and spatial scales are also incorporated and are defined within the context of each level. Temporal and spatial effects that occur on a broader scale would be called 'disturbance regime' such as the fire regime in grasslands, where the reoccurrence of the disturbance is every few years.

***Stability*** refers to how an ecosystem or community responds to disturbance. For ecosystems, stability would apply to populations or communities and their abiotic environment, such as analysis of nutrient dynamics (Barbour et al. 1999). For communities, stability is measured by determining how the community composition and diversity responds to disturbance (Barbour et al. 1999). Stability has two components, resilience and resistance, and the overall response to disturbance is determined by the interaction between the two (Barbour et al. 1999). On the ecosystem level, resilience is the 'ability of an ecosystem to return to predisturbance conditions' (Barbour et al. 1999), which may take a long time. Resistance is the 'ability of an ecosystem to resist changes in response to disturbance' (Barbour et al. 1999). These terms can also have community definitions. Community resilience is when the community returns to the same species composition after a disturbance, and community resistance is where species composition does not change due to disturbance (Tokeshi 1999). However, there are problems with the community resistance/resilience concepts. One problem is determining how much change in the community composition is needed before the community is considered 'disturbed'. Another problem is determining whether it is necessary for the community to return to its 'original' composition for stability to return (Tokeshi 1999). Also, community composition changes over time without disturbances (Tokeshi 1999), so these terms may be difficult to apply.

Although ecosystems may become unstable, they can still continue to function. How well an ecosystem responds to disturbance depends on its ***resilience***. Processes may be retarded, but if the ecosystem is resilient, then these processes can return to pre-disturbance conditions.

b) Assessing biodiversity

Assessing biodiversity has become an important issue because of the increased rate of loss of diversity due to anthropogenic activities. One argument used to support conservation is that preservation of biodiversity will maintain ecosystem functioning. Maintenance of biodiversity has become a surrogate for ecosystem function (Naeem 2002). For example, Naeem et al. (1995) found that higher diversity correlated with an increase in community respiration, productivity and nutrient retention in a mesocosm study, and therefore alteration of biodiversity can affect ecological processes.

Tilman et al. (1997a) examined how plant species diversity, functional diversity, and functional composition affect plant productivity, %N in plants, total N in plants, soil $NH_4$, soil $NO_3$, and light penetration. They found that functional diversity, but not plant diversity, significantly impacts these functional variables by positively affecting plant productivity and total N and negatively affecting soil $NO_3$, soil $NH_4$, plant %N, and light penetration. They also found that many species in monocultures have comparatively less biomass than when they are found in multifunctional group plots, which supports the hypothesis that higher diversity increases ecosystem productivity. They concluded that: 1) functional composition and diversity are significant determinants in grassland ecosystem processes and 2) not all plant species are equal, so the loss of one species may be more deleterious than another.

Higher species richness alone is not sufficient to explain the impact of biodiversity on ecosystem functioning. When examining competition in a resource model for plants, the variance of the model is explained more by species identification than species richness (Tilman et al. 1997a). Likewise, Hooper and Vitousek (1997) found that the identification of the functional groups explains more variance than species richness, and that species and combinations of species, rather than species richness, control yields and nutrients.

Another issue concerning how biodiversity affects ecosystem functioning is complementary effects versus selection effects (Hooper and Vitousek 1997, Cardinale et al. 2002). Complementary effect theory attempts to explain how resource use by organisms affects ecosystem processes (Cardinale et al. 2002, Loreau and Hector 2001). According to this theory, species diversity can increase while avoiding competition, and species are able to co-exist, especially in environments with limiting resources, by: 1) partitioning resources, where each species can use nutrients, water, or other resources differently instead of all species competing for or using the same resources; or 2) niche differentiation, where different species avoid using the same resources as other organisms in time and/or space (McKane et al. 2002). Loreau et al. (2001) found that feeding performance by caddisfly larvae improves in the presence of other taxa. They concluded that the increase in species diversity of other aquatic arthropods leads to interspecific facilitation.

In contrast, selection effect theory is applied when species diversity is correlated with the probability that a dominant species uses most of the available resources (Cardinale et al. 2002), so the formation of the community is heavily dependent on these dominant species. In a study where a mathematical model was used to test for complementary effects and selection effects on monocultures and mixed species of grasses, results explained by the

selection effect theory were not as reliable as by the complementary effect theory (Loreau et al. 2001). In complementary effect theory, the performance of communities can go beyond the additive effects of individual species (Loreau et al. 2001).

Schwartz et al. (2000) criticized studies that link greater biodiversity with an increase of ecosystem productivity. In both observational and experimental studies, conflicting results have been reported, with some studies having negative or no results and others that are variable through time and space (Schwartz et al. 2000). In addition, other problems with experimental studies include: 1) hidden manipulations such as weeding, which would change the diversity and composition of the experiment; 2) addition of species to poor environments, which probably would not occur in nature; and 3) extrapolation of results to the whole ecosystem when only one trophic level has been included (Schwartz et al. 2000). With theoretical models, the role of rare species may be missed in stabilizing ecosystems. Also, models may assess stability and function on the wrong scale; models may apply only at a local rather than an ecosystem scale.

Schwartz et al. (2000) concluded that where the relationship between biodiversity and ecosystem function are positive, the relationship is not linear as studies suggest, but that the function saturates after a few species or functional groups, creating more of an asymptotic relationship. For the 23 observational and experimental studies they examined where they could graph biodiversity against ecosystem function, they found that 60% of these studies produced the asymptotic graph. Although the relationship between biodiversity and ecosystem function is an important question, doubts about this relationship are prevalent because of conflicting results in finding this asymptotic relationship, and also because the number of species or functional groups to fulfill the functions of an ecosystem is not known.

However, as Loreau et al. (2001) suggested, biodiversity may not be as important in the maintenance of an ecosystem as it is in helping to facilitate changes in the environment.

Bengtsson (1998) argued that biodiversity is not mechanically linked to ecosystem functions. Simple measures of species richness assume that all species are equal in their function. This is unlikely to be true; therefore measuring diversity is pointless unless the function of species is known. He contends that knowing the species and their functions will explain the processes and stability of an ecosystem. However knowing the functions of all species is currently impossible. Even though all the functions of species are not known, linking biodiversity to ecosystem functioning remains important because more diverse ecosystems may include redundant species that could fulfill ecosystem functions when dominant species are lost. This would increase the probability of withstanding or rebounding from disturbances.

c) Rank/abundance curves

The shape of rank/abundance plots (a.k.a. dominance/diversity curve or Whittaker plots) is used to determine which species abundance model best describes the data (Magurran 2004). These rank/abundance plots are helpful in that: 1) different patterns of species richness are easily shown, 2) the relative abundance of species-poor communities is easily seen, and 3) emphasis is placed on the differences in evenness for contrasting communities (Magurran 2004).

Species abundance models can be categorized into two main groups – biological and statistical (Tokeshi 1999). The biological models are also called niche apportionment models and include the following: geometric series, broken stick, MacArthur fraction, dominance pre-emption, random fraction, dominance decay, random assortment, composite, and power

fraction (Tokeshi 1999). These biological models are based on the assumption that species divide the niche space among species that live in a community in different ways (Magurran 2004). These models have been criticized for possibly being too simplistic and confusing in terms of how the niches are apportioned, but they can be valuable tools for understanding niche differentiation (Magurran 2004).

Depending on which model fits the shape of the rank/abundance plot, interpretations can be made about how the niche space is divided in the community. Rank/abundance plots that fit a geometric series model often describe communities that are species-poor, such as those found in harsh environments or in early stages of succession (Magurran 2004). Those communities fitting the MacArthur's broken stick model (or random niche boundary hypothesis) are interpreted as having their species competing equally for one resource. However, MacArthur's broken stick model assumes that the niche space is partitioned simultaneously, which probably does not happen in nature (Magurran 2004).

Tokeshi developed a set of niche apportionment models that forgo the assumption of simultaneous niche partitioning of the broken stick model (Magurran 2004). Two of his models examine extreme cases when the least or most abundant species are invaded by new species (Tokeshi 1999). The dominance pre-emption model is where new species invade niche spaces occupied by the least abundant species in an existing community (niche fragmentation), or alternatively where a new species takes approximately half of a new niche space (niche filling) (Tokeshi 1999). In these cases, the dominant species remain so. The dominance decay model is a model of the other extreme where the largest niche space, instead of the smallest, is appropriated by new species (Tokeshi 1999).

Tokeshi has three models that examine how niche apportionment occurs when new species invade all potential niches and not just the ones occupied by the least and most abundant species. The MacArthur fraction model is similar to the broken stick model, but it assumes that the niche spaces are invaded sequentially rather than simultaneously; however, the same conclusions can be drawn from both models (Tokeshi 1999). In this model, the probability of a community being invaded depends on species abundance or niche size, so niche space of more abundant species will likely be invaded before less abundant species. This model implies a uniform distribution and may be applicable only to small communities with related species (Magurran 2004).

In the random fraction model, all species have the same probability of being invaded by a new species, so the abundance of species does not influence the chances of being selected (Tokeshi 1999). This model fits situations where new species compete for niche spaces randomly over an existing niche that is already occupied by an assemblage of species (Magurran 2004). Magurran (2004) finds this model to be innovative with a wide range of applications. The power fraction model is similar to the random fraction model, but it is used for species rich assemblages because most of the niche apportionment models are applicable to communities with small species assemblages (Magurran 2004).

The random assortment model assumes that there is no relationship, or a weak one, between niche apportionment and species abundance (Magurran 2004), so the abundances of species are independent of each other (Tokeshi 1999). This may be used for situations where communities are in a state a flux from major environmental changes and competition is not limited by species abundance (Magurran 2004).

The composite model is achieved by taking two or more of these niche apportionment models into account to describe how a niche is divided. Tokeshi realized that using only one model may be too simplistic for a community; however, knowing where to set the boundary between more and less abundant species may be problematic (Magurran 2004).

Statistical models were initially created so researchers could objectively compare species abundance between communities (Magurran 2004). Even though some of these models have been labeled as statistical, ecological implications have been drawn from these statistical models. For example, the log normal model is a statistical model, but the ecological implication is that it explains situations where new species come to a niche in a random order rather than in fixed intervals such as in the geometric series model (Magurran 2004). The log normal model has been found to fit many datasets and is commonly used (Magurran 1988). However this model has been criticized because it requires a large number of species so the log normal distribution may be a mathematical artifact of a large sample size and so may have few biological implications (Magurran 1988, Tokeshi 1999).

These models are useful in assessing plant communities based on species abundance distributions. Arctic ecosystems are considered harsh environments and, as expected, plant communities in the arctic fit the geometric series model (McKane et al. 2002). These species abundance models can be used to assess the root-associated fungal community and test if these communities fit models similar to their plant counterparts. These models will also be helpful in assessing how the root-associated fungal community responds to the direct, non-replacement succession (see below) found on Alexandra Fiord.

3. Methods to assess root-fungal communities

The techniques used in this study to examine the root-associated fungal communities included morphotyping, PCR-RFLP, T-RFLP, and DNA sequencing. The term 'root-associated fungal communities' will be used because some of the techniques do not differentiate mycorrhizal, endophytic, and pathogenic fungi. Because the techniques used are commonly applied for examining mycorrhizal fungal communities, much of the review will be based on this group of fungi.

Sporocarp collections and morphotyping have been commonly used to assess mycorrhizal fungal communities. However, sporocarp production was found to be an inaccurate estimate of composition of ectomycorrhizal fungi found on root tips below ground (Gardes and Bruns 1996a, Dahlberg et al. 1997, Jonsson et al. 1999), and corresponds to only approximately 20% (Jonsson et al. 1999) to 30% (Dahlberg et al. 1997) of the belowground ectomycorrhizal fungi. Often the most abundant sporocarps do not correspond with the most abundant mycorrhizae (Gardes and Bruns 1996a). Ectomycorrhizal fungal species may rarely, or never, fruit, or may produce small or hypogeous fruiting bodies that are missed in surveys, which could potentially lead to inaccurate estimation of mycorrhizal fungal species found on root tips.

Ectomycorrhizal communities described by morphotyping is limiting in that at least half of the species are completely unknown (Gardes and Bruns 1996a), especially if the morphotype comes from the field (Kårén et al. 1997). Morphotyping also requires a high level of skill (Kårén et al. 1997), and so often takes more time to learn than molecular based techniques (Dahlberg 2001). Better results in identification through morphotyping require more phenotypic characteristics, but then fewer samples can be examined (Horton and Bruns 2001). The efficiency of distinguishing mycorrhizal taxa improves with RFLP analysis over

morphotyping, circumventing phenotypic plasticity, where multiple species may be grouped as the same morphotype (Horton and Bruns 2001). Jonsson et al. (1999) distinguished 20 morphotypes from 7152 mycorrhizae but found 42 RFLP-types from 212 root tips that successfully amplified.

PCR-RFLP has been a useful tool in researching mycorrhizal fungal communities. The internally transcribed spacer (ITS) region of the nuclear-encoded ribosomal RNA (nrDNA) gene repeat is often used because: 1) it is readily amplified with fungal-specific primers, allowing amplification of fungal DNA from mixed genomes, such as plant and fungal DNA in ectomycorrhizal root tips (Gardes and Bruns 1996a) and 2) it is divergent enough for identifying species within a genus (White et al. 1990). The nrDNA is often used for fungal studies because DNA sequences are highly conserved among organisms, variability is high between species and minimal within a species (Egger 1995), and the ribosomal repeat is a multi-copy gene (Gardes and Bruns 1993), making it easier to amplify.

Studies involving PCR-RFLP of the ITS region have focused on comparing above and belowground fungal species composition (Gardes and Bruns 1996a, Dahlberg et al. 1997, Jonsson et al. 1999); describing differences in composition due to treatment or changing environments (Horton et al. 1999, Erland et al. 1999, Kernaghan 2001); or describing changes in composition due to succession (Nara et al. 2003). In addition to the confirmed lack of correlation between above and belowground fungal composition, these studies find that a few widespread species generally account for most of the abundance of mycorrhizal fungi (Gardes and Bruns 1996a, Dahlberg et al. 1997, Jonsson et al. 1999, Horton et al. 1999, Erland et al. 1999, Kernaghan 2001) and that spatial variation is a large determinant for species composition, at least for Swedish forests after a low intensity fire (Dahlberg 2001).

Terminal Restriction Fragment Length Polymorphism (T-RFLP) analysis is a relatively rapid and accurate, PCR-based tool to identify taxa (Avaniss-Aghajani et al. 1996, Martínez-Murcia et al. 1995). It has been used for examining microbial communities found in sludge (Marsh et al. 1998, Liu et al. 1997), termite guts (Liu et al. 1997), aquifer sand from groundwater (Liu et al. 1997), and for determining the effects of temperature on the microbial community in rice fields (Chin et al. 1999). A few more ectomycorrhizal fungal community studies have been based on T-RFLP analysis, such as analyzing how the increase of $CO_2$ would change the mycorrhizal fungal community (Klamer et al. 2002) and determining the soil vertical distribution of ectomycorrhizal fungi (Dickie and Koide 2002). T-RFLPs are helpful when organisms have indistinct morphologies (Avaniss-Aghajani et al. 1994), and this technique avoids creating time-consuming cloning of organisms, which may not work for all species (Bruce 1997).

T-RFLPs are similar to restriction length polymorphisms (Clement et al. 1998) in that they are both used to characterize the ITS region of the nrDNA for differentiating taxa in describing community diversity. However, the protocol for T-RFLPs differs from RFLPs in that each primer is fluorescently labeled with a different dye, and fragments are separated on a 6% polyacrylamide gel rather than an agarose gel (see Fig. 1). The fluorescence allows the samples to be detected by automated DNA sequencers/fragment analyzers and because of the polyacrylamide gel has higher resolution (Avaniss-Aghajani et al. 1996), allowing detection of fragments that are only 1 or 2 base pairs different (Tötsch et al. 1995). Once the ITS region is amplified with the fluorescent dye-labeled primers, restriction endonuclease enzymes are used to digest the region, as with the RFLP methodology. Instead of visually detecting multiple bands as with RFLP analysis, only one fragment, the terminal fragment, is

detected because the laser only detects the digested product with the fluorescent dye (Fig. 1). If both primers are labeled, two sets of results are generated: one from the forward primer and the other from the reverse primer. Multiple bands in PCR products, which indicate the presence of more than one organism, are problematic in RFLP analysis because assigning fragments to their respective fungus is difficult or impossible. In RFLPs, individual taxa are indicated by a unique pattern of multiple bands, but when multiple taxa of fungi are on the root, fragment patterns become too complex for analysis. T-RFLPs circumvent this problem by detecting only terminal fragments, so in theory, each fragment should represent a unique taxon. Several restriction enzymes may be needed to differentiate taxa that share restriction sites, but multiple restriction enzymes are used for RFLP analyses as well.

Clement et al. (1998) list potential problems with T-RFLPs: 1) PCR primers may differentially amplify certain species, therefore, measurements of relative abundance in a community may not be accurate; 2) unequal relative abundance may occur due to different optimum annealing temperatures for different species; 3) evidence of fragments may be limited by electrophoresis technology; and 4) accurate community analysis needs multiple digestive enzymes. The problems listed can also be applied to RFLP analysis as well, and cannot be resolved without more advanced technologies that reduce the number of samples that are analyzed.

T-RFLPs is a valuable tool in mycorrhizal fungal community analyses. Like bacterial systems, mycorrhizal fungi are often morphologically indistinct. In addition, this method circumvents the phenotypic plasticity of the mycorrhizal fungi on different hosts. Unlike RFLP analyses, this tool can detect and distinguish multiple mycorrhizal fungi on the same root tip.

Although RFLP and T-RFLP analyses are powerful tools for assessing communities, they are limited in identifying taxa. One way to identify taxa using RFLPs and T-RFLPs is to compare fragments with a database that already exists. However, comparing RFLP fragments with those in databases created by other researchers is often not feasible because restriction enzymes and primers are not standardized. DNA sequencing is important in filling this gap, especially when the goal is to identify unknown taxa. Some reasons why sequencing is more successful are: 1) there is a central database (GenBank) where scientists deposit their sequences, and 2) sequences are not restricted by choice of endonucleases or primers; as long as the unknown sequence has an overlapping segment in GenBank, then identification to at least order is plausible. The caveat with comparing sequences from GenBank is that submitted sequences are not checked for accuracy, so the identification of the sequences may not be reliable (Bridge et al. 2003).

4. Concepts in mycorrhizal fungal community ecology

Mycorrhizal fungal community ecologists often have the triple task of describing and interpreting the fungal community structure as well as extrapolating their results to the plant community. Some topics that are addressed in mycorrhizal fungal community ecology are complementary effects (e.g. Koide 2000, Perry et al. 1989), community structure (e.g. Horton and Bruns 1998, Dahlberg et al. 1997, Gardes and Bruns 1996a), and the role of mycorrhizae in plant communities' resistance to change (e.g. Horton and Bruns 1998). Mycorrhizal fungal community ecologists have approached the issue of complementary effects by examining facilitation and niche differentiation (e.g. Dickie and Koide 2002, Helm et al. 1999, Titus and del Moral 1998). Facilitation is an important concept in mycorrhizal community ecology because of the guild concept, where the diversity of the mycorrhizal

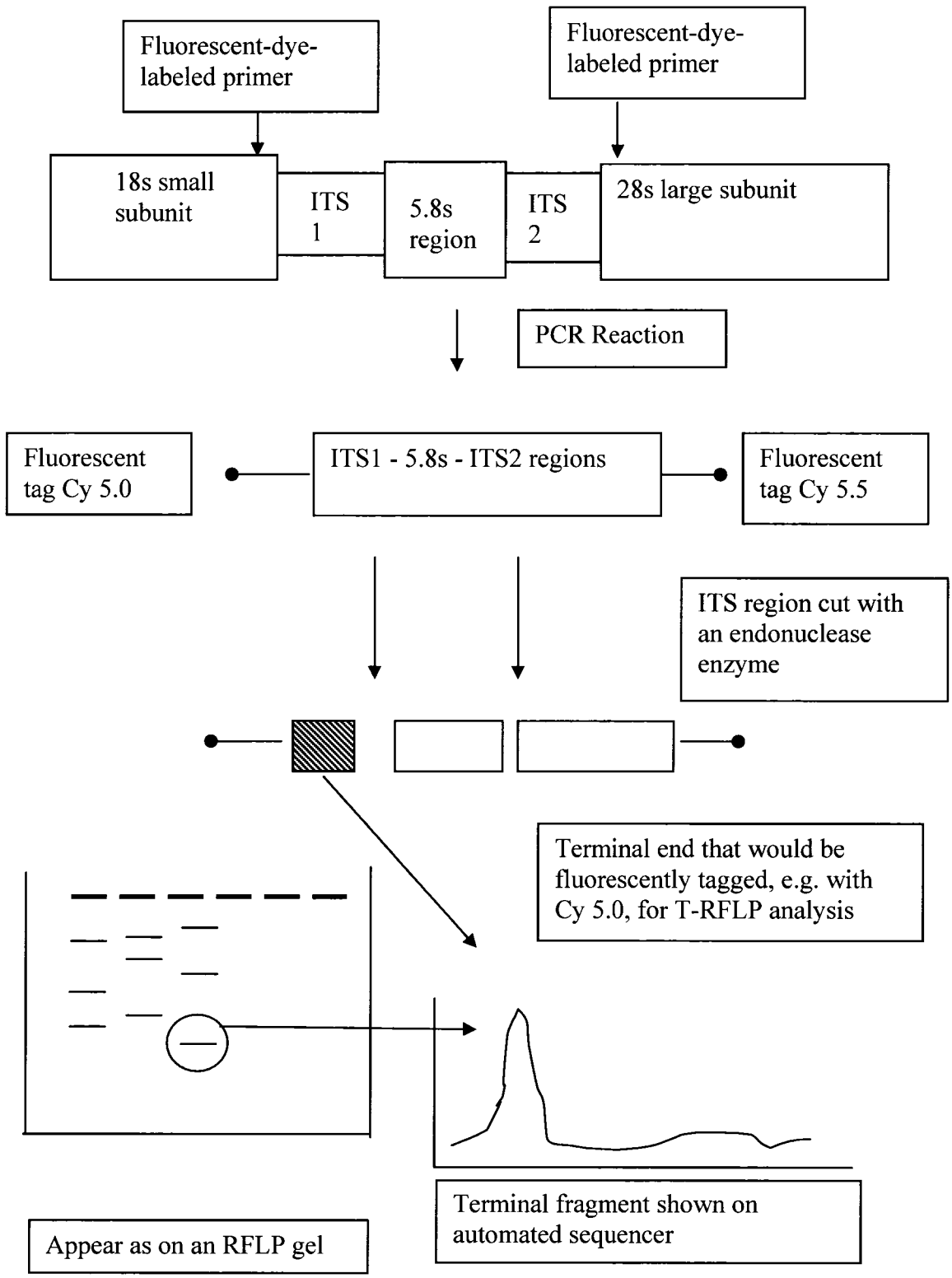


Fig. 1.1 Example of how T-RFLP is obtained in comparison to RFLP

fungal community and the plant community can stabilize their plant-soil ecosystem after disturbance or stress (Perry et al. 1989).

The benefits of linkages between plants by mycorrhizal fungi, are generally expressed by how plant communities may profit rather than by how the fungal communities may profit. Possible benefits for plants include: 1) seedlings may benefit by linking into a larger fungal network via fungal hyphae (Newman 1988); 2) interplant exchange of nutrients (Newman 1988); 3) interspecific competition between plants may be altered if nutrients are received from one central network (Newman 1988); 4) competition between plants may be reduced (Newman 1988); 5) nutrients from dying plants may pass directly to living plants (Newman 1988); 6) stabilization of succession patterns because some fungi could associate with both pre- and post-disturbance plant hosts (Horton and Bruns 1998); and 7) improved plant survival (Trappe and Luoma 1992).

Fungal linkages between plants have been demonstrated both in the laboratory and in the field. Phosphorus transfer between *Pinus sylvestris* and *Pinus contorta* via *Suillus bovinus* was found *in vitro* using $^{32}P$, in which the labeled phosphorus did not move only to the plants but throughout the whole fungal network (Finlay and Read 1986). Simard et al. (1997) showed a net transfer of carbon between *Betula papyrifera* and *Pseudotsuga menziesii* in the field. By shading *P. menziesii* seedlings and not *B. papyrifera*, they found that *P. menziesii* seedlings are sinks for carbon, and that carbon could transfer from a sink to a source or along a nutrient gradient. They also found that carbon exchange occurs between the two ectomycorrhizal plants (*B. papyrifera* and *P. menziesii*), but not with the arbuscular mycorrhizal plant *Thuja plicata*. Horton et al. (1999) suggested that linkages between ectomycorrhizal and arbutoid plants allow outplanted *P. menziesii* seedlings to survive in an

arbutoid stand of *Arctostaphylos*. They conclude that linkages probably do not exist between ecto- and arbuscular mycorrhizal because outplanted *P. menziesii* seedlings died in arbuscular stands of *Adenostoma.*

Koide (2000) offers two strategies for the role of complementarity in root colonization by arbuscular mycorrhizae. One strategy is where the fungi are complementary to each other. Although this would allow fungi to coexist on the same root, it does not explain why some antagonism happens between fungi (Koide 2000). The other strategy he proposes is that the function of the fungus is complementary to those of the plant, which would lead to redundant species on roots and may explain why some root-fungus relationships have a high level of specificity. The latter is similar to the application of coexistence theory to ectomycorrhizae, where plants select more beneficial fungi by lengthening or shortening time of root tip mortality (Hoeksema and Kummel 2003). These concepts may apply to ectomycorrhizal fungi as well, but competition (Wu et al. 1999), different life strategies, such as colonization rate and life spans (Hoeksema and Kummel 2003), must also be considered.

Another form of complementarity is niche differentiation. Although niche differentiation is one of the oldest explanations for biodiversity, it has not been tested in many ectomycorrhizal fungal communities (Bruns 1995). Recently, niche differentiation was used to explain the vertical distribution of ectomycorrhizal fungi. Dickie and Koide (2002) used cluster analysis and species diversity measures to differentiate six spatial patterns for fungi. The outcome of the distribution suggested that niche differentiation explained the vertical distribution of ectomycorrhizal fungi.

Only a few papers link mycorrhizal fungal diversity to ecosystem functioning. Van der Heijden et al. (1998) showed that higher species richness of arbuscular mycorrhizal fungi increased plant biodiversity, improved plant productivity, lengthened hyphal growth in the soil, increased phosphorus absorption of plants, and decreased the amount of phosphorus in the soil. They concluded that there is probably a feedback loop where the plant benefits from the increased amount of phosphorus, and the fungi prosper due to increased carbon, indicated by more hyphal growth.

Baxter and Dighton (2001) examined if higher diversity of ectomycorrhizal fungi would affect plant growth and nutrient acquisition. They concluded that ectomycorrhizal species diversity is more influential in plant biomass and nutrient uptake than the species composition or rate of colonization. Although a pioneering paper in linking mycorrhizal diversity to ecosystem function, this study examined a community with low diversity of mycorrhizal fungi (Leake 2001). This may limit applications due to its simplicity of being an *in vitro* study. Another confounding factor included using peat and vermiculite for the growth media, which adds excess nutrients. As a result of these short-comings, it may be premature to draw conclusions about the link between ectomycorrhizal fungi diversity and ecosystem functioning (Leake 2001).

a) Mycorrhizal fungal community structure of arctic and alpine systems

Examining mycorrhizal fungal communities in alpine and arctic systems is preferable because confounding factors due to direct human contact, such as logging or fire suppression, that hamper studies in temperate forests have minimal impacts upon these arctic and alpine systems (Trappe 1988). Another advantage is that these systems are thought to be relatively simple in comparison to mycorrhizal fungal communities of temperate systems (Read 1993).

Alpine and arctic systems share similar environmental stresses such as short growing seasons (Trappe 1988, Haselwandter 1987), low air and soil temperatures, and large seasonal and diurnal temperature fluctuations (Haselwandter 1987). Because of harsher environmental factors found in these systems, traits such as longevity and mycelial spread of individual fungal genets may be important (Gardes and Dahlberg 1996).

Dark-septate endophytes (DSE) are ubiquitous in both alpine and arctic systems (Bledsoe et al. 1990, Cázares 1992). Although Kohn and Stasovski (1990) reported no DSE were found on root tips of plants from Alexandra Fiord, samples of DSE from this area were found later (see Chapter 3). Hyaline septate hyphae that are reported and found on several plants may have the same ambiguous function of being either pathogenic or mutualistic, as has been found with DSE (Jumpponen and Trappe 1998).

Arbuscular mycorrhizae (AM) are scarce in the arctic (Bledsoe et al. 1990, Kohn and Stasovski 1990) and higher elevations in alpine systems (Haselwandter 1987). Although, Dalpé and Aiken (1998) found approximately 10% of *Festuca* species are associated with arbuscular mycorrhiza in the high Arctic, this is contrary to previous studies where no or very little AM was found (Bledsoe et al. 1990, Kohn and Stasovski 1990). The discrepancy between these may be due to the small sample size used by Bledsoe et al. (1990) and Kohn and Stasovski (1990) (Dalpé and Aiken 1998). Regardless, AM appear to be scarcer in arctic regions and in higher altitudes of alpine systems. Both AM and ectomycorrhizae have been found on *Salix* spp. in alpine studies (Trowbridge and Jumpponen 2004) while only ectomycorrhizae have been found in arctic *Salix* systems (Väre et al. 1992).

Because AM are scarce in the arctic in contrast to alpine systems, and DSE are common in both arctic and alpine, Bledsoe et al. (1990) suggested that DSE may replace the

functional role of AM in arctic systems. Jumpponen (1999) suggested that the DSE, particularly *Phialocephala fortinii*, may allow for transport of carbohydrates between plants through fungal linkages, as this function has been found for ectomycorrhizal fungi between *Betula* and *Pseudotsuga* (Simard et al. 1997). This was suggested because the same genet of *P. fortinii* is found on nine different plant species that are classified as ecto-, ericoid, and non-mycorrhizal (Jumpponen 1999).

Ericoid mycorrhizae are found on dwarf shrubs in the high arctic and nutritionally stressed alpine plant communities (Haselwandter 1987). Haselwandter (1987) suggested that ericaceous plants are capable of using more complex nitrogen sources such as proteins or amino acids, which is supported by findings that ericaceous plants take up amino acids in alpine regions (Michelsen et al. 1996). Ericoid mycorrhizal fungi have also been found to access N and P by producing enzymes that break down structural components of litter such as pectin and hemicellulose in plant cell walls, monophenols, tannins, polyphenols, and lignin (Smith and Read 1997). Although ectomycorrhizal fungi break down these structures as well, the production of these enzymes appears to be less common than by ericoid mycorrhizal fungi (Smith and Read 1997).

The mycoflora of alpine and arctic systems are similar in many aspects. Sporocarps of ectomycorrhizal fungi in both systems are sparse in comparison to lower elevation, temperate environments (Trappe 1988), which may be due to climatic factors which strongly influence fruiting (Gardes and Dahlberg 1996). Preliminary data from Alexandra Fiord indicate that several species, such as *Russula* sp., *Cortinarius* spp. and *Inocybe* sp., and *Cenococcum geophilum* are dominant. *Cortinarius* spp. are dominant species (comprising 20% of the abundance) on *Salix arctica* and *Dryas integrifolia* roots from the Canadian arctic

archipelago (Gardes et al. 2000). Contrary to the findings of Kohn and Stasovski (1990), *Cenococcum geophilum* was found in the present study as well as fruiting bodies of *Lycoperdon* and *Helvella*. *Lycoperdon* spp. are reputed to be mycorrhizal with *Picea abies, Pinus nigra, Pinus strobus, Pinus sylvestris, Pseudotsuga menziesii*, *Eucalyptus* spp., and *Quercus* spp. (Trappe 1962). *Helvella aestivalis* formed mycorrhizae with *Dryas octopetala*, and species of *Helvella* formed mycorrhizae with *Salix reticulata* under axenic conditions (Weidemann et al. 1998). *Helvella crispa* is reported to form mycorrhizae with *Fagus sylvatica* and *Quercus* spp., and *H. infula* with *Picea abies* (Trappe 1962).

b) Mycorrhizal fungal succession in relation to plant community succession

Glacier forefronts are commonly used for research on mycorrhizal fungi during primary succession. Primary succession is when pioneer species colonize virgin surfaces (Frankland 1998), and secondary succession is when the soil is nutrient poor after a disturbance (Smith and Read 1997). Mycorrhizal fungi may improve nutrient-poor conditions for latter species as detected by increasing diversity after volcanic disturbances (Titus and del Moral 1998) and glacial tills (Helm et al. 1999). This facilitative nature of mycorrhizal fungi is inferred by the successional pattern described for primary succession, which starts with non-mycorrhizal plants, AM plants, then ECM plants (Read 1993) and/or ericoid plants (Cázares 1992). Ectomycorrhizae are thought to colonize in older soils because they have access to nutrients contained in organic residues that are more abundant in later stages after accumulation of organic matter (Read 1993), such as the increase of nitrogen and organic matter (Jumpponen et al. 1998) along a chronosequence, which is a sequential change of related variables in certain properties, from an alpine glacial forefront.

Changes in carbohydrates supplied by the host (Dighton and Mason 1985), nitrogen availability (Baar 1996), and soil conditions (Termoshuizen 1991, Kranabetter and Wylie 1998) are some of the mechanisms suggested for primary succession to progress. For plant communities, primary succession can depend on life history traits, such as seed size and growth rate, maximum height of the plant, seed rain, and competition. Facilitation and initial site conditions are important for the rate of change and for species composition and productivity (Chapin et al. 1994). As found with plant communities, primary succession for mycorrhizal fungi probably is not dependent on a single variable. Several researchers found that changes in one variable are not enough to describe fungal succession (Termoshuizen 1991, Baar 1996, Helm et al. 1999, Kranabetter and Wylie 1998).

Svoboda and Henry (1987) described three types of succession: 1) directional, replacement succession with low resistance; 2) directional, non-replacement succession in high resistance environments; and 3) non-directional, non-replacement succession in extreme environments. In directional replacement succession, succession goes through seral stages with species replacement until a relatively stable ecosystem is reached. In directional, non-replacement succession, species are not replaced but live in co-existence with the invading species, which expand slowly. In these systems, in which polar semi-deserts are an example, there is enough space for expansion. Non-directional, non-replacement succession is found in extreme environments, such as polar deserts where few species survive. Several species may invade repeatedly but fail to establish permanently.

The lowlands of Ellesmere Island fit the directional, non-replacement succession description. The mycorrhizal guild system may play an important role in plant competition (Newman 1988, Horton and Bruns 1998) if co-existence between plants is typical and

expansion is slow. Although Kropp and Trappe (1982) suggested that pioneer plants may be more host-specific, the case on Ellesmere may be different because succession does not follow the replacement of plant species but the co-existence of additional species. Moving away from the glacial forefront, the plant community starts with *Papaver lapponicum* and *Luzula confusa. Salix arctica, Saxifraga oppositifolia, Cassiope tetragona*, and *Dryas integrifolia* eventually appear, and all six species are found not only on the glacier forefront but the rest of the lowlands of Alexandra Fiord. Van der Heijden and Vosatka (1999) showed that with AM, the increase of AM fungal composition and number leads to an increase in plant diversity as well because more variety of AM fungi allow different plants to establish themselves. Perhaps the increase of ectomycorrhizal and ericaceous mycorrhizal fungi will have a similar capacity of increasing plant diversity and stability.

Understanding succession on Ellesmere Island will be different from other studies, including those that are conducted in the Arctic. Previous successional studies in the Arctic occurred in the low arctic where trees still grow (Helm et al. 1999, Helm et al. 1996, Brubaker et al. 1995, Chapin et al. 1994) while Ellesmere Island is located in the high arctic where only low shrubs are found.

5. Global climate change: effects on aboveground plant community structure

Global warming is a complex type of disturbance because not only can it have direct effects on an organism or ecosystem, it can also lead to other disturbances. For example, warming has been linked to increase fire frequency (He et al. 2002) and more intense hurricanes (Shen et al. 2000). For this study, the effects of warming will be examined on the community level even though this disturbance is classified at the ecosystem or landscape

level, according to the hierarchical organization of disturbances described by Pickett et al. (1989).

Global circulation models predict that arctic systems will not only experience warming before other ecosystems, but also undergo the greatest increase in surface temperatures due to the doubling of $CO_2$ (Shaver et al. 1992, Oechel et al. 1993, Henry and Molau 1997). Climate change will have a more dramatic effect on the arctic than other forms of disturbance, mostly due to its spatial isolation, so findings from the arctic can be used to predict how other systems may respond (Shaver et al. 1992). By the year 2100, approximately 63% of biodiversity will be altered due to climate change in the arctic, compared to other human-induced disturbances such as changes in land use (15%), introduction of exotic species (4%), and changes in atmospheric $CO_2$ and/or nitrogen deposition (18%) (Chapin et al. 2000). Warming is expected to increase more in winter months (up to 17° C) than during the summer months (~4° C) (Oechel et al. 1993, Edlund 1992), thus lengthening the growing season of plants (Edlund 1992, Henry and Molau 1997) and altering plant communities through changes in the distribution of snow in the winter, persistence of snowbeds, and pattern of snowmelt (Edlund 1992). Global climate change will likely amplify in arctic regions due to positive feedback loops that include: 1) ice and snow melt that would decrease surface albedo; 2) stabilization of the atmosphere that may trap temperature anomalies near the ground surface; 3) cloud dynamics that may amplify change (Overpeck et al. 1997); and 4) the permafrost layer melting sooner (Oechel et al. 1993). Warming in the arctic affects lower latitudes by possibly changing river run-off and the circulation of the atmosphere, and increasing atmospheric concentrations of $CO_2$ and $CH_4$ (Overpeck et al. 1997, Henry and Molau 1997).

By understanding how plants established historically, predictions of how climate change will affect plant species evolutionarily and geographically may be more accurate (Murray 1995). For example, in the early Holocene period, warming increased the number of shrubs, which parallels the present spread of dwarf shrubs (*Salix* spp., *Betula nana,* and *Alnus crispa*) in Alaska (Sturm et al. 2001). This is indirect evidence that these regions in Alaska may adapt relatively quickly to climate change (Sturm et al. 2001). Also, fossil records of some species such as *Dryas integrifolia* and *Saxifraga oppositifolia* indicate that these plants have existed since the Tertiary period (Murray 1995) and, therefore, have survived temperature fluctuations for at least 1.8 million years.

Most of the present arctic flora established approximately 6000-3000 b.p. (Brubaker et al.1995) and originated from: 1) survivors from Tertiary forests, northern refugias from Quaternary glaciation, and Pleistocene migration from Asia; 2) plants that returned during interglacial and post-glacial time from unglaciated areas; and 3) newly evolved species from the Pleistocene and Holocene (Murray 1995). According to Late Quaternary pollen records, species found in the arctic tundra are thought to have expanded southward into much of Canada (Brubaker et al. 1995).

Arctic systems are carbon sinks. Current carbon sinks are the wet and moist tussock tundra of arctic systems (Oechel et al. 1993, Shaver et al. 1992). Arctic systems have three times more soil carbon than alpine systems but only 13% of the plant species richness, which indicates active accumulation of soil organic matter and little disturbance (Chapin and Körner 1995). Release of carbon to the atmosphere is predicted to be caused indirectly and not directly from the increase of temperature (Oechel et al. 1993). Researchers have suggested that the cause of the loss of carbon from arctic systems to the atmosphere is due to

enhanced drainage and soil aeration, decrease in the water table (Oechel et al. 1993, Shaver et al. 1992, Billings et al. 1983), and increase in respiration, especially from the soil microbial community (Schimel 1995, Billings et al. 1983) of which mycorrhizal fungal hyphae are thought to be a large contributor (Rygiewicz and Andersen 1994). Change in carbon storage is somewhat constrained by the nitrogen cycle because nitrogen is the primary limiting factor in arctic systems (Shaver et al. 1992, McKane et al. 1997).

With enhanced drainage and soil aeration, decomposition and release of carbon will likely occur in systems that have large amounts of carbon storage such as high latitudinal bogs, and boreal and arctic systems (Oechel et al. 1993). Billings et al. (1983) found that a 4-8° C warming decreased the net carbon storage in the wet sedge tundra rather than increased net primary production, which they attributed to greater increase in soil respiration. However, loss from carbon storage may be for the short-term, and eventually increase in above ground plant biomass may compensate for the carbon loss (Oechel et al. 1993).

Many factors influence the impacts of global climate change on above ground plant growth such as water availability, nutrient availability, summer warmth, snowfall, (Edlund 1992, Field et al. 1992), light, and $CO_2$ levels (Field et al. 1992). For arctic systems, warming may first impact individual plants, indicated by an increase of vegetation growth (Edlund 1992). Response by plant communities would depend on the combination of summer warming, snowfall in the winter, possible drought in the summer (Edlund 1992), and resource availability (Field et al. 1992). Global warming may result in major reorganization of plant communities (Brubaker et al. 1995); however these changes for the plant community may take centuries (Edlund 1992).

Several studies have examined the impacts of global warming on arctic plants *in situ* by manipulating temperatures with greenhouses (Hobbie and Chapin 1998, Havström et al. 1993) or open-top chambers (OTCs) (Henry and Molau 1997, Stenström et al. 1997, Jones et al. 1997) placed over plots. Open-top chambers have some advantages over closed greenhouse systems by allowing in more direct solar radiation; lessening the chance of overheating; allowing herbivores and pollinators to the plants; and avoiding decreased relative humidity (Marion et al. 1997). Problems of both systems include increasing temperature extremes rather than lowering the range of diurnal temperatures, altering of wind patterns around the plant, and disturbing the sites (Marion et al. 1997, Hobbie and Chapin 1998). Problems that are unique to OTCs consist of snow accumulation, disturbance by animals (Marion et al. 1997), and only a small area can be uniformly warmed (Shaver et al. 2000).

Table 1.1 summarizes experiments of warming on dominant plants of arctic systems that will be used in this present study. Experiments using greenhouses to increase air temperature find no significance of warming on *Cassiope tetragona* (Hobbie and Chapin 1998, Havström et al. 1993), which lead researchers to conclude that perhaps nutrients rather than temperature affect *C. tetragona* growth (Hobbie and Chapin 1998). Their findings are contrary to what is found when OTCs are used, where warming did increase different factors measuring plant growth (Henry and Molau 1997). This discrepancy may be because major changes in the arctic tundra from warming of the last glaciation have little similarity in different circumarctic sites (Brubaker et al. 1995), as the two studies are in Alaska and eastern Canada. Another explanation may be that the greenhouse experiments do not allow for enough time for temperature increase to show significant differences as is found with an

OTC study on *Salix arctica*, where Henry and Molau (1997) found no significance after two years but did after four years.

Table 1.1 Summary of experiments of warming on arctic plants used for present study.

| Plant | Reference | Location | Temperature Manipulation | Plant Growth | Predictions of Adaptation Due to Global Warming |
|---|---|---|---|---|---|
| *Cassiope tetragona* | Hobbie and Chapin (1998) | Alaska, USA | Greenhouse | Biomass decrease. | NA |
| | Havström et al. (1993) | Spitsbergen, Norway | Greenhouse | Significant increase from temperature for leaf indices and shoot growth index. | Will probably not migrate south as air temperature increases. |
| | Henry and Molau (1997) | Ellesmere Island, Canada | OTC | Significant increase in plant growth. | NA |
| *Saxifraga oppositifolia* | Stenström et al. (1997) | Ellesmere Island, Canada | OTC | Increase in flowering frequency and reproductive success. | Will probably be outcompeted by graminoids and forbs. |
| *Salix arctica* | Jones et al. (1997) | Ellesmere Island, Canada | OTC | Increase of plant growth after 3-4 years of warming. Increase\\ in seed production first years of warming. Stronger impact on male willows than females. | May adjust easily to global warming because it adapts to broad range of ecosystems. |
| *Dryas integrifolia* | Henry and Molau (1997) | Ellesmere Island, Canada | OTC | Significant but not strong increase in vegetative and reproductive phenologies, seed set and weight, germination. | NA |

NA: not available

6. Impact of global climate change on root fungal communities

Much of the research on global climate change on mycorrhizae has been indirect focusing on the effects of elevated $CO_2$. Results have been conflicting showing decreased (Fitter et al. 2000), increased, and no difference for ectomycorrhizal and AM colonization due to elevated $CO_2$ levels (Fitter et al. 2000, Treseder and Allen 2000). The impact of elevated $CO_2$ on mycorrhizal growth and colonization seems to depend on: 1) mycorrhizal fungal species because some species are more sensitive than others to elevated carbon (Fitter et al. 2000); 2) availability of N, where additional N can negate the effects of $CO_2$ on mycorrhizal biomass for some systems (Treseder and Allen 2000); 3) growth rate of plants because larger plants need more roots (Fitter et al. 2000, Treseder and Allen 2000); and 4) roots lengthening which would increase mycorrhizal colonization (Eissenstat et al. 2000).

In a review by Fitter et al. (2000), only two studies that examine the increase of temperature on AM colonization are published and no studies have currently been published on the effects on ectomycorrhizae. Perry et al. (1990) speculated on the role of mycorrhizal fungi in climate change in that plant species would migrate during climate change and that sharing mycorrhizal fungi would help with the transition.

Although there is a lack of experiments that examine the impact of global warming on mycorrhizae, the rhizosphere will probably be an important factor in how ecosystems adjust. The major effects of global warming may be from its impact on soil processes rather than the increase of biomass of plants in the tundra (Hobbie and Chapin 1998). Boone et al. (1998) suggested that the rhizosphere would be more sensitive to warmer temperatures than above-ground plant parts, and that variation in soil respiration is determined by responses of root respiration and heterotrophs to temperature change. Warmer soil temperature may influence

root growth, cell elongation, initiation of new lateral roots, increase in root respiration and ion uptake, interaction with water and nutrient availability, more N mineralization, less water availability, and earlier initiation of root growth in the spring (Pregitzer et al. 2000).

Although global warming is suspected to have significant impacts on the rhizosphere, much of implications have been speculative. In addition, information on the impacts on the mycorrhizal community, in particular ectomycorrhiza, is scarce.

### C. *Research objectives and hypotheses*

Alexandra Fiord provides an opportune site to examine root-associated fungal communities. The role of these fungi in plant establishment in primary succession increases the understanding of how plants and fungi adapt to nutrient-poor conditions. Future ecological conditions are examined by use of OTCs to simulate potential global warming scenarios.

This study will be one of the first to examine the impact of global warming on the root-associated fungal community. Because global warming impacts arctic systems more intensely than temperate environments, this may give insight to the role of root-associated fungi in facilitating changes to the plant community. Although global warming is suspected to have significant impacts on the rhizosphere, much of the implications have been speculative. This study examines how warming may impact the root-associated fungal community by using both PCR-based techniques and isolating fungi from root tips. Although there should be some overlap, the PCR-based techniques are more likely to favor mycorrhizal fungi; whereas, fungal isolations would favor faster-growing root endophytes that do not fit the morphological definition of mycorrhizae.

By examining the changes of the root-associated fungal community in a direct, non-replacement succession, insight will be gained as to how this community would adjust to a changing plant community. This type of succession also has the unique characteristic where the increase in biodiversity of plants happens *in vivo* while retaining all the same plants. This study can determine if the root-associated fungal community follows a similar trend. Also, this will be the first study to examine the root endophytic community for this type of succession.

Given what the literature indicates about fungal community structure, I expect that the root-associated fungal community, based on direct DNA extraction, will differ according to site and treatment (passive warming versus ambient). The following null hypotheses will therefore be tested:

$H_0$ *1.1 The root-associated fungal community, based on DNA directly extracted from root tips from* Salix arctica, *will not differ between warmed plots and ambient plots.*

$H_0$ *1.2 The root-associated fungal community, based on DNA directly extracted from root tips from* Salix arctica, *will not differ due to site.*

Because culture studies may reveal a different perspective than direct DNA amplification studies, I expecte that the root-associated fungal community will differ according to site, treatment (passive warming versus ambient), and host plant. Therefore, the following null hypotheses will be tested:

$H_0$ *1.3 The root-associated fungal community, based on cultures isolated from root tips, will not differ between warmed and ambient plots.*

$H_0$ *1.4 The root-associated fungal community, based on cultures isolated from root tips, will not differ according to the host plants* Cassiope tetragona, Dryas integrifolia, Salix arctica, *and* Saxifraga oppositifolia.

$H_0$ *1.5 The root-associated fungal community, based on cultures isolated from root tips, will not differ due to site.*

Because culture studies only assess culturable fungi, I expect that the fungal communities described by the two methods (direct DNA amplification from roots versus culturing) will be different (despite revealing the same patterns according to site, treatment, and host plant). Therefore, the following null hypothesis will be tested:

$H_0$ *1.6 The root-associated fungal communities described by the two methods (direct extraction versus culturing) will not differ.*

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The unusual directional, non-replacement succession pattern found in high arctic systems permits me to examine how diversity on different host plants varies along a chronosequence, without the confounding factor of host plant replacement. My objective was to examine how the root-associated fungal community changes during a directional, non-replacement primary plant succession, using *Cassiope tetragona, Dryas integrifolia, Luzula confusa, Papaver lapponicum, Salix arctica,* and *Saxifraga oppositifolia* as host plants Therefore, the following hypotheses for this objective will be tested:

$H_0$ *2.1 The root-associated fungal communities will not differ along a chronosequence.*

$H_0$ *2.2 The root-associated fungal communities will not differ according to host plant.*

D. ***Thesis organization***

This thesis starts with a literature review to provide background on the concepts, theories, and techniques associated with this thesis. This provides more comprehensive information that may not be covered in subsequent chapters.

The next two chapters address the first research objective, using two different methods that collectively provide a more complete assessment of the root-associated fungal community. One method, directly amplifies fungal DNA from root tips, and the second involves isolations of fungi from roots. These two methods have described different fungal communities found on the same plant host in previous studies. Chapter 2 addresses how warming will impact the root-associated fungal community detected by direct extraction of fungal DNA from root tips of one host species and covers hypotheses 1.1-1.2. Chapter 3 addresses the question based upon fungal cultures from several host species, aseptically isolated from plant roots, and will cover hypotheses 1.3-1.5.

Chapter 4 covers research objective 2 and chapter 5 is a synthesis of the research findings. This synthesis will cover hypothesis 1.6, and will attempt to tie the three studies together. The final chapter is a summary of the thesis.

## II. Impact of warming on the frequency and diversity of the root-associated fungal community on *Salix arctica* from the Canadian High Arctic

### A. *Introduction*

Global circulation models predict that arctic systems experience will experience a greater effect of global warming before other ecosystems due to increased surface temperature and $CO_2$ levels (Shaver et al. 1992, Oechel et al. 1993, Henry and Molau 1997), decreased surface albedo, alterations in cloud dynamics that may amplify change (Overpeck et al. 1997), and melting of the permafrost layer (Oechel et al. 1993). Plant communities may change due to lengthening of the growing season (Edlund 1992, Henry and Molau 1997) by increasing air and soil temperature (Oechel et al. 1993, Edlund 1992) and altering water distribution by changing the dispersal of snow, increasing the persistence of snowbeds, and modifying the pattern of snowmelt (Edlund 1992).

The objective of this study was to examine the impacts of experimental warming on the root-associated fungal community of *Salix arctica* in the Canadian high arctic. Although there have been numerous studies on the impact of experimental warming on arctic plants (e.g. Chapin et al. 1995, Henry and Molau 1997, Hobbie and Chapin 1998, Jones et al. 1997, Sturm 2001), no studies have examined how warming may impact the root-associated fungal community even though the rhizosphere may play an important role in plant response (Hobbie and Chapin 1998, Boone et al. 1998). To date, only one study has examined the impact of warming on an ectomycorrhizal fungal community from Douglas-fir (*Pseudotsuga menziesii* Mirb. Franco) seedlings (Rygiewicz et al. 2000), where they found warming to increase species richness. Perry et al. (1990) speculated that the mycorrhizal fungal community may help facilitate migration of plants. Hobbie and Chapin (1998) suggested that

the major effects of global warming will result from the influences of increased soil temperatures and the subsequent soil processes. Increased soil temperature may influence root growth by increasing cell elongation, initiating new lateral roots, increasing root respiration and ion uptake, interacting with water and nutrient availability, increasing N mineralization, decreasing water availability, and initiating root growth in the spring earlier (Pregitzer et al. 2000). Other studies have indirectly examined the impact of warming on mycorrhizal fungi by studying how $CO_2$ fluxes may impact the community (Oechel et al. 1993). Some researchers have suggested that the main cause of the loss of carbon from arctic systems to the atmosphere is enhanced drainage and soil aeration, decrease in the water table (Oechel et al. 1993, Shaver et al. 1992, Billings et al. 1983) and increase in respiration, especially from the soil microbial community (Schimel 1995, Billings et al. 1983) of which mycorrhizal fungal hyphae are thought to be a large contributor (Rygiewicz and Andersen 1994).

We chose three distinct sites, a lowland site, highland granitic site, and highland dolomitic site, to see how soil type interacts with warming to influence the abundance, composition, and biodiversity of these communities. In order to address these questions, we used terminal restriction fragment length polymorphism (T-RFLP) analysis, a technique commonly used in prokaryotic systems and but only used recently in mycorrhizal community analysis (Dickie et al. 2002, Klamer et al. 2002).

## B. *Materials and methods*

### 1. Study Site

Samples were collected from three sites at Alexandra Fiord on Ellesmere Island, Nunavut, Canada, 78° 53'N, 75° 55'W. One site was located on the lowland (valley bottom)

and two on a mountain plateau. The lowland mesic site was at or near sea level and enclosed by 450–700 m-high plateaus to the east and west, a glacial forefront to the south, and the ocean to the north. The climate at the lowland site was relatively warm due to frequent sunny skies, relatively warm air masses from the west and south (Labine 1994), and reflection of sunlight from the surrounding cliffs and ocean (Freedman et al. 1994). The soil was granitic, which is mostly composed of quartz, feldspar, and mica. The highland sites were located on top of the western plateau at 450 m. These sites were xeric with sparse vegetation and have desert and semi-desert arctic conditions (Batten and Svoboda 1994), which accounted for the decrease of diversity of vascular plants by approximately 40% when compared to lowland areas (Batten and Svoboda 1994). The two highland sites were distinguished by soil type, dolomitic, which is distinguished by high amounts of calcium magnesium carbonite ($CaMg(CO_3)_2$), and granitic for the other site.

2. Vegetation

Alexandra Fiord has been described as a 'polar oasis' because it comprises an 8-$km^2$ pocket of arctic shrubs, mosses, lichens, and sedges nested within vast ice fields. *Dryas integrifolia* Vahl, *Cassiope tetragona* (L.) D.Don, and *Salix arctica* Pall. are the most prominent (Freedman et al. 1994) of the 92 species of vascular plants found on the lowlands (Ball and Hill 1994) as well as the less vegetated and less diverse uplands (Batten and Svoboda 1994).

The lowland site has been described as a dwarf shrub-cushion plant community (Muc et al. 1994) and is dominated by *Salix arctica* and *Dryas integrifolia*, along with *Saxifraga oppositifolia* L., *Cassiope tetragona, Papaver, Pedicularis*, sedges, and mosses. The granitic upland site, described as a *S. arctica-C. tetragona* dominant community (Batten and Svoboda

1994) includes *S. oppositifolia* and *D. integrifolia*. The upland dolomitic site is a *S. oppositifolia*-dominated community (Batten and Svoboda 1994) with no *D. integrifolia* present.

To simulate the increased temperatures predicted by global climate models, open-top chambers (OTCs), which covered 0.8 $m^2$ and were 0.3 m high, were used to increase air temperatures by 1-4° C, the predicted temperature range of global maean increases for the middle of the 21$^{st}$ century for the Canadian high arctic due to climate change (Henry and Molau 1997). Three 1-m diameter OTCs were placed on each of the three sites; in 1995 (GHR Henry 2000, pers. com.) for the lowland site, and 1993 for the two highland sites (Stenström et al. 1997). Plants that were found between 0.5 m and 1.5 m from the OTCs were harvested as controls for a total of 18 plots.

3. Field collection

Two specimens of *Salix arctica* were harvested from each of the plots in August 2000. Plants and surrounding soil were kept in Ziploc® bags (18 x 20 cm) in a permafrost cooler while in the field and in a 4° C refrigerator once back at UNBC until processed.

Two 300 gram samples of soil were collected in August 2001 from each plot. Soil was collected no more than 1 m from the harvested plant. Soils were dried and separated in a 2-mm sieve to remove rocks from the samples. One hundred grams from each of the two replicates were mixed and sent to the Ministry of Forests, Research Branch Laboratory, Analytical Chemistry Section in Victoria, BC for the following analyses: pH in water, total C and N using combustion elemental analysis, cation exchange capacity and exchangeable cations using 0.1 N barium chloride extraction, available $NH_4$-N and $NO_3$-N extracted with 2N KCl, and available P using the Mehlich III protocol.

Fungal sporocarps from Alexandra Fiord were collected and tentatively identified to genus; however, no sporocarps were found in any of the plots per se. A 2- x 2- $mm^2$ piece was extracted from each sporocarp and stored in 50% EtOH for DNA extraction. The remaining sporocarp tissue was dried for storage.

4. Sampling from roots

Plant roots were immersed in water for at least 24 hours at 4° C. The root systems were gently cleaned with water and collected in a 0.5 mm sieve (No. 35 USA standard testing sieve, W.S. Tyler, Inc.). Root systems were placed on a numbered grid for random selection of root tips. Numbers from a random-numbers table were used to select grids for sampling. The root tip that traversed or was closest to the grid was selected, and a 2-cm root section was sampled. Fifteen root tips were randomly selected, described morphologically, placed in a 1.5 ml microcentrifuge tube, and frozen at -40° C until DNA was extracted. Root tips were assessed morphologically (morphotyping) based upon characteristics such as color, tip ramification, and presence and absence of rhizomorphs, loosely following the techniques of Agerer (1987-1998) and Goodman et al. (1995). An additional forty root tips were randomly selected, frozen, lyophilized, and stored at -40° C for further DNA extractions if needed. Of these additional tips, approximately 15 were later extracted to increase number of samples. The remaining root system was frozen and lyophilized. Preparation for DNA extraction was done first for the highland sites because the plants did not appear as robust as at the lowland site. Plant tissue from stems or leaves was also sampled to determine if plant DNA would amplify with the chosen primers.

5. DNA Extraction and ITS-T-RFLP analysis

DNA of root tips was extracted using the CTAB protocol of Gardes and Bruns (1996b), which was modified by excluding the freeze-thaw procedure and by including another purification step of adding phenol:chloroform-isoamyl alcohol (1:1) (Lee and Taylor 1990). Individual frozen tips were ground in 300 µL of 2X CTAB buffer (100 mM Tris at pH 8, 1.4 M NaCl, 20 mM EDTA, 2% CTAB, and 0.2% β-mercaptoethanol) and then incubated at 65° C for one hour. Equal volumes of phenol: chloroform-isoamyl alcohol (24:1) were added, vortexed, and centrifuged at 13,000 rcf for 15 minutes. The supernatant was then transferred to fresh tubes. A second wash of chloroform-isoamyl alcohol (24:1) was added (v/v), vortexed and centrifuged for 5 minutes. Again the supernatant was removed and placed in fresh tubes. Nucleic acid was precipitated by adding 500 µL of isopropanol and incubated for at least 3 hrs at -20° C. This was centrifuged for 15 min, and the isopropanol was removed. The remaining pellet was washed with 70% ethanol and then centrifuged for 10 minutes. The ethanol was removed and allowed to evaporate before the pellet was resuspended in 50 µL of TE-8 buffer. Plant tissue and sporocarps were extracted with the same protocol except the extra phenol:chloroform-isoamyl alcohol purification step was not included, and pellets were resuspended in 100 µL of TE-8 buffer instead of 50. All extractions were stored in -20° C until use.

Amplification of the nrDNA ITS region was done using 10X Buffer (200 mM Tris-HCl [pH 8.4], 500 mM KCl) (Invitrogen), 2X dNTPs, 25 mM $MgCl_2$, 0.5 µM of ITS 1 (White et al. 1990) dye labeled with Cy 5.0, 0.5 µM of ITS 4 (Gardes and Bruns 1993) dye labeled with Cy 5.5, 5 U of Platinum Taq DNA Polymerase (Invitrogen), and 0.10X DNA template . The following program was used for PCR on a MJ Research thermocycler PTC-100: 94 °C (4 min); 48° C (1 min); 72° C (2min); [94 °C (30 sec); 48° C (30 sec); 72° C (1

min 30 sec) x 34 cycles]; 72°C (6 min 30 sec). Samples were run on a 0.7% agarose gel to confirm amplification. Unsuccessful amplifications were redone with no dilution and 1:10 dilutions of the DNA template.

Digestions using either *Alu*I or *Hin*fI (Invitrogen) and 6 µL of the PCR product were completed in 10 µL reactions following the manufacturer's recommendations and incubated at 37° C for at least 3 hours. One microliter of the digested samples was mixed with 1.65 µL of loading dye mixture that contained formamide and two sets of internal markers at 101, 200, and 351 bp; one set labeled with Cy 5.0, the other with Cy 5.5. The samples containing the loading dye mixture were denatured at 80° C for two minutes and then quenched on ice. Two microliters were then loaded onto a 6% polyacrylamide gel with the laser power set at 50%, temperature at 53° C, and current at 1250 V and ran for 60 min. on an OpenGene System Long Tower Sequencer (Bayer International). Fragments for each primer-enzyme combination (i.e. ITS 1-*Alu*I, ITS 1-*Hin*fI, ITS 4-*Alu*I, and ITS 4-*Hin*fI) were determined using GeneObjects 3.1 fragment analysis software.

6. Matching root tips with sporocarps

Terminal restriction fragments (T-RFs) from root tips were compared to those from sporocarps using TRAMP (T-RFLP analysis matching program) (Dickie et al. 2002) to check for matches. However, because most of the root tips exhibited multiple fragments, determining T-RFs for individual genotypes was difficult. Identification of T-RFs for individual genotypes was attempted by matching all possible combinations of at least three fragments against the known fragments from sporocarps.

7. Data analysis

Frequencies of genotypes generated from the T-RFLP analysis were tabulated for each enzyme-primer combination. First, genotypes were binned and the average of the binned numbers was used to identify that genotype, then the number of genotypes in each bin was entered into frequency tables. Each different primer-enzyme combination was treated as an independent database for determining if site and treatment had changed the root-associated fungal communities; therefore all analyses except for the ordination analyses were done in quadruplicate.

Non-metric multidimensional scaling (NMS) was used to analyze the frequency tables generated from the four primer-enzyme combinations using PC-ORD, version 4.25 (McCune and Mefford 1999) as an initial exploratory tool to visualize the overall effects of site and treatment on genotype frequency. The four individual primer-enzyme frequency tables were merged into a single table for the ordination, and Beal's transformation was used to alleviate problems associated with databases containing numerous zeroes (McCune and Grace 2002). The following parameters for NMS were used: Sorensen was used for measuring distance; the configuration for the first run was randomly selected; for all subsequent runs the best configuration from the previous run was used, as recommended by the program; 40 runs were conducted; dimensionality was assessed by examining the scree plot; and 50 randomized runs were used in the Monte Carlo test.

Canonical correspondence analysis (CCA) in PC-ORD (McCune and Mefford 1999) was used to examine the influence of soil properties (i.e. pH, CEC, available $NH_4$, available $NO_3$, available P, and C:N ratio, and exchangeable cations) in explaining the differences in genotype occurrence among sites. Genotype frequencies were transformed by adding one (to remove zero frequencies), and soil properties were transformed by $\log_{10}$ except for pH and

C:N ratio. Log transformation was used on soil properties because results from the different properties greatly varied so the log transformation compressed high values and spread the low values (McCune and Grace 2002). A Monte Carlo test was used to test for linear relationships between the genotype abundance and soil properties. This test was used because a large number of reiterations were needed to gain a more precise p-value (McCune and Grace 2002). The ordination diagram was based on LC (linear combination) scores, which are linear combinations of soil properties (McCune and Grace 2002).

To further investigate the impacts of site and treatment on genotype frequency, a nested, two-way analysis of variance (ANOVA) in Statistica, vers. 6.1 (StatSoft) was used. Because the number of fungi extracted from root tips varied from each plant specimen, genotype frequency was standardized by:

[1] $\frac{total\ number\ genotypes\ per\ plant\ specimen_a}{total\ number\ of\ root\ tips\ used\ from\ plant\ specimen_a}$. This standardization assumes that genotypes were not present in unsuccessful amplifications. The statistical model used was:

[2] $y_{ijkl} = \mu + S_i + T_j + R_{k(j)} + P_{l(jk)} + (ST)_{ij} + \varepsilon_{(ijkl)m}$, where

$y_{ijkl}$ = genotype frequency

$S_i$ = effect of site, i = 1,2,3;

$T_j$ = effect of treatment (OTC or control), j = 1,2;

$R_{k(j)}$ = replication effect, k = 1,2,3 nested within the *j*th treatment.

$P_{l(jk)}$ = plant specimen effect nested within the *k*th replicate and *j*th treatment, l = 1,2.

ANOVA was also used to test if warming affected soil properties, which could explain some of the variation due to site or treatment. The model used was:

[3] $y_{ijk} = \mu + S_i + T_j + R_{k(j)} + S_i*T_j + \varepsilon_{(ijk)j.}$, where

$y_{ijk}$ = the amount (ppm for P and available N) , concentration (exchangeable cations and CEC), or ratio (C:N ratio, pH) of soil properties;

$S_i$ = effect of site, i = 1,2,3;

$T_j$ = effect of treatment (OTC or control), j = 1,2;

$R_{k(j)}$ = replication effect, k = 1,2,3 nested within the treatment.

Genotype diversity was investigated by examining genotype abundance curves, genotype richness, and evenness according to site and treatment. Genotype abundance curves were made by taking the natural log of genotype frequency and plotting it against the arithmetic ranking of frequency. Once a model was chosen from the genotype abundance curves, niche apportionment analyses were conducted using PowerNiche, an Excel-based macro that uses niche division algorithms (Drozd and Novotny 1999), which helps determine if abundance is associated with random fraction, power fraction, broken stick, or other niche apportionment models based on the works of Tokeshi, Sugihara, and MacArthur (see Magurran 2004). The selection and division exponents were varied for each test of the power model with 250 replications. Different models were tested by changing the selection (= k) and division exponent (= m), which is indicative of which niche apportionment model is chosen, e.g. to test for the broken stick model, one is chosen for both exponents (i.e., k = 1, m = 1). Other than the broken stick model, the random fraction model (k=0, m=1), the power fraction model (0<k< 1, m=1), and Sugihara's sequential breakage model (k=0, m=0:25:0:75) were tested (Drozd and Novotny 1999).

Genotype richness and genotype evenness were tested using EcoSim (Gotelli and Entsminger 2001). Genotype richness measured the number of genotypes in a rarefied sample, i.e. different samples had the same abundance level before the number of different

genotypes was counted (Gotelli and Entsminger 2001). Hurlbert's probability of interspecific encounter (PIE) index was used to measure evenness (Gotelli and Entsminger 2001). ANOVA was performed for both richness and evenness using the model:

[4] $y_{ijk} = \mu + S_i + T_j + R_{k(j)} + S_i*T_j + \varepsilon_{ijk(j)}$, where

$y_{ijk}$ = genotype richness (number of different genotypes) or evenness (probablilty),

$S_i$ = effect of site, i = 1,2,3;

$T_j$ = effect of treatment (OTC or control), j = 1,2;

$R_{k(j)}$ = replication effect, k = 1,2,3 nested within the treatment.

Similarity in community structure was examined using Sorensen's quantitative index and analysis of molecular variance (AMOVA). Sorensen's quantitative index was used to examine the similarity of community composition between warmed and ambient plots in each site using EstimateS vers. 6.0b1 (Colwell 1997). This index examines the similarity between communities by comparing genotype frequency distributions, and gives more weight to frequent genotypes (Magurran 1988). For each plot, the average Sorensen's quantitative index for all four primer-enzyme combinations was calculated.

Variations in genotype frequency due to site, treatment, and within plots were tested with AMOVA using Arlequin, vers. 2.0 (Schneider et al. 2000). These parameters were also tested without rare genotypes (those found less than 5% of the sample). Although AMOVA is normally used for population studies, it was used here to test for variation in genotype frequency. Genotype frequency variation between plants was analyzed using a Euclidean distance measure and Ward's method for linking groups for cluster analysis in PC-ORD (McCune and Mefford 1999).

Changes in genotype composition were examined by tracking the relative frequency of dominant genotypes, (i.e. genotypes that occurred on more than five root tips for a plot). Genotypes that were dominant for one plot were assessed in all plots for each primer-enzyme combination.

C. ***Results***

Overall 1105 extractions were attempted; on average 30 extractions per plant. For the four primer-enzyme combinations, 1058 genotypes were found for the ITS 1-*Hin*fI, 1047 for ITS 4-*Hin*fI, 985 for ITS 1-*Alu*I, and 894 for ITS 4-*Alu*I. None of the aboveground plant tissues amplified with the given primers. No sporocarp T-RFs matched the fragments from root tips.

Site was the main determining factor in differentiating genotype composition and frequency according to the results from NMS and cluster analysis (see Figs. A2.1 and A2.2). Whereas genotypes clustered according to site, there were no clear patterns of differentiation of genotypes by temperature (i.e. warmed or ambient). The root-associated fungal community from the granitic site was more similar to the community found on the lowland site than on the dolomitic site. The variation was largest for the dolomitic site, as indicated by the higher scattering of the plots than the other two sites. The overall variance was explained by $r^2$ =0.894 with two axes; axis 1 explained 64.9% of the variance and axis 2 explained 24.5%. Thirty-seven iterations were needed for the final solution. Final stability of the model was met with the final stress = 12.1 (p= 0.0196) and final instability = 0.00363.

1. Soil properties

The soil conditions at the different sites were distinguished by CCA. The lowland site had higher amounts of Fe, K, and $NO_3$ than the other two sites (see Fig. 2.1); the

dolomitic site had higher C:N ratio, pH, CEC, and Ca; and the granitic site had higher levels of $NH_4$. For the final CCA analysis, Al, Ca, and K were excluded because of their high correlation with other variables: Al with $NO_3$ ($r = 0.91$), Ca with CEC ($r = 0.94$), and K with $NO_3$ ($r = 0.91$). The dimensions used for the final analysis were 18 plots and 95 genotypes for the main matrix and 18 plots and 8 variables for the environmental factors matrix. Three axes were interpreted; the first axis explained 15.8% of the variance, the second 12.7%, and the third 8.2%. Results from the Monte Carlo test indicate that genotype occurrence and soil properties were related as indicated by eigenvalues that were higher than expected by chance: 0.071 ($p = 0.02$) for axis 1 and 0.057 ($p = 0.01$) for axis 2.

Results from ANOVA on the different soil properties confirm the outcome from the CCA analysis. Site, not warming, was important in distinguishing the different soil properties except for Al, Na, and Fe, which were too variable to interpret (see Fig. A2.3 Table A2.1). Soil properties did not change due to warming. The soil properties for the lowland and granitic sites were more similar to each other than to the dolomitic site. The dolomitic site was differentiated from the others by higher CEC, Ca, pH, and C:N ratio (Fig. A2.3 c, d, i, j) and with lower K, Mn, $NO_3$, and P (Fig A2.3 f, h, k, m). The granitic site had higher $NH_4$ than the other two sites, but had a lower amount in the warmed plot compared to the ambient one (Fig. A2.3 l). There was lower Mg on the lowland site than the granitic (Fig. A2.3 g).

2. Genotype frequency

The genotype frequency was significantly lower for the dolomitic site compared to the other two sites ($p = 0.003$, $p < 0.001$, $p < 0.001$, and $p = 0.007$ for ITS 1-*Hin*fI, ITS 4-*Hin*fI, ITS 1-*Alu*I, and ITS 4-*Alu*I respectively) for each of the primer-enzyme combinations.

Genotype frequency also increased significantly in warmed plots ($p = 0.017$, $p = 0.029$, $p = 0.003$, and $p < 0.001$ for ITS 1-*Hin*fI, ITS 4-*Hin*fI, ITS 1-*Alu*I, and ITS 4-*Alu*I respectively) (see Fig. A2.4, Table 2.1). However, after further examination of the least square means with their confidence intervals, only the warmed plots of the granitic site had significantly higher frequency than the ambient plot (see Fig A2.4). There were no significant interactions between site and treatment for any of the primer-enzyme combinations, and there were no significant differences between the two plant specimen replicates.

The distribution patterns of genotypes were log normal for most of the treatments on each site (see figs. A2.5-A2.7). Further analyses with the power niche model indicated that these graphs fit the power fraction model for the lowland and granitic sites and broken stick for dolomitic site for all primer-enzyme combinations except for ITS 4-*Alu*I (see Table 2.2). ITS 4-*Alu*I differed from the other datasets in that the broken stick model fit best for the lowland control and OTC plots and for the granitic control plot.

3. Diversity

Genotype richness (i.e. the number of unique genotypes) varied according to site ($p$ 0.002 ITS1-*Hin*fI, $p < 0.001$ ITS 4-*Hin*fI, $p = 0.038$ ITS1-*Alu*I, and $p < 0.001$ ITS 4-*Alu*I) (see Fig. A2.8, Table A2.2) but did not change due to warming. Genotype richness was lowest on the dolomitic site, averaging 10 unique genotypes for the control and 20 genotypes for the OTC plots. Genotype richness was about the same for the lowland and granitic sites with the average of 26 unique genotypes for the control and 32 for the OTC. Genotype evenness did not change due to warming and was significantly different for site according to the ITS 1-*Hin*fI analysis ($p = 0.012$), where the granitic site had lower evenness, and ITS 4-*Alu*I ($p = 0.026$), where the lowland site had higher evenness (see Fig. A2.9, Table A2.3).

Table 2.1 2-way, 2-stage nested ANOVA for the effects of site, treatment, treatment replicate, and plant specimen replicate on genotype frequency

| Source of variation | Effect (fixed/ random) | Degrees of freedom | Sum of squares | | Mean square | | F | | p-value | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | ITS1-*Hin*fI | ITS4-*Hin*fI | ITS1-*Hin*fI | ITS4-*Hin*fI | ITS1-*Hin*fI | ITS4-*Hin*fI | ITS1-*Hin*fI | ITS4-*Hin*fI |
| Intercept | Fixed | 1 | 34.077 | 33.614 | 34.077 | 33.614 | 766.403 | 670.630 | <0.001 | <0.001 |
| Site | Fixed | 2 | 1.825 | 2.517 | 0.912 | 1.259 | 7.742 | 11.509 | 0.003 | <0.001 |
| Treatment | Fixed | 1 | 0.696 | 0.562 | 0.696 | 0.562 | 15.661 | 11.215 | 0.017 | 0.029 |
| Site * Treatment | Fixed | 2 | 0.209 | 0.007 | 0.105 | 0.004 | 0.886 | 0.034 | 0.428 | 0.966 |
| Replicate (treatment) | Random | 4 | 0.178 | 0.200 | 0.044 | 0.050 | 0.612 | 1.171 | 0.670 | 0.411 |
| Plant specimen (replicate) | Random | 6 | 0.436 | 0.257 | 0.072 | 0.043 | 0.616 | 0.391 | 0.715 | 0.876 |
| Error | | 20 | 2.357 | 2.187 | 0.118 | 0.109 | | | | |

Table 2.1 2-way, 2-stage nested ANOVA for the effects of site, treatment, treatment replicate, and plant specimen replicate on genotype frequency (cont'd)

| Source of variation | Effect (fixed/ random) | Degrees of freedom | Sum of squares | | Mean square | | F | | p-value | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | ITS1-*Alu*I | ITS4-*Alu*I | ITS1-*Alu*I | ITS4-*Alu*I | ITS1-*Alu*I | ITS4-*Alu*I | ITS1-*Alu*I | ITS4-*Alu*I |
| Intercept | Fixed | 1 | 29.507 | 24.832 | 29.507 | 24.832 | 1122.557 | 2666.863 | <0.001 | <0.001 |
| Site | Fixed | 2 | 2.918 | 1.592 | 1.459 | 0.796 | 13.268 | 6.539 | <0.001 | 0.007 |
| Treatment | Fixed | 1 | 1.03 | 0.866 | 1.034 | 0.866 | 39.356 | 92.986 | 0.003 | <0.001 |
| Site * Treatment | Fixed | 2 | 0.170 | 0.017 | 0.085 | 0.009 | 0.773 | 0.071 | 0.475 | 0.931 |
| Replicate (treatment) | Random | 4 | 0.105 | 0.037 | 0.026 | 0.009 | 0.283 | 0.112 | 0.879 | 0.974 |
| Plant specimen (replicate) | Random | 6 | 0.557 | 0.499 | 0.0928 | 0.083 | 0.844 | 0.683 | 0.551 | 0.665 |
| Error | | 20 | 2.200 | 2.435 | 0.110 | 0.122 | | | | |

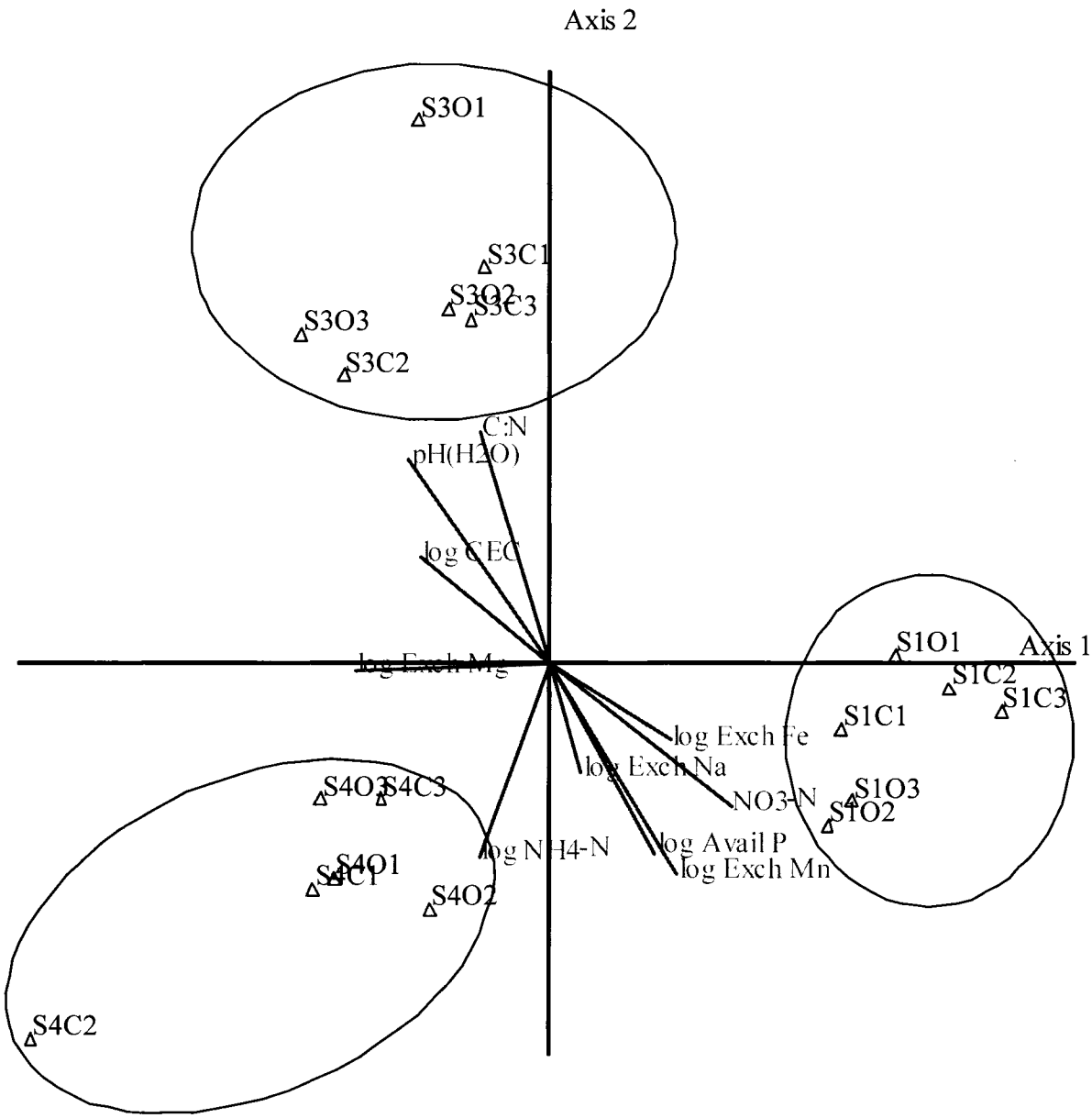



Fig. 2.1. CCA on warming treatments and site with soil properties as biplots.

Image rotated at 15°. Coding is as follows: first two characters represent site, S1 (lowland), S3 (highland dolomitic), S4 (highland granitic); the last two characters signify the treatment and the replicate of that treatment, e.g. C1 would be replicate one of control, O3 would be replicate three of OTC. Site is clustered together with the lowland and highland granitic sites clustering closer together than the lowland and highland dolomitic sites. Soil properties are the biplot vectors radiating from the center. Biplots were used to assess the relationship between genotype and soil properties.

Table 2.2 Summary of Tokeshi's model used to describe the rank abundance curves at each plot. PF = power fraction model, BS = broken stick model. OTC = open-top chamber

| Primer-Enzyme Site | ITS 1-*Alu*I | ITS 4-*Alu*I | ITS 1-*Hin*fI | ITS 4-*Hin*fI |
|---|---|---|---|---|
| Lowland control | PF | BS | PF | PF |
| Lowland OTC | PF | BS | PF | PF |
| Dolomitic control | BS | BS | BS | BS |
| Dolomitic OTC | BS | PF | BS | BS |
| Granitic control | PF | PF | PF | PF |
| Granitic OTC | PF | PF | PF | PF |

However, genotype evenness was not significantly different when analyzed for the other three primer enzyme combinations.

4. Community composition

Based on the Sorensen's quantitative index, the similarity of the genotypes from the root-associated fungal communities between the control and the OTC for each site ranged from 52%- 65% for the lowland site, 35%-55% for the dolomitic site, and 42%-66% for the granitic site. The similarity was generally lower between sites, which ranged from 26%-59% (see Table 2.3).

Results from AMOVA indicate that most of the variation in genotype frequency is explained by within plot variation (variation within plots averaged 0.95 for all four primer enzymes - 0.85 without rare genotypes).

When comparing the increase in genotype frequency from control to OTC, most of the additional genotypes came from increases in the frequency of dominant genotypes (the three genotypes most frequently found in each plot) (see Table A2.4). For the lowland site, the dominant genotypes remained dominant from the control to the OTC plot, except when analysis was done with ITS4-*Alu*I. The dominant genotypes remained dominant from the control to the warmed plots also in the dolomitic site for all primer-enzyme pairs except for

ITS1-*Alu*I. For the granitic site, results were less conclusive. When analyzed with ITS1-*Hin*fI and ITS1-*Alu*I, the relative frequency of genotypes from the control to the OTC increased from genotypes that had low relative frequencies (1-2%) to mid-ranged frequencies (8-9%). When the granitic site was analyzed with ITS4-*Hin*fI and ITS4-*Alu*I, the dominant genotypes remained dominant between the ambient and warmed plots.

Approximately 7-11 genotypes were found on all plots; 6-8 genotypes were found only on the lowland and granitic site; 0-5 genotypes were found on the two highland sites; and 0-1 genotype was found only on the dolomitic site. Only 0-2 genotypes increased in relative abundance from the control to the OTC plot; 0-1 genotypes decreased in all plots; 0-5 genotypes increased from the control to OTC on the lowland and granitic sites; and 1-3 genotypes decreased from the control to OTC on the lowland and granitic sites.

D. ***Discussion***

Genotype composition, cumulative frequency, and richness differed primarily according to site and not by warming. The dolomitic site had the lowest cumulative frequency and richness, while the granitic and lowland sites were higher and more comparable to each other. Reasons for the low richness and abundance for the dolomitic site include a high C:N ratio, which is not conducive to the breakdown of organic matter, and low levels of $NH_4$, $NO_3$, and P. $NH_4$ and $NO_3$ are forms of nitrogen that are absorbed by ectomycorrhizal fungi, which are either assimilated into the fungal mycelia or transformed into glutamine and transported to plants (Martin et al. 2000), making available nitrogen a possible limiting factor for these dolomitic sites. The low frequency of genotypes found on the dolomitic plots was not due to an effect of storage since these samples were the first to be prepared for DNA extraction.

Table 2.3 Sorensen's quantitative index for treatments per site differentiated by primer-enzyme combinations

For *Hin*fI:

| **ITS 4** ITS 1 | Lowland Control | Lowland OTC | Highland Dolomitic Control | Highland Dolomitic OTC | Highland Granitic Control | Highland Granitic OTC |
|---|---|---|---|---|---|---|
| Lowland Control | 0 | **0.65** | **0.28** | **0.39** | **0.41** | **0.5** |
| Lowland OTC | 0.61 | 0 | **0.28** | **0.39** | **0.56** | **0.59** |
| Highland Dolomitic Control | 0.33 | 0.3 | 0 | **0.51** | **0.32** | **0.29** |
| Highland Dolomitic OTC | 0.32 | 0.37 | 0.59 | 0 | **0.34** | **0.28** |
| Highland Granitic Control | 0.43 | 0.45 | 0.33 | 0.39 | 0 | **0.66** |
| Highland Granitic OTC | 0.4 | 0.44 | 0.33 | 0.34 | 0.42 | 0 |

Table 2.3 Sorensen's quantitative index For *Alu*I

| **ITS 4** ITS 1 | Lowland Control | Lowland OTC | Highland Dolomitic Control | Highland Dolomitic OTC | Highland Granitic Control | Highland Granitic OTC |
|---|---|---|---|---|---|---|
| Lowland Control | 0 | **0.52** | **0.29** | **0.3** | **0.36** | **0.35** |
| Lowland OTC | 0.62 | 0 | **0.29** | **0.29** | **0.35** | **0.44** |
| Highland Dolomitic Control | 0.18 | 0.2 | 0 | **0.55** | **0.31** | **0.33** |
| Highland Dolomitic OTC | 0.23 | 0.23 | 0.35 | 0 | **0.26** | **0.33** |
| Highland Granitic Control | 0.25 | 0.35 | 0.24 | 0.3 | 0 | **0.5** |
| Highland Granitic OTC | 0.35 | 0.36 | 0.25 | 0.3 | 0.56 | 0 |

Dolomitic sites are characterized by having $CaMg(CO_3)_2$; the high pH of 8 was due to excess Ca and Mg. The high concentration of Ca on these sites also explains the low availability of P because Ca reacts with P to create calcium phosphate, which is insoluble. Although not statistically significant, P levels dropped to negligible amounts in the warmed plots on the dolomitic site, where the warmer temperatures may have accelerated the chemical reaction to form calcium phosphate. The lack of available P could explain the paucity of plants and mycorrhizal fungi present at this site. However, some ectomycorrhizal fungi can solubilize calcium phosphate (Martin et al. 2000) so that fungi that can fulfill this role may have a competitive advantage over other fungi that cannot solubilize calcium phosphate. This could play an important role in plant establishment and maintenance on this dolomitic site.

Genotype frequency tended to be greater on the warmed plots compared to the ambient ones but was significantly different only at the granitic site. Genotype diversity did not increase due to warming. The lack of significance to the response of warming could be compounded by different plant age, plant size, sampling effects, amount of time plots were allowed to warm, or the increase in soil temperature was too small to affect the root-associated fungal community. Chapin et al. (1995) found significant increases in plant biomass after nine years of experimental warming in the low arctic. Even though our plots have been warmed for 5-7 years, this may not have been sufficient to detect significant changes. This may be particularly true for arctic *Salix* species because any excess energy goes to seed production, so it may take a few years for the benefits of excess energy to be realized belowground (Jones et al. 1997). Further, *Salix* may be constrained in its response to warming due to its tightly controlled meristem activity (Arft et al. 1999). Researchers also

found an increase in vegetative growth in the 1st year of experimental warming, but the effect was no longer significant by the 4th year, which may be due to limitation of resources other than temperature, soil nutrients, and decrease in litter quality indicated by a high C:N ratio (Arft et al. 1999). There was no significant difference between warmed and ambient plots for soil properties in the present study, which may indicate that our plots had acclimated to temperature change. Some reasons as to why the increase of genotype frequency from ambient to warmed conditions was not found on the lowland and dolomitic site include:: 1) the granitic site had harsher conditions than the lowland site, so the lowland site may be more resistant to change; and 2) the dolomitic site was too harsh for many root-associated fungal species to survive

In cases where amplification was unsuccessful, the distinction between true 'zeroes' and failed amplification of a genotype was impossible to demonstrate conclusively. The assumption that genotypes were not present in unsuccessful amplification was made because numerous attempts were made to amplify these samples with and without diluting the DNA template. Also, the results of the least squared means were more consistent between the four different primer-enzyme combinations than by assuming that unsuccessful amplifications were null. The alternative assumption, that the failed amplifications were nulls, would have favored plots for which amplification was difficult, i.e. the dolomitic site.

Our finding that there was no change in genotype richness due to warming is contrary to the study by Rygiewicz et al. (2000) where experimental warming was found to increase the genotype richness on Douglas-fir (*Pseudotsuga menziesii*) seedlings grown in environmentally controlled chambers. This is probably due to difference of the host plants and their ecology. Douglas-fir grows in temperate forests, where the growing season is

longer the seedlings had more time to react to passive warming in the same time frame. Other reasons include that seedlings were used for their study while we sampled mature shrubs; the age of the plant may determine what fungi are colonizing the roots (Fleming et al. 1984); and that we used field samples, which would increase the range of possible fungi that may colonize the roots. The lack of change in genotype richness may indicate that our sites were more stable and less susceptible to warming (Rygiewicz et al. 2000, Kårén and Nylund 1997), which is reasonable considering these plant communities have adapted to past warming events.

Similarity in community structure was greatest within sites as supported by results of Sorensen's quantitative index and the ordination analyses. Although the results from AMOVA indicate that the genotype composition is the same between sites and treatments, by examining the ordination graph from NMS (Fig A2. 1), the dolomitic site had high variation, which could have overshadowed any significant differences that the AMOVA may have found. The large variation found on the dolomitic sites as compared to the other two is probably there was less diversity. The same number of root tips was sampled, but amplification was not as successful as the other two sites. Perhaps sampling from many different plants would have captured greater diversity for the given sites (Gehring et al. 1998). However, this was not possible because many of the OTCs had only a few plants in them, and these ecosystems have low plant diversity and abundance.

Part of the reason for studying arctic sites was the assumption that these ecosystems would have a 'simpler' mycorrhizal community (Read 1993). This was not the case; these systems had 25-35 genotypes for the lowland and granitic sites, which is comparable to other studies using T-RFLP analysis such as Klamer et al. (2002) who found between 19-35

genotype in an oak shrub community, and Dickie et al. (2002) who observed 26 genotypes in a 60 year old pine plantation, as well as studies using RFLP analysis in temperate forests (Kårén and Nylund 1997, Gardes and Bruns 1996a, Horton and Bruns 1998, Dalhberg et al. 1997). Our findings were less comparable to other arctic and alpine studies. We had comparable numbers of genotypes associated with *S. arctica* with an alpine study, where 34 genotypes were found on four plant species and with an arctic site in Greenland where 137 genotypes were found on 10 plant species; however;our results were less comparable to one study where 60 ectomycorrhizal fungi were found on *S. herbacia* (Gardes and Dahlberg 1996).

Arctic plant systems have been reported to conform to a geometric series model (McKane et al. 2002), which is commonly associated with species-poor, harsh environments, or species found in early succession (Magurran 2004). Arctic plant communities fit the geometric series model because of their paucity compared to temperate ecosystems; only 4% of known vascular plants are found in the arctic (Chapin and Körner 1994). In contrast our mycorrhizal fungal communities, including the dolomitic site, have a log normal distribution, which is usually associated with more diverse ecosystems such as temperate and tropical forests (McKane et al. 2002), and found in ectomycorrhizal fungal communities of plant communities with more extensive root systems than arctic shrubs such as Douglas-fir, bishop pine (*Pinus muricata*), *Arctostaphylos* spp., ponderosa pine (*Pinus ponderosa*), Norway spruce (*Picea abies*), and western hemlock (*Tsuga heterophylla*) (Horton and Bruns 2001).

The log normal model has been criticized for possibly being a statistical artifact without any biological meaning (Tokeshi 1999). Further testing based upon Tokeshi's analyses indicated that the power fraction model best explained the distribution of genotypes

in the lowland and granitic sites. Although Tokeshi's models often discuss the mechanisms by which a niche can be fragmented for different species, these models can also be used to describe how a niche can be filled (Tokeshi 1999, Magurran 2004). The latter fits better with mycorrhizal fungi because once a niche is occupied by a mycorrhizal fungus, another fungus is challenged to outcompete and take the existing mycorrhizal fungus, which would be difficult due to the intimate contact of the Hartig net and mantle. This is not necessarily true for ectendomycorrhizas where ectomycorrhizas can outcompete established ectendomycorrhizas (Wilcox 1971) or DSE, which have little or no mantle; niche fragmentation in these systems may be more easily visualized. In the power fraction model, abundant species are more likely to successfully invade or inhabit niche space (Tokeshi 1999) or in this case colonize a segment of root. This may help explain the patchiness of species found on root tips, where 1-2 dominant types are found in one area but may not be dominant or present a few centimeters away (Horton and Bruns 2001). These clusters of fungi may outcompete other fungi and dominate a certain niche. When examining the increase in cumulative frequency of genotypes due to warming, the dominant genotypes, for the most part, were the ones that increased in number, more so than the rare or subdominant genotypes. However, this is probably not the whole picture as niche allocation for dominant species may be different than for rare and subdominant species. Tokeshi's composite model takes into account more than one niche allocation model because the assemblage of species probably requires more than one process (Tokeshi 1999). The random assortment model may be more appropriate to explain the resource use of subdominant and rare species. In this model, invading species exploit resources not used by existing species (Tokeshi 1999).

The broken stick model best fits the dolomitic sites. The broken stick model is usually based on one resource, evenly divided among species (Magurran 1988). This may be applied to the dolomitic sites because this site is limiting to both the plant and fungal community as seen by its limited diversity compared to the other sites. Those fungi that tolerate the limitations of this site may be sharing rather than competing for what little resources are available.

Applying niche apportionment concepts to mycorrhizal fungal communities is difficult because the availability of a niche may be determined by the host plant. Hoeksma and Kummel (2003) suggested that plants may increase mortality of root segments that are colonized by mycorrhizal fungi that are consuming too much carbon from the plant. Another possibility would be that this niche has yet to be filled; more fungi may colonize these roots. Perhaps because the conditions are harsher on the dolomitic site, colonization is retarded in comparison to the other two sites.

Magurran and Henderson (2003) suggest that communities can be divided into two groups, core genotypes and occasional genotypes. Core genotypes are persistent and abundant while occasional genotypes are infrequent and low in abundance. Most of the dominant genotypes remained so in both ambient and warmed plots and can be classified as core genotypes. That we were able to distinguish between core and occasional genotypes, and the persistence of the dominant genotypes, may indicate that these few dominant genotypes contribute the most to ecosystem function, a hypothesis suggested by Walker et al. (1999). These dominant genotypes, whose functions are assumed to be very different from each other, may maintain ecosystem functioning (Walker et al. 1999), which may explain the lack of changes in soil properties between the warmed and ambient conditions. They also

suggest that the many occasional genotypes may be functionally similar to the few dominant genotypes, so if the dominant genotype is lost, then hypothetically, the abundance of an occasional genotype will increase to fill the void, thus adding to the resilience of the ecosystem (Walker et al. 1999). This may be an important factor for these sites. The lowland and highland granitic sites may prove to be more resistant to warming because of their high diversity, while the dolomitic site, with its low genotypic richness, may be less resistant to change.

T-RFLPs proved to be a useful tool for analyzing root-associated fungal communities by providing a community fingerprint for the different sites and treatments. It allowed us to analyze multiple fungi that colonize single root tips, and to distinguish morphologically indistinct tips. However, this technique was not without problems.

One of the difficulties was interpretation of the four datasets that were produced; results with different enzyme-primer combinations did not always concur. The differences between the primer-enzyme combinations may be because the restriction site for one of the enzymes may be in a more variable region, which may explain why the forward primer would detect more rare genotypes in the warmed plots than the reverse primer. ITS4-*Alu*I had the fewest genotypes compared to the other three combinations, which suggests that this combination cuts in a more conserved area so detection of genotypes is less discriminating. In addition, the dye used to label the reverse primer, Cy 5.0, had a weaker reading than the Cy 5.5 dye, which labeled the forward primer. More bands labeled with the Cy 5.0 dye may have been too weak to be detected. The combination of the weaker dye and the more conserved region may explain why the reverse primers, and especially *Alu*I, showed fewer genotypes than the other three primer-enzyme combinations.

Another factor affecting the reliability of T-RFLP analysis is the generation of pseudo-fragments, PCR artifacts where secondary structures are formed from single stranded DNA. Single stranded DNA may be created by intrastrand annealing (Egbert and Friedrich 2003). These single strands may form palindromic secondary structures so that the restriction enzyme would still have the required double strand to cut (Egbert and Friedrich 2003). Creation of these secondary structures is more common for some restriction enzymes than others (Egbert and Friedrich 2003), which may be the case with *Hin*fI. More fragments were generated from the *Hin*fI enzyme than *Alu*I, which could have resulted in more variation and noise than the *Alu*I digestions. Klamer et al. (2002) also found *Hin*fI to produce more fragments than the other endonucleases that he used (*Taq*I and *Mse*I).

Finally, genotype frequency classifications may be misleading for mycorrhizal fungal communities because mycorrhizal root tips tend to be clustered, so sampling may miss or overestimate genotypes. For example, there were some dominant fungi that were found only on 1-2 plants from the same plot (e.g., a genotype found 18 times from one plant), so they may appear to be dominant genotypes but are not distributed among all of the plots.

How global warming will affect the root-associated fungal community on *Salix arctica* is difficult to predict. These communities are relatively stable as indicated by the lack of change in genotype richness, but if the trend towards increasing frequency of dominant genotypes continues, then changes in the root-associated community may become more apparent. Because the soil properties did not change much for the granitic and lowland sites, these communities may remain more stable. The dolomitic site will likely experience a decrease in plants due to its increase in C:N ratio and possibly reduced available phosphorus.

Because of the changes in these conditions, this site may not increase in plant or fungal richness.

## III. How host plant, warming, and site affect the culturable root-associated fungal community from the Canadian High Arctic

### A. *Introduction*

Fungi isolated from within root tips represent a different community than one determined using PCR-based methods. Cultures tend to favor fast-growing saprotrophs or endophytes over mycorrhizal fungi, and those species isolated from roots were rarely found in PCR-based (e.g. RFLPs, sequencing, T-RFLPs) studies (Kernaghan et al. 2003). Allen et al. (2003) also found different communities based on direct PCR and isolating fungi from ericoid plant roots, noting that *Sebacina* was the dominant fungus with direct PCR but was not found in any of their cultures. Part of the problem with culturing is that complete information on the nutritional requirements for ectomycorrhizal fungi is not known, so isolating ectomycorrhizal fungi is very difficult, and success is low. With surface sterilization of the root tips the endophytic fungal community seems to flourish when cultured. Endophytic hyphae may not be as abundant as ectomycorrhizal fungi, and as a result they are not easily amplified. Although ectomycorrhizal fungi can be classified as endophytic fungi, the distinction used here is that 'endophytic fungi' do not have a mantle and Hartig net while 'ectomycorrhizal fungi' do.

Dark-septate endophytes (DSE) are ubiquitous in both arctic and alpine systems (Bledsoe et al. 1990 Gardes and Dalberg 1996, Cázares 1992) and are found associated with ecto-, ericoid, and non-mycorrhizal plants (Jumpponen 1999). Hyaline septate hyphae have been reported and found on several plants and may have the same ambiguous function as DSE of being either pathogenic or mutualistic (Jumpponen and Trappe 1998). Because arbuscular mycorrhizae (AM) are scarce in the arctic, in contrast to alpine systems,

(Haselwandter 1987, Bledsoe et al. 1990), DSE may replace the function of AM fungi (Bledsoe et al. 1990). In addition, *Phialocephala fortinii* Wang & Wilcox, a commonly found DSE from arctic and alpine environments, is thought to transport carbohydrates (Jumpponen 1999), a function demonstrated by mycorrhizal fungal linkages (Simard et al. 1997).

The objective of this study was to test for the impacts of site, host, and warming on the root endophytic fungal community using culture-dependent techniques combined with morphological and molecular identification. Molecular identification included grouping taxa according to RFLP patterns and DNA sequencing. Dissimilarity matrices, which were used for clustering the RFLP patterns, were also the basis of a method to ordinate the data using the Phi index, which is a diversity index.

## B. ***Materials and methods***

### 1. Field collection

To assess the root endophytic fungal community found on these roots, two specimens each of *Dryas integrifolia* Vahl, *Salix arctica* Pall., *Cassiope tetragona* (L.) D.Don, and *Saxifraga oppositifolia* L. from each open top chamber (OTC) and ambient plot were destructively harvested in late July, 2000 along with surrounding soil and then placed in Ziploc ® bags. To increase the sample size for identification by sequencing or culture morphology, extra specimens of *C. tetragona* and *D. integrifolia* were harvested from the designated plots; these samples were not used to statistically test for differences according to treatment or plot. The samples were stored in a permafrost cooler while in the field and placed in a 4° C walk-in cooler at University of Northern British Columbia until they were processed.

2. Sampling from roots

Soil was loosened from plant roots by immersing in water for at least 24 hours at 4° C. Roots were gently cleaned with water and were collected in a 0.5 mm sieve (No. 35 USA standard testing sieve, W.S. Tyler, Inc.). Segments of roots were randomly selected by placing the root system on a numbered grid. Root segments that intersected a randomly selected numbered grid were sampled. Fungi were isolated from five of these randomly selected root tips. From each root section, 3-6 mm was excised, surface-sterilized with 30% $H_2O_2$ following Danielson (1984), and placed on modified Melin Norkrans (MMN) agar (Marx 1969) with streptomycin sulphate and chlorotetracycline added to minimize bacterial growth. The protocol of Monréal et al. (1999) was used for root tips that were from the ericaceous plant *Cassiope tetragona*. These tips were plated on 1/3 concentration potato dextrose agar. All plates were incubated at room temperature (20-22° C). Fungi were purified by re-isolation from each colony onto fresh agar plates. Once these re-isolated cultures grew, a 0.5 mm plug was taken for DNA extraction and frozen at -20° C. Two more plugs were placed on separate agar slants for storage at 4° C until being sent to Dr. R. Currah at the University of Alberta for identification based on morphology.

3. DNA extraction and ITS-RFLP analysis

DNA from cultures and sporocarps, and plant tissue from stem and leaves, was extracted using the CTAB miniprep protocol of Gardes and Bruns (1996b) except samples were not freeze-thawed prior to crushing. All DNA samples were reconstituted in 100 µL of TE-8 buffer.

The ITS region of the nrDNA was amplified in a 35 µL reaction containing 10X buffer, 2X dNTPs, 25 mM $MgCl_2$, 0.45 µM of the primer pair ITS1/ITS4 , and 2.5 U of Platinum Taq (Invitrogen). PCRs were done on MJ Research PTC-100 thermocyclers with

the following program: 94 °C (30 sec); [93 °C (35 sec); 50° C (53 sec); 72° C (30 + 5 sec/cycle) x 34 cycles]; 72°C (5 min). PCR amplification was confirmed on a 0.7 % agarose gel. Unsuccessful amplifications were redone with 1:50 dilutions of the DNA template.

PCR products were digested with the endonucleases *Alu*I, *Hin*fI (Invitrogen), and *Mbo*II (Fisher), as suggested by manufacturers, using 8 µL of the PCR product in a 15 µL reaction with 10 U of enzyme/µL for 5 hrs at 37° C. Digested products were separated on a 1% low-melting point agarose/1.5% agarose 10X TBE gel containing ethidium bromide and photographed using a Biophotonics 2000i imaging system (BioCan Scientific). RFLP patterns and fragment sizes were determined using Gene Profiler version 4.02 (Scanalytics) with a 1 KB ladder standard (Invitrogen).

4. Data analysis

Cluster analysis using the "Neighbor" algorithm in PHYLIP (Felsenstein 2004) was performed using Gene Profiler based upon the total number of polymorphic bands as the distance measure. Based on the resulting tree (not shown), samples representing commonly occurring RFLP groups were selected for sequencing.

In order to ordinate treatments without subjective assignment of isolates into taxon groups, we developed an ordination method using a distance measure based upon the Phi index (Mah et al. 2001). To calculate the Phi index for each plot, a pairwise distance matrix based on Dice's index [(2 x the number of common bands)/ (2 x the number of common bands + number of polymorphic bands)], was generated for each plot database using Gene Profiler, with a 5% match tolerance to account for inter-gel variation in band sizes. From this distance matrix, the Phi Index, was calculated by squaring each distance, summing the distances for each sample, and dividing the summation by $n$-1. The totals of the different

distances for each sample were then summed and divided by $n$ (Mah et al. 2001, Khetmalas et al. 2002):

$$[1] \qquad \Phi = \frac{\sum_{j=1}^{n}\left[\frac{\sum_{i=1}^{n} d_{ij}^2}{n-1}\right]}{n}.$$

A Phi distance was calculated for pairs of treatments by combining the two databases, then recalculating the Phi index of the merged database. If the two merged databases were identical, then the minimum possible Phi distance value can be estimated as a weighted average of the two individual Phi index scores:

$$[2] \qquad \text{Min}_{(1,2)} = \frac{[Phi_1 * (n_1^2 - n_1)] + [Phi_2 * (n_2^2 - n_2)]}{(n_1 + n_2)^2 - (n_1 + n_2)},$$

If the two merged databases shared no RFLP fragments, then the maximum Phi index value can be estimated as

$$[3] \qquad \text{Max}_{(1,2)} = \frac{[Phi_1 * (n_1^2 - n_1)] + [Phi_2 * (n_2^2 - n_2)] + 2(n_1 * n_2)}{(n_1 + n_2)^2 - (n_1 + n_2)}.$$

The Phi index of the merged database must fall between the minimum and maximum values estimated from the Phi index values of the two unmerged databases, therefore a standardized Phi distance can then be calculated as:

$$[4] \qquad \text{Phi dist}_{(1,2)} = \frac{Phi_{1,2} - Min_{1,2}}{Max_{1,2} - Min_{1,2}}.$$

The Phi distances were placed in a distance matrix, and a non-metric multidimensional scaling (NMS) ordination was run with a starting configuration using the standard Guttman-Lingoes and 50 iterations in Statistica vers 6.1 (StatSoft).

Nested, two-way analysis of variance (ANOVA) in Statistica was used to test for the impacts of site and treatment on species richness and cumulative frequency. The following model was used for host plants that had successful isolation from all plots:

$y_{ijk} = \mu + S_i + T_j + R_{k(j)} + S_i*T_j + \varepsilon_{ijk(j)}$, where

$y_{ijk}$ =species richness or cumulative frequency

$S_i$ = effect of site, i = 1,2,3;

$T_j$ = effect of treatment (OTC or control), j = 1,2;

$R_k$ = replication effect, k = 1,2,3;

A nested, three-way ANOVA was conducted to test for differences in the cumulative frequency for *P. fortinii* that were identified according to morphology. Although morphological identification was done on all isolates, including replicates made for storage, for this analysis only one representative of each sample was included. The model used for the analysis was:

$y_{ijkl} = \mu + P_i + S_j + T_k + R_{l(k)} + P_i*S_j*T_k + S_j*T_k + \varepsilon_{ijkl(k)}$, where

$y_{ijkl}$ = cumulative frequency of *P. fortinii*,

$P_i$ = effect of host plant, i = 1, 2, 3, 4, and the other variables are as in the above model.

5. DNA sequencing

Sequencing was done following the manufacturer's instructions for the Thermo Sequenase Cycle Sequencing Core Kit (Amersham Life Science). Samples were first amplified with primer pair ITS1 (White et al. 1990) and NL8 (Egger 1995) with the program: 94° C (35 sec); [93° C (35 sec); 54° C (53 sec); 72° C (2 min 10 sec) x 35 cycles]; 72 °C (3 min), and amplification was confirmed on 0.7% agarose gels. Amplicons were purified with Qiaquick PCR purification kit (Qiagen) according to manufacturer's instructions. Cycle

sequencing was done by adding PCR product to 3 U of Thermo Sequenase, 0.34 pmol/μL of Cy 5.0 labeled primer (for forward primers) or Cy 5.5 labeled primer (for reverse primers), 10X sequencing buffer diluted to one-tenth the volume, 15% dimethylsulfoxide, sterile $dH_2O$, and 0.1 mM of one the four ddNTPs; cycle sequencing was completed for each of the four ddNTPs for every sample. Cycle sequencing used the following program: 94° C (2 min); [94° C (20 sec); annealing temp (45 sec); 70° C (varied) x 25 cycles]; [94° C (25 sec); 70° C (2 min) x 15 cycles]; 72° C (6 min). Annealing temperature and the first extension time varied according to the primer used: ITS1 (52-54° C, 75 sec); ITS4 (54° C, 1 min); NL5 (50° C, 70 sec); NL6c (52° C, 75 sec); NL7 (50 24° C, 70 sec); and NL8 (52° C).

Samples were run on an OpenGene System Long Tower Sequencer (Bayer International). Three microliters of PCR products were added to 4.5 μL of formamide loading dye, denatured between 70 to 80° C for 2 minutes, quenched on ice, and loaded onto a 6% polyacrylamide gel. Samples ran for 50 min. with the temperature on sequencing control set at 50° C, gel voltage at 1250V, and laser power for 50%.

Sequences were edited in Sequencher (Gene Codes, corp). These edited sequences were compared in GenBank (BLAST search <www.ncbi.nlm.nih.gov>) to identify fungi that were most closely related to the sequence and to ensure that sequences were fungal. Edited sequences were imported into MacVector (Oxford Molecular) and were aligned with the most closely related sequences downloaded from GenBank. Sequences downloaded included *Epicoccum nigrum* AF455455, *Colispora elongata* AY148102 Leaf litter Ascomycete AF502763, *Epacris microphylla* root associate AY268216, Dothideales sp. AY465446, Fungal endophyte MUT 2723 AF373055, Fungal endophyte MUT 585 AF373051, Ascomycete sp. AJ279473, *Phoma glomerata* AY618248 (Fig. 3.4); *Zalerion varium*

AF169303, *Glarea lozoyensis* AF169304, *Hymenoscyphus epipiphyllus* AY348581, *Hymenoscyphus monotropae* AF169309, *Lachnum bicolor* AJ430394, *Phialocephala virens* AF486132, *Xenochalara juniperi* AF184889, *Phialophora* sp. AY465463, *Cadophora* sp. AY371512, *Phialophora finlandia* AF486119, axenic ericoid root isolate AJ430119, ectomycorrhiza cf. *Hymenoscyphus ericae* (= *Rhizoscyphus ericae* (Zhang and Zhuang 2004)) AJ430150, *Hymenoscyphus* sp. AY354244, ectomycorrhizal isolate (Helotiales) AJ430410, *Cadophora luteo-olivacea* AY249069, *Leptodontidium orchidicola* AF214578, *Cadophora gregata* AY249071, *Cadophora malorum* AY249063, *Cadophora* sp. AY371506, ectomycorrhiza (cf. *Phialocephala fortinii*) AJ430214, *Phialocephala fortinii* crypt sp. AY347405, *Phialocephala fortinii* AY078138 (Fig 3.5); *Inocybe angustispora* AY380360, *Favolaschia* cf. *sprucei* AF261420, *Favolaschia* cf. *calocera* AF261419, *Panellus serotinus* AF518633, *Ripartites metrodii* AF042012, *Mycena haematopoda* AJ406590, *Mycena leaiana* AF261411, *Omphalina rivulicola* U66451, *Collybia tuberosa* AY639884, *Clitocybe subvelosa* AY647208 (Fig. 3.6); *Phlebia lindtneri* AF141623, *Ceratobasidium goodyerae-repentis* AY243523, *Uthatobasidium fusisporum* AF518664, *Thanatephorus cucumeris* AF354062, *Ceratobasidium* sp. AGO AF354094, *Ceratobasidium* sp. AGL AF354093, *Inonotus weirii* AY059040, *Phlebiopsis gigantea* AF141634 (Fig 3.7); *Alternaria helianthi* AY154713, *Ampelomyces* sp. AY293794, Ascomycota sp. AJ301960, *Byssoascus striatosporus* AB040688, *Cadophora luteo-olivacea* AY249087, *Cadophora malorum* AY249086, *Cenococcum geophilum* AY112935, *Chalara fungorum* AF222462, *Chalara kendrickii* AF222464, *Chalara longispes* AF222466, *Chalara microchona* AF222468, *Chalara parvispora* AF222473, *Chalara strobilina* AF222477, *Cryptosporiopsis* sp. AY442321, *Cryptosporiopsis ericacea* AY442323, *Cudonia lutea* AF433139, ericoid

mycorrhizal sp PPO-7 AY599245, ericoid mycorrhizal sp PPO-8 AY599246, ericoid mycorrhizal sp. AY599244, ericoid mycorrhizal sp. PPO-5 AY599243, ericoid mycorrhizal sp. PPO-2 AY599240, euascomycete RFLP type A AF127116, euascomycete RFLP type B AF127117, euascomycete RFLP type C AF127118, *Fabrella tsugae* AF356694, *Graphium rubrum* AY266313, *Hymenoscyphus ericae* (= *Rhizoscyphus ericae* (Zhang and Zhuang 2004)) AY284122, *Hymenoscyphus* sp. UBC tra 1436 AY219881, *Iodosphaeria* sp. AF452045, *Lachnum* cf. *bicolor* AY544674, *Lachnum virgineum* AY544646, *Lecythophora* sp AF353607, *Leptosphaeria doliolum* U43475, mycorrhizal sp. AF081443, *Mycosphaerella mycopappi* U43480, *Oidiodendron tenuissimum* AB040706, *Phialocephala fortinii* AF326082, *Phialocephala dimorphospora* AF326081, *Phialophora gregata* AF222502, *Phialophora* sp. AF156922, *Phoma herbarum* AY293788, *Phoma* sp. 199 AY293785, *Pleomassaria siparia* AY004341, *Pleospora herbarum* U43476, *Rhytisma acerinum* AF356696, *Setomelanomma holmii* AF525678, *Shiraia bambusicola* AB105798, *Trematosphaeria heterospora* AY016369 (Fig 3.8).

Neighbor-joining analysis was used for all trees, with 1000 repetitions for bootstrap values. The Kimura 2-parameter model was used for the distance measure, the transition:transversion ratio was estimated, and gaps were distributed proportionally. Bootstrap values greater than 50 were reported. Identifications from samples that were sequenced were applied to those samples with matching RFLP patterns. Those that clustered according to RFLP data were verified by viewing composite gels of likely samples in Gene Profiler database, vers. 4.02 (Scanalytic, Inc.). These samples were then compared to those identified based on morphological traits.

C. ***Results***

1. ITS-RFLPs

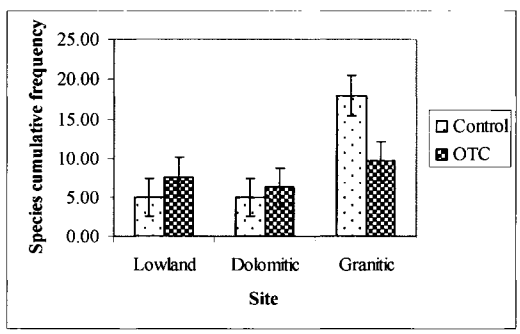
Out of 720 isolated cultures, 432 were amplified successfully and digested with all three restriction enzymes for RFLP analysis. For statistical analyses, 30 of the isolates were deleted for a total of 402. These isolates were not included for statistical analyses because they were from plants that were not sampled within the experimental design (i.e. they were sequenced to increase the chances of identification by providing more isolates from the harsher highland sites). The species cumulative frequency for each plot is illustrated in Table 3.1. Isolating fungi from root tips was most successful for *Salix arctica* and *Saxifraga oppositifolia*. The average number of isolates was highest from *S. arctica* growing in control plots at the granitic site ($p=0.02$) (Fig 3.1). Although not statistically significant, more isolates from *S. oppositifolia* were also recovered from the granitic site, averaging 15 isolates from both plant specimens for the granitic site compared to six for the other two (Fig. 3.2). Fungal isolation was particularly unsuccessful for *Cassiope tetragona* from the warmed plots for all three sites, as well as for the control from the dolomitic site; fungi were only successfully isolated from one plot on the granitic site. In contrast to *Salix arctica* and *Saxifraga oppositifolia*, no fungi were successfully isolated from roots of *Dryas integrifolia* from the granitic site.

Results from NMS showed that the site was the most influential factor in differentiating the root endophytic fungal community, more so than warming or host plant (Fig 3.3). The lowland site had a wider range of variation when compared to the other two sites. The dolomitic site also had large variation, but the isolates were more similar at this

Table 3.1 Number of successful samples isolated from root tips based on RFLPs.

| Treatment / Site | OTC Replicate | | | | Control Replicate | | | |
|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | Sum (cumulative frequency) | 1 | 2 | 3 | Sum (cumulative frequency) |
| Lowland | | | | | | | | |
| *C. tetragona* | 0 | 0 | 8 | 8 | 2 | 8 | 6 | 16 |
| *D. integrifolia* | 4 | 6 | 7 | 17 | 10 | 5 | 6 | 21 |
| *Salix arctica* | 9 | 10 | 4 | 23 | 5 | 4 | 6 | 15 |
| *Saxifraga oppositifolia* | 5 | 8 | 3 | 16 | 10 | 1 | 6 | 17 |
| **Sum (species abundance)** | **18** | **24** | **22** | **64** | **27** | **18** | **24** | **69** |
| Dolomitic | | | | | | | | |
| *C. tetragona* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *D. integrifolia* | 5 | 3 | 5 | 13 | 2 | 8 | 5 | 15 |
| *Salix arctica* | 7 | 2 | 8 | 17 | 4 | 4 | 7 | 15 |
| *Saxifraga oppositifolia* | 3 | 1 | 8 | 12 | 4 | 2 | 17 | 23 |
| **Sum (species abundance)** | **15** | **6** | **21** | **42** | **10** | **14** | **29** | **53** |
| Granitic | | | | | | | | |
| *C. tetragona* | 0 | 0 | 0 | 0 | 10 | 0 | 0 | 10 |
| *D. integrifolia* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Salix arctica* | 17 | 6 | 5 | 28 | 24 | 16 | 12 | 52 |
| *Saxifraga oppositifolia* | 16 | 11 | 16 | 43 | 8 | 20 | 13 | 40 |
| **Sum (species abundance)** | **33** | **17** | **21** | **71** | **42** | **36** | **25** | **103** |

(a) *Salix arctica*




(b) *Saxifraga oppositifolia* p=0.017

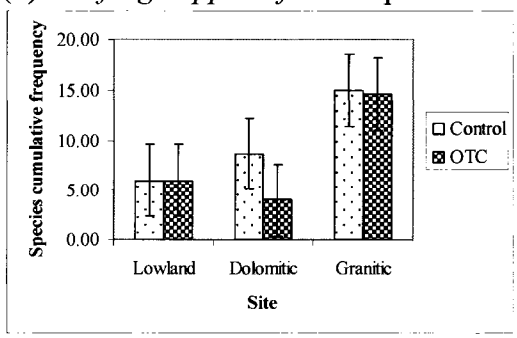
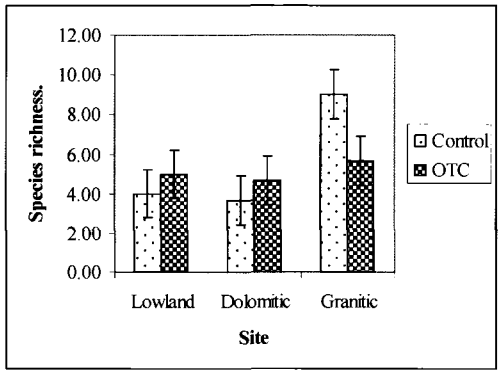



Fig. 3.1 Species cumulative frequency for *Salix arctica* and *Saxifraga oppositifolia* on different sites. LSM ± SE

(a) *Salix arctica* p=0.02




(b) *Saxifraga oppositifolia*

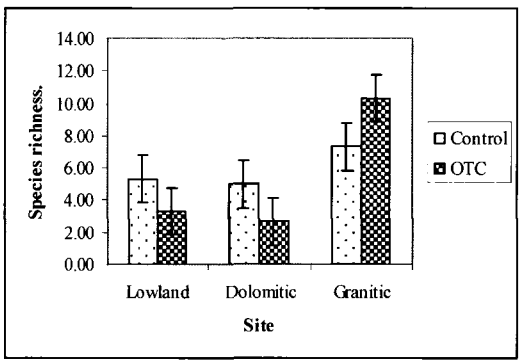



Fig. 3.2 Species richness for *Salix arctica* and *Saxifraga oppositifolia* on different sites. LSM ± SE.

site than in the community from the lowland site. The genotypes within the granitic site clustered tightly together in the ordination and appeared to have a similar composition. Species richness ranged from 0-13 for *Cassiope tetragona*, 0-18 for *Dryas integrifolia*, 11-27 for *Salix arctica*, and 10-31 for *Saxifraga oppositifolia* (Table 3.2) for each of the two replicate plants for each treatment. Because fungi were not recovered from all of the sites for *Cassiope tetragona* and *Dryas integrifolia*, ANOVAs were only done for *Salix arctica* and *Saxifraga oppositifolia*. There were significantly more different types of cultures isolated from *Salix arctica* in the granitic site (p=0.02) (Table 3.2) than the other two sites. Although not statistically significant, more diversity in fungal species was found (Table 3.1, Table 3.3) in the granitic control than the other plots for *Salix arctica*. For *Saxifraga oppositifolia*, diversity was greatest (p=0.017) in the granitic site, and a trend showing higher cumulative frequency in the granitic site was found as well. None of the plant tissues amplified with the primer pair ITS1/4.

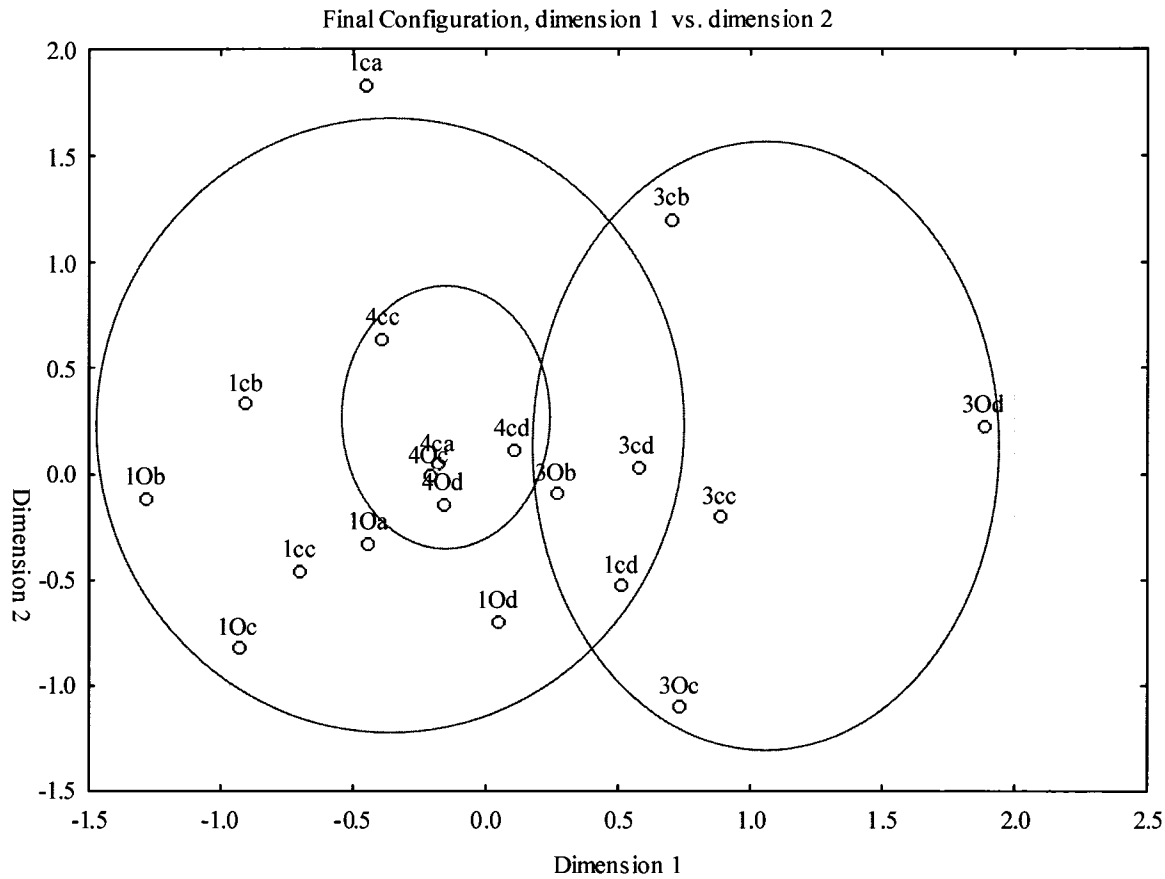



Fig. 3.3 NMS for host plant and warming treatments per site based on phi distances. The first number represents the site where 1 = lowland, 3 = highland dolomitic, and 4 = highland granitic; the 2nd letter is c for control or O for OTC, and the last letter represents the plant, a = *Cassiope tetragona*, b = *Dryas integrifolia*, c = *Salix arctica*, and d = *Saxifraga oppositifolia.*

Table 3.2 Species richness of cultures according to host plant, site, and treatment based on RFLPs

| | OTC | | | | Control | | | |
|---|---|---|---|---|---|---|---|---|
| Treatment / Site | 1 | 2 | 3 | Total for species | 1 | 2 | 3 | Total for species |
| Lowland | | | | | | | | |
| *C. tetragona* | 0 | 0 | 6 | 6 | 1 | 6 | 6 | 13 |
| *D. integrifolia* | 2 | 5 | 7 | 16 | 8 | 5 | 5 | 18 |
| *S. arctica* | 5 | 7 | 3 | 15 | 5 | 4 | 4 | 13 |
| *S. oppositifolia* | 4 | 3 | 3 | 10 | 10 | 1 | 5 | 16 |
| Dolomitic | | | | | | | | |
| *C. tetragona* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *D. integrifolia* | 4 | 3 | 5 | 13 | 2 | 6 | 5 | 13 |
| *S. arctica* | 5 | 2 | 7 | 16 | 4 | 2 | 5 | 11 |
| *S. oppositifolia* | 3 | 1 | 6 | 10 | 4 | 1 | 10 | 15 |
| Granitic | | | | | | | | |
| *C. tetragona* | 0 | 0 | 0 | 0 | 9 | 0 | 0 | 9 |
| *D. integrifolia* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *S. arctica* | 10 | 6 | 5 | 21 | 11 | 10 | 6 | 27 |
| *S. oppositifolia* | 11 | 9 | 11 | 31 | 8 | 7 | 7 | 22 |

Table 3.3 Species richness of cultures according to host plant, site, and treatment based on identification made morphologically.

| | OTC | | | | Control | | | |
|---|---|---|---|---|---|---|---|---|
| Treatment / Site | 1 | 2 | 3 | Total for species | 1 | 2 | 3 | Total for species |
| Lowland | | | | | | | | |
| *C. tetragona* | 0 | 0 | 1 | 1 | 0 | 4 | 3 | 7 |
| *D. integrifolia* | 3 | 6 | 1 | 10 | 3 | 4 | 5 | 13 |
| *S. arctica* | 3 | 4 | 3 | 10 | 5 | 2 | 5 | 12 |
| *S. oppositifolia* | 8 | 10 | 5 | 23 | 9 | 2 | 10 | 21 |
| Dolomitic | | | | | | | | |
| *C. tetragona* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *D. integrifolia* | 4 | 2 | 4 | 10 | 0 | 3 | 4 | 6 |
| *S. arctica* | 3 | 1 | 3 | 7 | 2 | 4 | 1 | 7 |
| *S. oppositifolia* | 3 | 3 | 4 | 10 | 5 | 2 | 6 | 12 |
| Granitic | | | | | | | | |
| *C. tetragona* | 0 | 0 | 0 | 0 | 6 | 0 | 0 | 6 |
| *D. integrifolia* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *S. arctica* | 8 | 7 | 6 | 21 | 8 | 8 | 7 | 23 |
| *S. oppositifolia* | 12 | 8 | 8 | 28 | 8 | 14 | 8 | 30 |

2. DNA sequencing

Of the 50 samples that were sequenced, 43 produced high quality results for analysis. Approximately 330 bp were used for phylogenetic analysis of the ITS region (ITS) and 350 bp of the nuclear large subunit rRNA gene (LSU). Five trees were formed based on primer pairs and alignment consensus, which were the ITS and the Dothideales; ITS and the Helotiales; LSU and the Ceratobasidiomycetes; LSU and the Agaricales; and LSU and ascomycetes (Figs 3.4-3.8 respectively). Sequencing was able to classify 37.9% of the samples to at least Order.

The ITS – Dothideales tree had two unknown samples (see Fig. 3.4). One of the unknown samples was affiliated with *Colispora elongata* although there was <50% bootstrap support, and the other unknown sample was nested in the Dothideales.

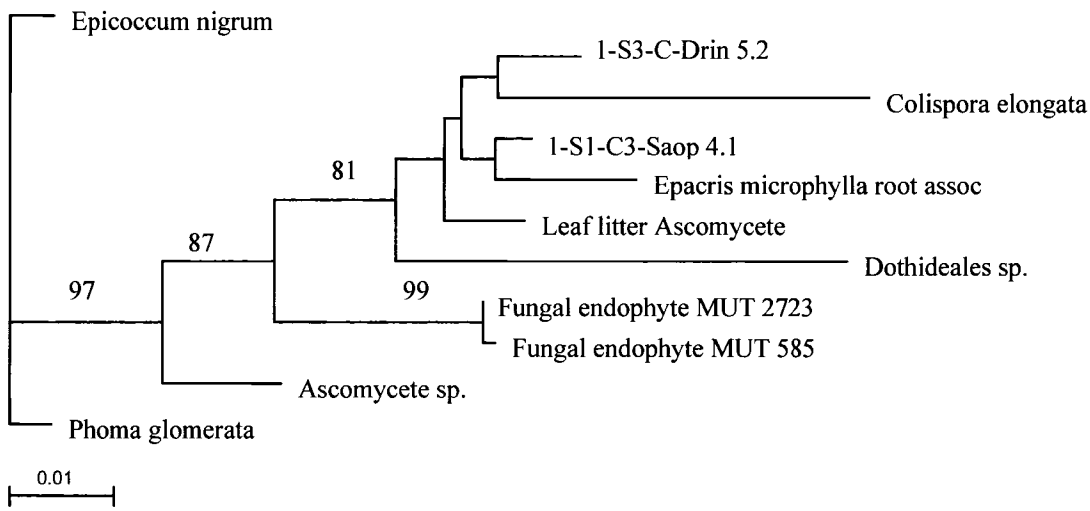



Fig. 3.4 Neighbor-joining best tree based on ITS and the Dothideales. Bootstrap values (1000 replications) >75 are reported.

All unknown cultures from the ITS – Helotiales (Fig. 3.5) were found to be affiliated with *Cadophora, Phialocephala fortinii*, or *Hymenoscyphus*. One sample was associated with species of *Cadophora* with a bootstrap value of 100%; three cultures were clustered with *P. fortinii* supported by a bootstrap value of 81%; and two samples were affiliated with *Hymenoscyphus* even though the bootstrap value was <50%.

Cultures affiliated with basidiomycetes were associated with the Agaricales, which had <50% bootstrap support, and with *Ceratobasidium* (77% bootstrap value) and (Fig. 3.6 and 3.7 respectively). From the LSU -ascomycete tree (Fig. 3.8), four cultures were affiliated

with *Cryptosporiopsis* (99% bootstrap value); five cultures with *Mycosphaerella* (85% bootstrap); three with *Phoma* (84% bootstrap); two with *Cadophora* (88% bootstrap); three with *Hymenoscyphus* (90% bootstrap); and four with *Phialocephala* (95% bootstrap). Identities of ten cultures were not resolved from this analysis. Not all samples were resolved to genus and are identified by their family names. One sample could not be resolved even to order and was left as an unknown.

3. Culture morphology

Culture morphology was based on 1347 cultures (usually two tubes for each isolate). Forty percent of the cultures were identified to genus, which included Acremonium, Cryptosporiopsis, Geomyces, Leptodontidium, Monodictys, Phialocephala fortinii, Scytalidium, Sebacina, Staphylotrichum, Trichocladium, Trichoderma, Trichosporiella, or Xenosporium. Of these, Phialocephala fortinii was the most abundant taxon isolated, accounting for 85.7% of the identified cultures. Of the unknowns, cultures with hyaline hyphae were the most abundant (23%) followed by other isolates with dark hyphae other than

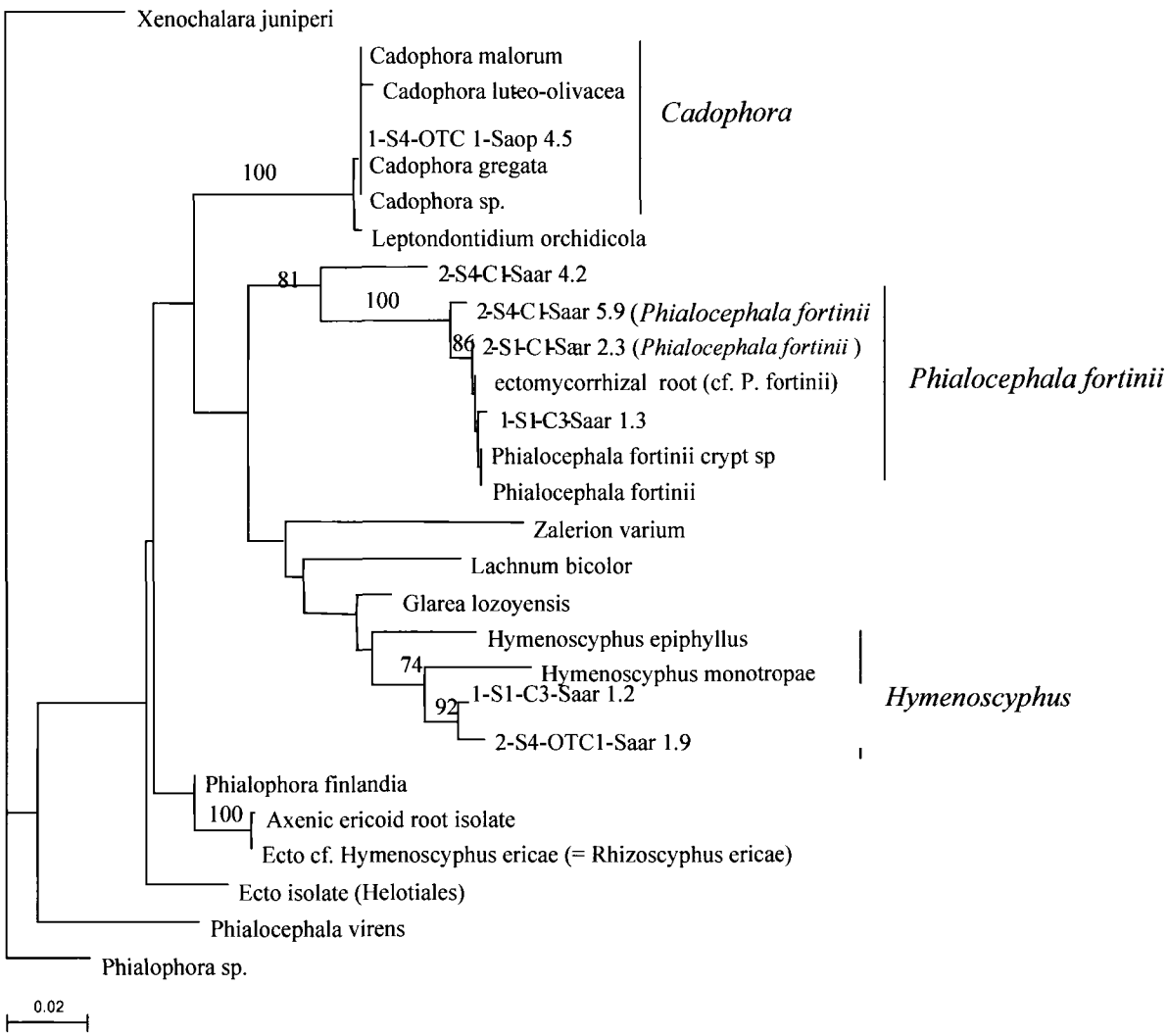



Fig. 3.5 Neighbor-joining best tree based on ITS and the Helotiales. Bootstrap values (1000 replications) >75 are reported. Names in parenthesis after samples are identifications based on morphology.

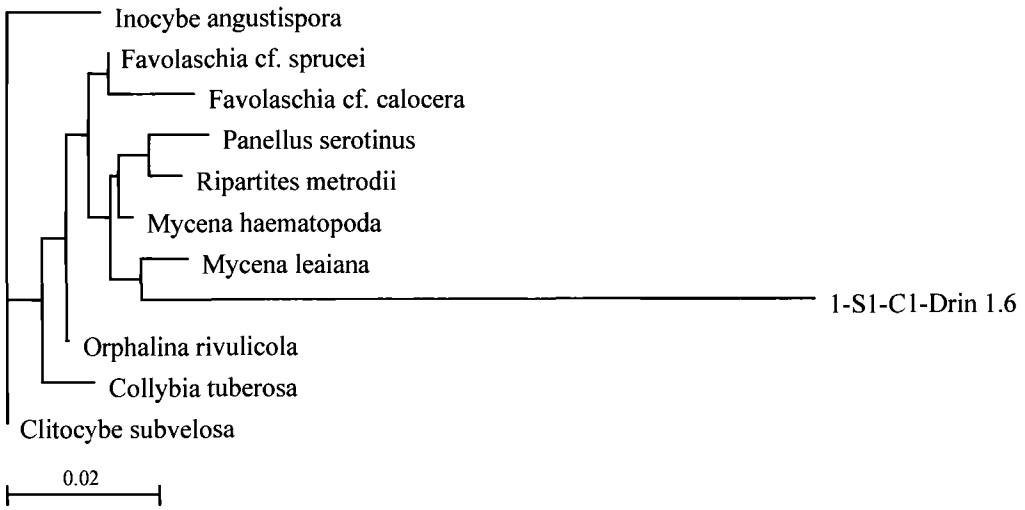



Fig. 3.6 Neighbor-joining best tree based on LSU and the Agaricales.

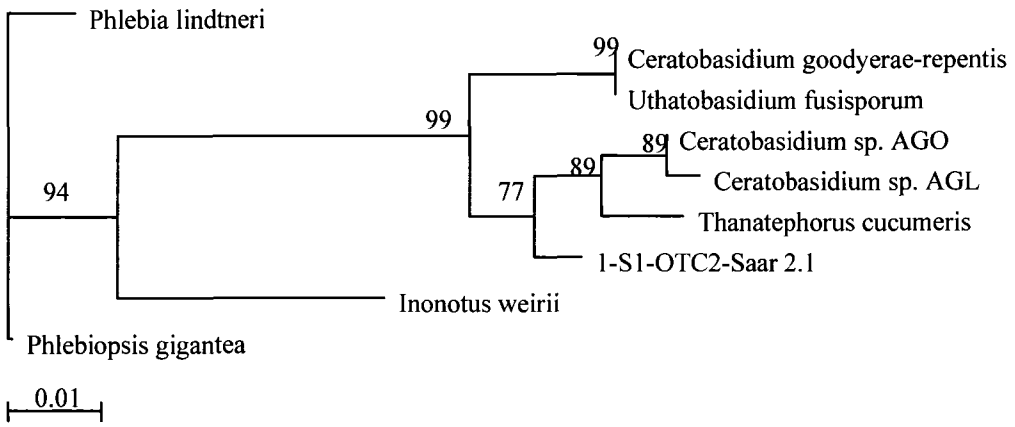



Fig. 3.7 Neighbor-joining best tree based on LSU and the Ceratobasidiomycetes. Bootstrap values (1000 replications) >75 are reported.

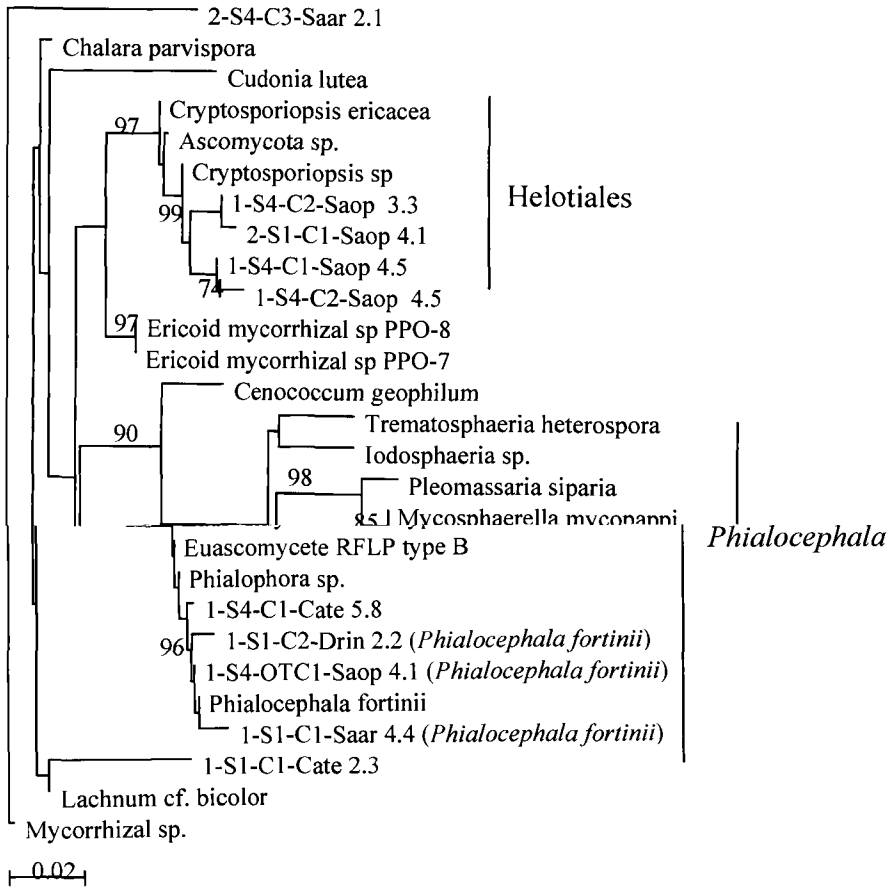



Fig. 3.8 Neighbor-joining tree based on LSU and ascomycetes. Species in parentheses are based on morphology. Bootstrap values (1000 replications) >75 are reported.

*P. fortinii* (15%).

Success of isolation of *Phialocephala fortinii* differed according to host plant species ($p < 0.001$), site ($p < 0.001$), and from which site the host plant was taken ($p < 0.001$). *P. fortinii* was found on *Salix arctica* more than other host plants (60.5%), but the number of successful isolations plummeted for *S. arctica* on the dolomitic site (Fig 3.9). The cumulative frequency of isolates for the lowland OTC plots was as high as 34, but only 1-2 isolates were found on the dolomitic plots. With *Saxifraga oppositifolia* as the host, isolation of *P. fortinii* was most successful from the granitic site (~ 6 isolates), whereas the number of

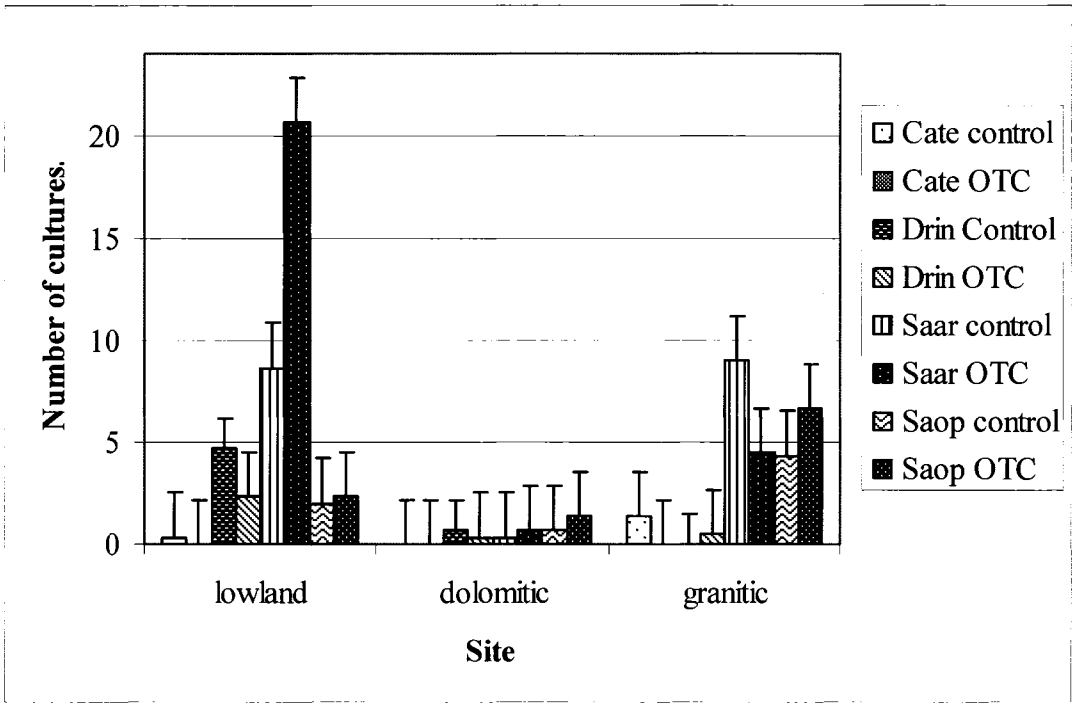



Fig 3.9 Abundance of *Phialocephala fortinii* based on number of morphologically identified cultures differentiated by host plant and treatment. Cate = *Cassiope tetragona*, Drin = *Dryas integrifolia*, Saar = *Salix arctica*, and Saop = *Saxifraga oppositifolia*. OTC = open top chamber, and control refers to ambient conditions.

isolates was low (less than 2) on both the lowland and dolomitic sites. Isolation of *P. fortinii* from *Cassiope tetragona* was low from all sites. Isolation from *Dryas integrifolia* was most

successful from the lowland site with 14 isolates from the control and seven from the OTC plots. Isolating *P. fortinii* from the two highland sites was not very successful, with only two from the control and one from the OTC from the dolomitic site and no isolates from the granitic site.

4. Identifications based on molecular techniques and morphology

When combining sequence and morphological data, 193 (44.7%) of the cultures remained unknown or inconclusive, 63 (14.6%) were identified as *Phialocephala fortinii*, 44 (10.2%) as members of the Helotiales, 29 (6.7%) closest to *Mycosphaerella*, 23 (5.3%) closest to members of *Cryptosporiopsis*, 21 (4.9%) closest to *Hymenoscyphus*, 12 (2.8%) closest to *Phoma,* 9 (2.1%) closest to *Ceratobasidium*, 10 (2.3%) closest to *Cadophora*, 9 (2.1%) as members of the Dothideales, 7 (1.6%) closest to *Geomyces*, 4 (0.9%) as members of the Agaricales, 2 (0.5%) closest to *Colispora*, 2 (0.5%) closest to *Trichoderma*, and 1 each (0.2%) closest to *Monodictys, Penicillium, Sebacina,* or *Trichocladium.* Samples whose morphological identifications were incongruent for the two replicates were considered inconclusive. Samples identified based on molecular data and corresponding morphological identification are listed in A3.1-A3.12.

Morphological identification as *Phialocephala fortinii* matched 34 of 52 samples (65.4%) that were shown to be affiliated with *P. fortinii* from sequence/RFLP analyses (Table A3.1). An additional five isolates that were affiliated with the Helotiales based on sequence analysis were morphologically identified as *P. fortinii* (Table A3.2). When isolates with RFLP patterns that matched these five Helotiales were included in the *P. fortinii* complex, an additional 15 samples were included in the *P. fortinii* complex. Morphological identification also suggested that 11 samples that could not be identified from

sequence/RFLP analyses were *P. fortinii* (Table A3.3). Each of these 11 samples had a unique RFLP pattern. There were seven different RFLP types based on identification by sequence/RFLP and morphological analyses, which would total 18 different RFLP patterns for all putatively identified *P. fortinii*. There were some discrepancies between molecular and morphological identifications. *P. fortinii* was identified morphologically for samples that were affiliated with *Mycosphaerella* and members in the Dothideales according to the molecular analysis. The RFLP patterns for these samples matched others in the same cluster but were identified differently morphologically.

There were also discrepancies between the two identification methods for *Hymenoscyphus, Mycosphaerella*, and the Dothideales. One sample, identified morphologically as *Leptodontidium*, was affiliated with *Hymenoscyphus* based on sequence data, and belonged to an RFLP pattern that had a wide range of morphological descriptions (Table A3.4). Another discrepancy included a sample that was identified morphologically as *Phialocephala fortinii*, whose RFLP pattern matched two samples that were affiliated with *Hymenoscyphus* according to sequence data. In total, four RFLP types were found for this group.

Twenty-nine samples were affiliated with *Mycosphaerella*, which formed five different RFLP patterns (Table A3.5). Two discrepancies between morphological and molecular techniques were found; one sample was identified as *Phialocephala fortinii* and the other *Staphylotrichum*, both of which have RFLP patterns matching several samples associated with *Mycosphaerella*.

Members that were shown to be affiliated with the Dothideales by the molecular methods had some incongruity with the morphological identification (Table A3.6). Two

samples were morphologically identified as *Phialocephala fortinii* (Helotiales) and one as *Acremonium*, a member of the Sordariomycetes. For all the isolates that grouped in the Dothideales, there was only one RFLP pattern, and all the samples were isolated only from *Saxifraga oppositifolia*.

Ten samples were affiliated with *Phoma* according to sequence and RFLP data, with two different RFLP patterns. Morphologically, *Monodictys* and *Trichocladium* were identified for four of these samples (Table A3.7). *Phoma* belongs to the mitosporic Ascomycetes, while both *Monodictys* and *Trichocladium* are teleomorphs that are members of the Sordariomycetes. Two of the *Monodictys* had the same RFLP pattern as *Trichocladium* and other members of the *Phoma* clade, while the third *Monodictys* had a RFLP pattern that matched the second RFLP type for *Phoma*.

Four samples were associated with *Cryptosporiopsis* from sequence analysis, but when samples with matching RFLP patterns were included, the number of samples increased to 23 (Table A3.8). Three of these samples were supported by morphology. There were two RFLP types for this clade, which had only a difference of 50 bp for one of the fragments in *Mbo*II; all other fragments in the three enzymes matched.

Ten samples were affiliated with *Cadophora* according to the molecular analysis, with three RFLP types. Three of these samples were morphologically identified as *P. fortinii* (Table A3.9).

There were only two groups of cultures associated with Basidiomycetes, the Agaricales and *Ceratobasidium* (Tables A3.11-A3.12). All the morphological identifications were labeled as 'hyaline sterile', with the exception of one of the replicates, which was inconclusive.

Most of the samples that were not identified by sequencing/RFLP data remained unknown based on morphology, but a few samples were identified. Seven unknown samples that were morphologically identified as *Geomyces* were probably the same species because they all had the same RFLP pattern. Although three samples were identified as *Trichocladium*, each had a distinct RFLP pattern, which may be an artifact of the small number of samples.

D. ***Discussion***

In this study, site was the most influential factor in distinguishing the root endophytic fungal community composition, more so than warming or host plant, which was evident from the NMS analysis. Most of the members consisted of DSE, of which *Phialocephala fortinii* was the most common. This was similar to the findings of Stoyke et al. (1992) when they isolated fungi from 10 different subalpine host plants, but contrary to Kohn and Stasovski (1990) who reported no DSE from the same study area. In concurrence with previous research (Kohn and Stasovski 1990, Stoyke et al. 1992), hyaline septate fungi were found in the present study. Thirty-one different species were found on the granitic - OTC plot based on RFLP analysis and 30 species with morphological identification, which was comparable to the species richness of isolated root endophytes found in temperate grasslands (Wilberforce et al. 2003).

Differences in species cumulative frequency depended on site and host plant but not due to passive warming. The dolomitic site had significantly fewer isolates than the other two sites. This may be in part due to the soil conditions of this site, where CEC, pH, Ca, and C:N ratio were higher than from the other two sites (see Chapter 2), the pH substantially higher, pH 8, versus pH 6 for the other two sites. Interestingly the host plants *Salix arctica*

and *Saxifraga oppositifolia* yielded the most isolates from the granitic site, but at the lowland site, *Dryas integrifolia* and *Cassiope tetragona* produced the most isolates.. Although the granitic site had more isolates than the other two sites, it was the most homogenous, as indicated by the tight clustering found in CCA. This may be because isolates from the granitic site were from *S. arctica* and *S. oppositifolia*, with the one exception of isolates from *C. tetragona* that were found on one of the plots; whereas, the other sites had isolates from the other host plants. The high variability from the dolomitic site may be due to the low number of isolates that were found from this site.

Even though root tips from *Cassiope tetragona* were isolated using a protocol that supposedly favors ericaceous plants, the success rate was low when compared to *Salix arctica* and *Saxifraga oppositifolia*. Fungal isolates from *Dryas integrifolia*, a reputed ectomycorrhizal plant, were also low. The incubation temperature may also have been too high. Although unlikely, the possibility that isolates from *D. integrifolia* and *C. tetragona* may favor colder conditions than those found on the other host plants is possible.

Species richness tended to be higher for most plots when assessed by RFLP analysis compared to morphological identification, which was in part because cultures morphologically identified as *Phialocephala fortinii* had 18 different RFLP types. Many of the cultures that were identified as *P. fortinii* had unique RFLP types; therefore, the ITS region for these *P. fortinii* isolates was highly polymorphic, as were strains from Europe and North America (Harney et al. 1997, Grünig et al. 2002a). Our results were contrary to studies that found morphological differences but no or little RFLP differences for the rDNA ITS region (Addy et al. 2000, Jumpponen 1999). Although Addy et al. (2000) covered a larger geographic range, we had 70 isolates of *P. fortinii* compared to their 33 isolates and 34

for Jumpponen (1999), which may partially explain the wide range of RFLP types in our study. Our strains may have more characters that were polymorphic as Grünig et al. (2002a) found with their isolates, which had over five times more polymorphic characters than Addy et al. (2000).

*Phialocephala fortinii* is part of a species complex composed of cryptic species, which are species that are morphologically similar but have unique genotypes (Grünig et al. 2004, Piercey et al. 2004). Cryptic species are often found in morphologically asexual fungi that are genetically isolated (Taylor et al. 1999), which would likely be the case for these *P. fortinii* from Ellesmere Island. Grünig et al. (2004) even found different cryptic species on the same root and clusters of cryptic species that were morphologically indistinct but had high diversity at the population level. In addition, genetic variation of *P. fortinii* increases at higher latitudes (Ahlich and Sieber 1996, Piercey et al. 2004); which is consistent with the high number of RFLP types found for *P. fortinii* at our site. However, some samples may have been misidentified, which is particularly true for those with RFLP patterns that matched other species, and samples with unique RFLP patterns that were not supported by molecular analyses. This inflated the number of ribotypes and may be an indication of phenotypic plasticity for culture identification.

Most of the *Phialocephala fortinii* were isolated from *Salix arctica.* Although *P. fortinii* has been reported to be non-host specific, it was isolated more frequently from *S. arctica* and *Saxifraga oppositifolia* than from *Cassiope tetragona* and *Dryas integrifolia.* This difference may be a combination of two factors: 1) *P. fortinii* is found commonly on roots of ectomycorrhizal plants (Stoyke et al. 1992, Jumpponen 1999, Addy et al. 2000, Grünig et al. 2001), which would explain its absence on the ericoid *C. tetragona*, and 2)

although *D. integrifolia* is an ectomycorrhizal plant, the root systems from *Salix arctica* and *Saxifraga oppositifolia* were larger. Also, *P. fortinii* grew more frequently on the granitic and lowland sites. The number of isolates decreased significantly from plants grown on the dolomitic site, perhaps due to the different soil chemistry properties found on this site. Surprisingly, isolates from *Saxifraga oppositifolia* did not follow the same trend as those from *Salix arctica*. Even though the abundance of *P. fortinii* was comparable from the two hosts from the granitic site, there were significantly more isolates found on *Salix arctica* on the lowland site. Therefore, both host plant and site appear to affect the abundance of *P. fortinii*.

Twenty fungal isolates were potentially affiliated with *Cryptosporiopsis*. Only three of these samples were identified morphologically; the other samples were identified from RFLP patterns. These isolates varied in color, suggesting that morphological differences could be due to different stages of development. Verkley et al. (2003) found that *Cryptosporiopsis* isolates from Ericaceous plants that grow on MEA and oatmeal agar change colors at different stages of development; even though our isolates were grown on MMN, this change could occur on this medium as well. Also identification could have been hampered because macroconidia are rarely produced in culture (Verkley et al. 2003). Although Verkley et al. (2003) found that isolation of *Cryptosporiopsis* is rare after surface sterilization, we were successful even though we surface sterilized the roots, as did Ahlich and Sieber (1996), who isolated *C. radicola* from *Fagus sylvatica, Abies alba, Picea abies,* and *Pinus sylvestris*. *Cryptosporiopsis* has teleomorphs in *Pezicula* and *Neofabracea*. However, our sequences were based on the primer pair for the large subunit of the rRNA gene and sequences for this region were not available in GenBank for *Pezicula* and

*Neofabracea.* Therefore, we could not resolve the teleomorph with which our samples were affiliated.

Five isolates were affiliated with *Mycosphaerella* with an 85% bootstrap value and an additional 23 samples were included with matching RFLP patterns. *Mycosphaerella* has not been reported from roots in Arctic and Antarctic systems, but *Cladosporium*, which has a *Mycosphaerella* teleomorph (Wirsel et al. 2002) and is described as an oligotrophic, melanized fungus (Wirsel et al. 2002, Gunde-Cimerman et al. 2003), is commonly found in the Arctic and Antarctica. In particular *C. sphaerospermum, C. herbarum* (Gunde-Cimerman et al. 2003, Bergero et al. 1999), and *C. cladosporoides* (Widden and Parkinson 1979, Robinson 2001, Tosi et al. 2002) are found. *Cladosporium* has been reported to have a wide range of functions, from saprotrophic, to epiphytic and endophytic in aboveground plant tissues, and pathogenic and mycoparasitic (Held et al. 2005, Wirsel et al. 2002, David 1997). Our samples were probably pathogenic or were soil fungi that were not killed by surface sterilization, because there have been no reported cases of root endophytic *Cladosporium.* However, this needs to be investigated further.

Other common fungi found in the Arctic include *Geomyces* and *Phoma* (Bergero et al. 1999, Robinson 2001, Tosi et al. 2002). We found seven samples that were morphologically identified as *Geomyces* with matching RFLP patterns. Ten samples were affiliated with *Phoma* after sequencing and RFLP analyses. Three of these samples were morphologically identified as *Monodictys*. This discrepancy may be due to *Phoma* having *Monodictys* – like conidia. When Grondona et al. (1997) described *Pyrenochaeta dolchi*, which belongs to *Phoma* section *Paraphoma*, they found this species as having *Monodictys* – like conidia on lateral branches and also stated that the conidia were similar to *Phoma cava*

and *Phoma tracheiphla*. These fungi were found in soil by previous researchers (Bergero et al. 1999, Robinson 2001) or from moss (Tosi et al. 2002). Because we surface sterilized our roots, these fungi may be endophytes colonizing the root or saprotrophs not adversely affected by the hydrogen peroxide used for sterilization.

Our fungal community may be overly representative of psychrotrophic fungi. Psychrotrophic and psychrophilic fungi can grow at 0 °C, but psychrotrophic fungi can grow above 20°C, while psychrophilic fungi have an optimum growth temperature at 15 °C or below and cannot grow above 20°C (Robinson 2001). We grew our cultures at room temperature, which may have excluded some of the psychrophilic fungi. Another factor in determining what fungi were successfully isolated is pH, because it influences the growth of cultures (Taber and Taber 1984). Our isolates were grown on MMN, which has a pH of 5 to 6. However, we found later that the pH of our sites was higher at 6 to 8. The high pH of 8 was from the dolomitic site, which may help explain the low number of fungi that successfully isolated from roots.

Discrepancies between morphological and PCR-based identification can be the result of two factors. The morphological identification may be incorrect, or the identity from the sequence database may be incorrect. Bridge et al. (2003) found that approximately 20% of sequences available in GenBank are unreliable for the ITS region of rDNA for *Amanita* and *Phoma* and the rRNA small subunit for members of the Helotiales. Our findings, however, were probably accurate because many of the similar taxa downloaded for analysis had more than one sequence for a species. Problems can arise if the unknown sequence is closely matched to an incorrectly identified sequence. Knowledge of phylogenetic relationships and taxonomy is important to find these possible misidentified sequences.

Overall, warming and host plant did not have a noticeable impact on the composition of the root endophytic fungal community, which was dictated primarily by site. These plots have only been warmed for approximately five years, which may be too short a time to find differences, especially in the arctic where processes are much slower than those found in temperate forests. Examining the fungal communities after another 5 to 10 years may show more effects of warming on the root endophytic fungal community. Although host specificity was not found for the overall community, *Phialocephala fortinii* was preferentially isolated from *Salix arctica* and *Saxifraga oppositifolia* over other host plants.

Our study identified many of the isolates; however, most of the isolates remain unidentified. Even samples that have been sequenced remained unknown, which implies that there are many fungi that have yet to be identified in these ecosystems. The role of these fungi was not determined although many have speculated that root endophytes play an important role for nutrient uptake by plants in harsh environments. Bioassay studies using some of these isolates would help elucidate some of the roles of these fungi.

## IV. The root-associated fungal community along a directional, non-replacement succession chronosequence from the Canadian High Arctic

### A. *Introduction*

Facilitation is a type of complementary effect, a theory to explain how resource use by organisms can direct ecosystem processes (Cardinale et al. 2002, Loreau and Hector 2001). In this theory, species diversity can increase while avoiding competition, and species are able to co-exist especially in environments with limiting resources by: 1) partitioning resources, where each species can use nutrients, water, or other resources differently instead of all species competing for or using the same resources; or 2) niche differentiation, where different species avoid using the same resources as other organisms by means of time and/or space (McKane et al. 2002). In contrast, selection effect theory is where species diversity is correlated with the chance that a dominant species uses most of the available resources (Cardinale et al. 2002), so the formation of the community is heavily dependent on this one central species.

Facilitation is thought to play an important role for the primary succession of mycorrhizal fungi in that nutrient poor conditions are improved by pioneer species, as indicated by increasing diversity of arbuscular mycorrhizal fungi after volcanic disturbances (Titus and del Moral 1998), and of ectomycorrhizae on glacial tills (Helm et al. 1999), and by the increase of nitrogen and organic matter along a chronosequence from a sub-alpine glacier (Jumpponen et al. 1998). This facilitative process is also inferred by the successional pattern in the mycorrhizal status of plants, which begins with non-mycorrhizal plants, to arbuscular mycorrhizal, ectomycorrhizal (Read 1993), and/or ericoid mycorrhizae (Cázares 1992). Given that complementary effect is the main theory to explain resource use by the root-

associated fungal community, selection effect theory cannot be totally discarded. The possiblility that one or two dominant species may use most of the resources and influence how the community structure will develop subsists.

The objectives of this study were to examine how the mycorrhizal fungal community composition, species diversity and abundance change according to host plant and chronosequence in a directional, non-replacement succession. Directional, non-replacement succession has been used to describe a type of plant succession, where species found in the youngest plots are also found in older plots; they are not replaced by different plant species (Svoboda and Henry 1987). This type of succession serves as a natural laboratory where the increase of biodiversity can be examined. This study is the first to our knowledge that examines the root-associated fungal community in this type of succession. Also, previous research on succession in the arctic occurred in the low arctic where trees are present (Helm et al. 1999, Helm et al. 1996, Brubaker et al. 1995, Chapin et al. 1994). Ellesmere Island is located in the high arctic where only low shrubs grow.

## B. *Materials and methods*

### 1. Study site

Samples were collected from the receding western lobe of the Twin glacier located at the south end of an 8-$km^2$ lowland high arctic plant oasis on Ellesmere Island, Nunavut, Canada (78°53'N, 75°55'W) at the end of July, 2001. The western lobe of the Twin glacier, which started to advance since the Little Ice Age and began to recede approximately 1960, is diminishing approximately 10 m/yr (GHR Henry, pers. comm.). Plots were placed within zones delineated by the time since exposure: 1990 to present [labeled 1990 plots], 1980-90 [1980 plots], 1970-80 [1970 plots], and 1960-70 [1960 plots]. The area before 1959 was not

covered by the western lobe of the glacier (GHR Henry, pers. comm.), so plants collected from this area were used as controls.

2. Field collection

Plants were selected that fulfilled the directional, non-replacement succession model (i.e. once the plant appeared within the chronosequence, it was present for all remaining chronosequence zones including the control). The plants chosen were *Luzula confusa* Lindeb., *Papaver lapponicum* (Tolm.) Nordh., *Salix arctica* Pall., *Saxifraga oppositifolia* L., *Cassiope tetragona* (L.) D.Don, and *Dryas integrifolia* Vahl. *Luzula confusa* and *P. lapponicum* were first present in the 1990 plots (Y90), and they were the dominant plants for 1990, 1980, and 1970 plots. *S. arctica* first appeared in the 1980 plots (Y80), became more abundant and larger in the 1970 plots (Y70), and was dominant in the 1960 plots (Y60). *S. oppositifolia* was first noted in the 1970 plots, and *C. tetragona* and *D. integrifolia* in the 1960 plots. The control (<Y50) was dominated by *C. tetragona* and *S. arctica*. *Epilobium latifolium* Pursh was also found, but only in the 1980 plots. Moss was present in all chronosequence zones. Three plant specimens of each target species were collected, and when possible, approximately 300 g of soil were collected adjacent to the plant samples. Both plant and soil samples were stored in a permafrost cooler while in the field and at 4° C at UNBC until processed.

3. Sampling from roots

Soil was loosened from plant roots by storing roots in water for at least 24 hours at 4° C. A 0.5 mm sieve (No. 35 USA standard testing sieve, W.S. Tyler, Inc.) was used to capture roots that were gently cleaned with water. Fifteen root tips were randomly sampled by placing root systems on a numbered grid. Grid locations were selected with a random

number generator, and 2-cm segments of root closest to the grid location were selected. Root segments were described morphologically based on color, tip ramification, and presence and absence of rhizomorphs (Agerer 1987-1998, Goodman 1995). Tips were then placed in a 1.5 ml Eppendorf tube and frozen in a -40° C freezer until DNA extractions were done. Remaining root systems were frozen and lyophilized. To test if primers would amplify plant DNA, plant tissue from stems or leaves was also collected.

4. Root microscopy

Root tips of *Papaver lapponicum* and *Luzula confusa* were cleared and stained according to Brundrett et al. (1996) to confirm that they were non-mycorrhizal as reported by Kohn and Stasovski (1990). If roots of other species appeared to be non-mycorrhizal when randomly selecting for DNA extraction, a subsample was collected for clearing and staining as well. Roots were cleared by autoclaving in 10% KOH for 30 min for *P. lapponicum*, 60 min for *L. confusa*, and 80 min for *Saxifraga oppositifolia* and *Cassiope tetragona.* Cleared roots were rinsed with water and then stained by autoclaving for 15 min in 0.03% Chlorazol Black E (CBE) in lactoglycerol. Roots were stored and mounted in lactoglycerol and observed microscopically.

5. DNA extraction and ITS-T-RFLP analysis

Extraction of root endophytes followed the CTAB protocol of Gardes and Bruns (1996b), modified by excluding the freeze-thaw process and including an extra purification step with phenol:chloroform-isoamyl alcohol (1:1) (Lee and Taylor 1990). DNA was resuspended in 50 µL of TE-8 buffer. Plant tissue was extracted with the same protocol except the extra phenol:chloroform-isoamyl alcohol purification step was not included, and

DNA was resuspended in 100 μL of TE-8 buffer instead of 50. All extractions were stored in -20° C until use.

The ITS region of the rRNA gene was amplified using a PCR cocktail composed of 10X Buffer (200 mM Tris-HCl [pH 8.4], 500 mM KCl) (Invitrogen), 2X dNTPs, 25 mM $MgCl_2$, 0.5 μM of ITS 1 (White et al. 1990) dye-labeled with Cy 5.0, 0.5 μM of ITS 4 (Gardes and Bruns 1993) dye-labeled with Cy 5.5, 5 U of Platinum *Taq* Polymerase (Invitrogen), and 2.2 μL of DNA template. PCR amplification was accomplished using the following program on a MJ Research thermocycler PT-100: 94 °C (4 min); 48° C (1 min); 72° C (2 min); [94 °C (30 sec); 48° C (30 sec); 72° C (1 min 30 sec) x 34 cycles]; 72°C (6 min 30 sec). Successful amplification was verified on a 0.7% agarose gel. For all unsuccessful amplifications, amplifications were retried with the DNA templates diluted to 1:10.

The endonucleases *Alu* I and *Hinf* I (Invitrogen) were mixed with 6 μL of the PCR product in a 10 μL volume following the manufacturer's recommendations and incubated at 37° C for at least 3 hours. Digested product was mixed with formamide loading dye (1:1.65 μL) then denatured at 80° C in a heat block for 2 min and quenched on ice before 2 μL were loaded onto a 6% polyacrylamide gel. The formamide loading dye contained two sets of internal markers at 101, 200, and 351 bp, labeled with Cy 5.0 for one set and Cy 5.5 for the other. Samples were run for 60 min. on an OpenGene System Long Tower Sequencer (Bayer International), with the laser power set at 50%, temperature at 53° C, and current at 1250 V. GeneObjects 3.1 fragment analysis software (Bayer International) was used to determine fragments for each primer-enzyme combination (i.e. ITS 1-*Alu* I, ITS 1-*Hinf* 1, ITS 4-*Alu* I, and ITS 4-*Hinf* I).

6. Matching root tips with sporocarps

Fragments generated from root-associated fungi were compared with those of sporocarps that were collected from Alexandra Fiord in 2000 using TRAMP (T-RFLP analysis matching program) (Dickie et al. 2002). Multiple fragments were found on many of the root tips; therefore, a database using every combination of fragment sizes from the four primer-enzyme combinations was made to be tested with fragment sizes from sporocarps.

7. Statistical analysis

Frequency tables of genotypes generated from the T-RFLP analysis were made for each enzyme-primer combination. To adjust for inter-gel variation, genotypes were manually binned. These average binned fragment genotypes were used to construct frequency tables, which were based on cumulative frequency of the genotypes for each replicate of treatment per site. The different primer-enzyme combinations were used as individual datasets for examining if root-associated fungal communities were more influenced by time of exposure or by host plant; therefore all analyses except for the ordination analyses were done in quadruplicate (once for each enzyme using forward [Cy5] and reverse [Cy5.5] fragments). Genotypes that matched fragments from plant tissue (+/- 3 bp) of *Saxifraga oppositifolia* and *Papaver lapponica* were deleted.

The frequency tables made for the four primer-enzyme combinations were merged into a single frequency table to use for non-metric multidimensional scaling (NMS) with PC-ORD, version 4.25 (McCune and Mefford 1999). NMS was used to examine the effects of time of exposure and host plant on species frequency. Beal's transformation was applied to the merged data to alleviate problems associated with datasets that contain numerous zeroes (McCune and Grace 2002). The following parameters for NMS were: Sorensen was used for

measuring distance; selection for the configuration of the first run was random, but scores for the third configuration were used for the consequent runs as recommended by the program; 15 runs were conducted; dimensionality was assessed by examining the scree plot; and 30 randomized runs were used in the Monte Carlo test.

Canonical correspondence analysis (CCA) was used to test for correlations between soil properties and differences in fungal communities using PC-ORD (McCune and Mefford 1999). Transformation of genotypes was done by adding one to each frequency to alleviate the problems associated with too many zeroes in databases. Soil properties (pH, CEC, available $NH_4$, available $NO_3$, available P, and C:N ratio, and exchangeable cations) were transformed with log base 10 for each property except for pH and C:N ratio. Linear relationships between the genotype abundance and soil properties were tested with the Monte Carlo test. The ordination diagram was based on LC (linear combination) scores; these scores are linear combinations of soil properties (McCune and Grace 2002).

To examine how communities would group according to time exposed for a given host plant, a hierarchical clustering was done using Ward's method of clustering based on a Euclidean distance matrix. Ward's method is a space-conserving method and minimizes chaining, which is the addition of single items to existing groups so distinct branches are lost from dendrograms (McCune and Grace 2002).

Effects of soil properties were further examined by simple regression, where

[1] $\mu\{\text{soil properties}|\text{year of exposure}\}=\beta_0 + \beta_1 \text{yr of exp} + \varepsilon$.

A regression was done for each of the 13 soil properties: exchangeable Al, exchangeable Na, exchangeable Fe, exchangeable K, exchangeable Ca, CEC, exchangeable Mg, exchangeable Mn, pH, C:N ratio, available P, available $NO_3$-N, and available $NH_4$-N. To test for the

possible changes in soil properties due to presence of plants, simple regressions were done, where

[2] $\mu\{\text{soil properties}|\ \text{host plant}\}=\beta_0 + \beta_1 y\ \text{host plant} + \varepsilon$.

Host plant was limited to plots in which they were present, e.g. to test for all chronosequence zones, only *Luzula confusa, Papaver lapponicum*, and *Salix arctica* were used; whereas for the control and Y60 all host plants were used because all were present in these plots.

Genotype diversity was examined using two indices (richness and evenness) and rank abundance curves. Richness was determined as the number of different genotypes for a given plot. Evenness was determined using EcoSim (Gotelli and Entsminger 2001) for each genotype where Hurlbert's probability of interspecific encounter (PIE) index was applied to measure evenness. Simple regression was done to test for differences of richness and abundance (the number of individuals in a plot) for each host plant at each succession zone. Niche differentiation was analyzed by examining rank-abundance curves. The natural log of abundance of species was graphed against the arithmetic ranking of abundance. To test for similarities in community composition, Sorensen's quantitative similarity index was used in EstimateS, vers.7 (Colwell 1997). Communities from the same host plant as well as different host plants were tested for shared species.

## C. ***Results***

Overall, 53% of the 930 attempted extractions were successful for all four primer-enzyme combinations. Successful amplification differed according to host plant and primer-enzyme. Fungi extracted from roots from *Papaver lapponicum* and *Luzula confusa* were most successful (see Table 4.1) and *Saxifraga oppositifolia* the least. When examining the number of extractions per number of attempts, the percentage of successful amplification was

comparable for all primer-enzyme combinations. When compared to total number of genotypes generated, up to 16% more genotypes were obtained with the forward primer than the reverse. Although there were no genotypes that matched with sporocarps, there were matches with sequenced cultures of *Phialocephala fortinii*. From microscopic analysis, fungi colonizing *Papaver lapponicum* and *Luzula confusa* were endophytic fungi with runner hyphae and microsclerotia present, characteristics of dark septate endophytes. *Cassiope tetragona* and *Saxifraga oppositifolia* also had runner hyphae and microsclerotia present for those samples examined with clearing and staining.

Fungal communities, determined by NMS and CCA, were distinguished by host plant and not by how long the soil had been exposed in the chronosequence (see Figs. A4.1 and 4.1). For both analyses, the root-associated fungal community found on *Papaver lapponicum*, *Luzula confusa*, and *Cassiope tetragona* were distinct from each other. The communities found on *Salix arctica,* and *Dryas integrifolia* were more tightly grouped. In NMS, the fungal community from *Saxifraga oppositifolia* clustered with *L. confusa*. The overall variance in NMS was explained by a cumulative $r^2$ =0.925 with three axes; axis 1 explained 47.5% of the variance, axis 2 explained 15.4% of the variance, and axis 3 explained 29.6% of the variance. The final solution required 58 iterations. Final stability of the model was met with the final stress = 8.01 (p= 0.03) and final instability = 0.00009.

Soil was not a determining factor in distinguishing root-associated fungal communities that are separated according to host plant. From the CCA, available P was more strongly associated with the *Luzula confusa* clade, and nitrate was higher for soil surrounding *Papaver lapponicum* and *L. confusa*. However, results from the Monte Carlo test indicate that there was no relationship between soil variables and the main matrix

Table 4.1. Number of successful extractions according to the number of attempts at extraction and total number of genotypes for each host plant.

| | ITS 1-*Hin*fI | | ITS 4-*Hin*fI | | ITS 1-*Alu*I | | ITS 4-*Alu*I | |
|---|---|---|---|---|---|---|---|---|
| | # of successful extraction per # of attempts | # of extractions per total # of genotypes | # of successful extraction per # of attempts | # of extractions per total # of genotypes | # of successful extraction per # of attempts | # of extractions per total # of genotypes | # of successful extraction per # of attempts | # of extractions per total # of genotypes |
| *Luzula confusa* | 61% | 82% | 63% | 76% | 61% | 82% | 63% | 75% |
| *Papaver lapponicum* | 79% | 92% | 78% | 81% | 77% | 88% | 78% | 81% |
| *Salix arctica* | 48% | 67% | 46% | 59% | 44% | 62% | 44% | 55% |
| *Saxifraga oppositifolia* | 18% | 41% | 17% | 29% | 18% | 41% | 18% | 30% |
| *Cassiope tetragona* | 29% | 61% | 24% | 45% | 31% | 63% | 30% | 55% |
| *Dryas integrifolia* | 49% | 60% | 51% | 59% | 48% | 57% | 48% | 59% |

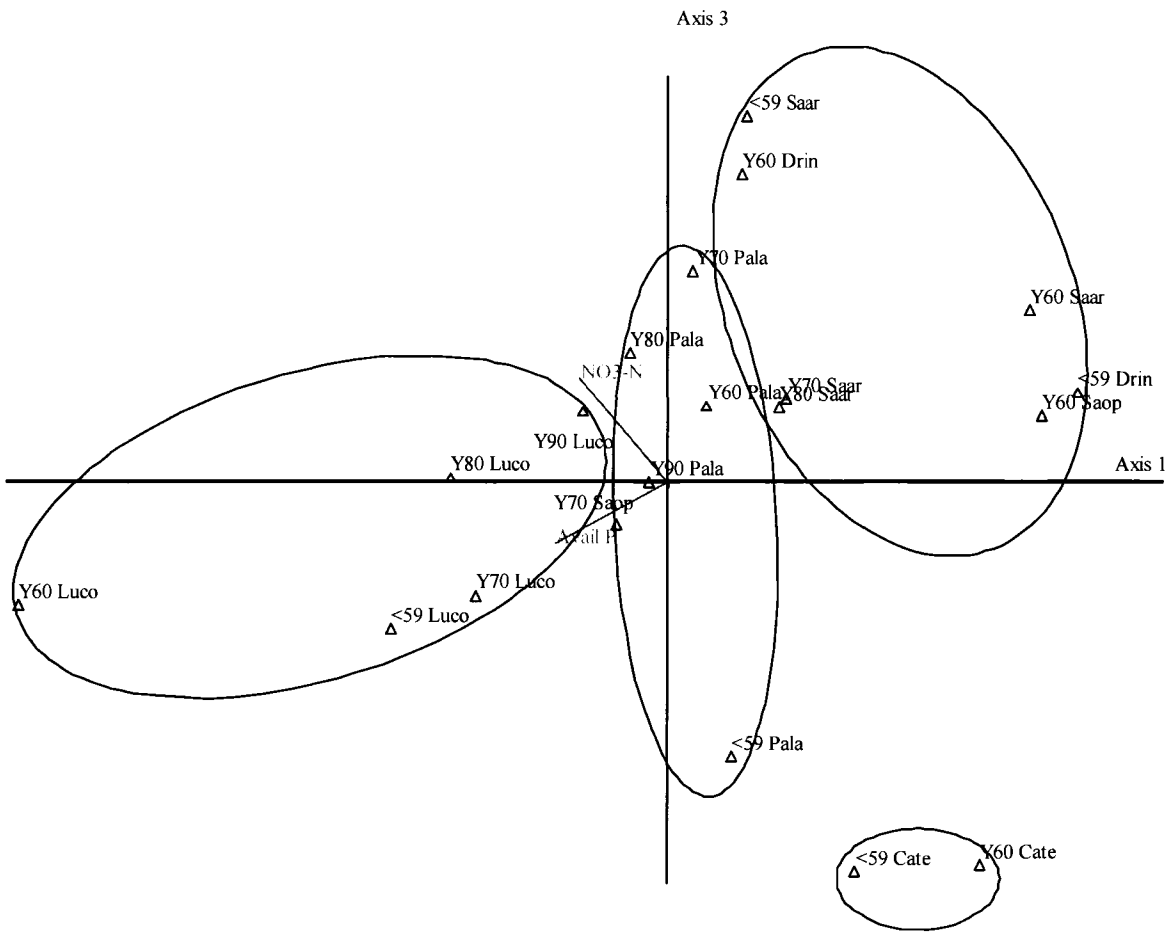



Fig 4.1 Canonical Correspondence Analysis for effects of host plant and chronosequence zone. Rotated 345°. Number preceded by 'Y' represent the earliest time of exposure from the glacier. <Y59 is the control, or refugia plot that was not covered by the glacier studied. Plants are: Cate=*Cassiope tetragona*, Drin = *Dryas integrifolia*, Luco=*Luzula confusa*, Pala=*Papaver lapponicum*, Saar=*Salix arctica*, Saop=*Saxifraga oppositifolia.* Biplots were used to examine the relationship between soil properties and genotypes.

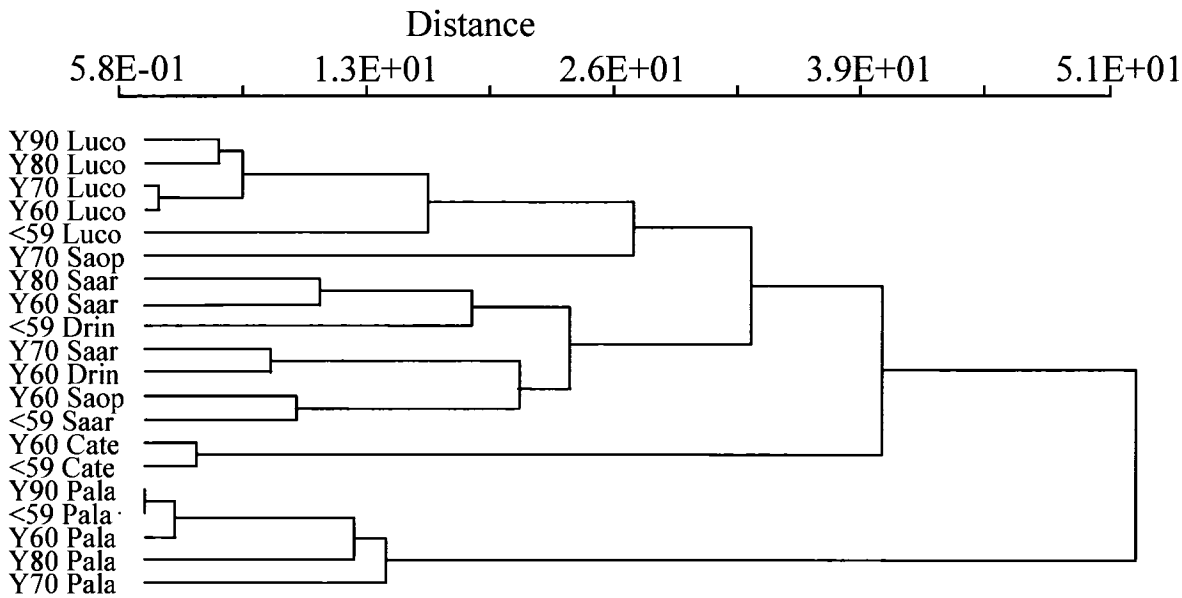



Fig 4.2 Cluster analysis using Ward's method based on Euclidean distances. Key same as in Fig. 4.1

because the eigenvalue occurrence could be explained by chance; eigenvalue for axis 1=0.106 (p=0.3); axis 2=0.059 (p=0.57); axis 3=0.047 (p=0.38). Calcium and magnesium were omitted from the final analysis because of their high correlation with CEC (Ca: r=0.97, Mg: r=0.962). The final dimensions used were 20 plots for both the main (plot) and secondary (soil properties) matrices with 288 species and 11 soil properties. Three axes were interpreted, with axis 1 explaining 19.7%, axis 2 10.3%, and axis 3 8.7% of the variance. One of the differences between the two ordination analyses is that in CCA, both fungal communities from *S. oppositifolia* do not cluster with *Luzula confusa* as in the NMS analysis; one community associated with *S. oppositifolia* grouped more closely with *S. arctica* and *D. integrifolia.*

Results from our cluster analysis supported those found from the ordination analyses and were closer to the results from CCA (see Fig. 4.2). Plots clustered by host plant with the exception of *Salix arctica* and *Dryas integrifolia* grouping together, Y70 *Saxifraga oppositifolia* branching with *Luzula confusa*, and Y60 *S. oppositifolia* clustering in the *S. arctica-D. integrifolia* cluster.

Some of the soil properties either increased or decreased as the glacier receded (see Fig. A4.2). Concentrations of Al ($p=0.0005$), K ($p=0.013$), and Fe ($p=0.006$) increased as the glacier receded, and Mg ($p=0.03$), CEC ($p=0.09$) and $NO_3$ ($p=0.009$) decreased. Although not significant, P followed a trend where amounts increased as the glacier receded, and Na and Ca tended to decrease. Amounts of $NH_4$ were lower in the glaciated areas than in the control. The pH was higher in the glaciated areas with a pH ranging from 4.2-4.5 compared to a pH in the control of 5. The C:N ratio increased from Y90 to Y80, decreased from Y80 to Y70 with the introduction of *Salix arctica*, and gradually increased with older plots. There were no differences in soil properties of soil samples collected near different host plants. Data from soil samples that were collected around *Dryas integrifolia* from the control and from around *Papaver lapponicum* in Y90, were deleted because less soil was collected from these plots, which skewed the results.

Genotype richness increased as the glacier receded for *Luzula confusa* for the reverse primer datasets (see Fig. A4.3). The regressions testing species richness against time for both ITS 4-*Hin*fI and ITS4-*Alu*I were significant ($p=0.005$ and $p=0.02$ respectively). Although regressions based on the datasets of the forward primer were not significant, they had a similar trend where richness increased with increasing time since exposure. Genotype cumulative frequency also increased significantly for *L. confusa* as the glacier receded

(p=0.008, p=0.003, p=0.02, p=0.02 for ITS1-*Hin*fI, ITS4-*Hin*fI, ITS1-*Alu*I, and ITS4-*Alu*I respectively) (see Fig. A4.4). The richness (Table A4.1), evenness, and cumulative frequency of the communities on the other host plants did not change significantly with time. Species evenness did not change significantly between plots for any community.

Similarity based on Sorensen's quantitative similarity index was highest for communities found on the same host plant except for *Dryas integrifolia*, which had a higher similarity with *Salix arctica* at 25% (Table A4.2). The root-associated fungal communities found on *Luzula confusa* for all 5 plots were more similar than fungal communities found on other host plants according to Sorensen's quantitative index, where 40%-60% of the communities were similar, depending on which primer-enzyme was used for analysis. For *Papaver lapponicum* 32%-40% of the fungal communities from five plots were similar; for *S. arctica* 41%-48% of the fungal communities found on four plots were similar; for *Saxifraga oppositifolia* 28% of the fungal communities found on three plots were similar; for *Cassiope tetragona* 26% of the fungal communities found on two plots were similar, and *D. integrifolia* 15% of the fungal communities found on two plots were similar.

The resource allocation for the fungal communities differed according to its host plant. On *Luzula confusa,* the community from Y90 fit the geometric series model and those from the control were described by the log normal model, with the communities in the other plots falling between the two extremes (Fig A4.5). Information for *Papaver lapponicum* was more inconclusive and depended upon the enzyme-primer (Fig A4.6); most of the communities from the different succession zones fit the log normal model, but some were described by the geometric series model. Most of the communities of *Salix arctica* fit the log normal model, but results with *Alu*I found that the geometric series model described

communities from Y70 plots (Fig A4.7). The communities on *Saxifraga oppositifolia* fit the geometric series model except in the Y70 plot, where the log normal model better described the community (Fig A4.8). The time of exposure made a difference for resource allocation for communities found on *Cassiope tetragona* (Fig A4.9). In the most recently exposed plots, the community associated with *C. tetragona* fit the geometric series model. However, in the older plots, the communities fit the log normal model. For *Dryas integrifolia*, the log normal model fit communities found in <Y59 plot (Fig A4.10). For communities in the Y60 plot, either the log normal or geometric series model described the community depending on the primer; communities described by the forward primer fit the geometric series model and by the reverse, the log normal model.

D. ***Discussion***

Root-associated fungal community composition depended more on the mycorrhizal status of the host plant rather than how long plant communities have colonized the area since the glacier retreated. The root-associated fungal communities found on the ectomycorrhizal plants, *Salix arctica* and *Dryas integrifolia*, grouped together in both the ordination analyses and cluster analysis. The root-associated fungal communities found on the only ericoid plant, *Cassiope tetragona*, were separated from the other plants. The root-associated fungal communities on *Saxifraga oppositifolia* clustered with either the communities found on *Luzula confusa* or with the *S. arctica* - *D. integrifolia* clade. The mycorrhizal status of *S. oppositifolia* at Ellesmere Island has been reported to be ectomycorrhizal (Kohn and Stasovski 1990), but runner hyphae and microsclerotia, characteristic of septate endophytes, were observed in its roots in the present study. Therefore, its tendency to group with the ectomycorrhizal plants and non-mycorrhizal, endophytic plants was not surprising.

The roots of *Papaver lapponicum* and *Luzula confusa* had both runner hyphae and microsclerotia, which indicated that both of these host plants were colonized by septate endophytes, which included *Phialocephala fortinii*. Interestingly, the communities on these host plants were distinct from each other as indicated by the two ordination analyses, the cluster analysis and the low similarity index from the Sorensen's quantitative index. Because septate endophytes are thought to be ubiquitous and not host-specific (Jumpponen and Trappe 1998, Wilberforce et al. 2003, Harney et al. 1997), finding similar communities on both hosts was expected. There are at least three explanations for this dissimilarity between the two communities: The first explanation is that the root-associated communities may have contained a higher proportion of parasitic or pathogenic fungi, which generally exhibit higher levels of host specificity than expected. Parasitic fungi were found from soil extractions taken from a glacial terminus (Jumpponen 2003).. Latent pathogens grow along and over the root surface, eventually the hyphae enter the stele when the plant decreases its resistance, and invade senescent cortical cells (Garrett 1981). Another explanations is that the distribution of host plants may be correlated with an environmental variable, e.g. soil moisture, that we did not measure, but which affected the composition of the root-associated community. The third possibility is that there may be more host-specificity in the root-associated community than previously recorded.

This possibility that there is more host specificity of DSE than previously thought, which would also indicate that this group is very diverse in order to have two communities distinct from the other. Genotype richness was highest for the septate endophyte communities found on *Luzula confusa* and *Papaver lapponicum*. Number of potential genotypes for *L. confusa* reached as high as 39, and its range was comparable to the number

of fungal endophytes from grasslands (Wilberforce et al. 2003). Although many of the members were thought to be *Phialocephala fortinii* from microscopic investigations, the morphological features of *P. fortinii* may not be limited to this species and may have broad taxonomic affiliations to taxa that have not been identified (Schadt et al. 2001). The high diversity of DSE may also be attributed to: 1) *P. fortinii* is a multi-species complex that contains cryptic species that have been found to occupy the same root tip (Grünig et al. 2002b); 2) additional DSE were present that fit the DSE description but were not culturable; or 3) intraspecific variation, which has been reported for the ITS region for *P. fortinii* (Harney et al. 1997). Identifying the taxa involved would help clarify the richness and distinction between the two communities.

Species richness was comparable to other fungal community studies based on RFLP and T-RFLP for other host plants. However, similarity between communities of different host plants was low. Even for communities with the same host plant but on different specimens, the similarity index was low, with only a couple of incidences where the similarity was close to 60% on *L. confusa*. The low similarity suggests that spores were aerially deposited (Jumpponen 2003), with ramets establishing from different sources (Grünig et al. 2002a). The high similarity of 60% found on *L. confusa* may be from chance. High similarity would suggest that the fungi are vegetative; however, *L. confusa* have small root systems so the chances of a root system from one plant contacting another would be small.

As the plant community became more diverse along the chronosequence, soil properties began to change. No host plant significantly altered soil properties, which indicates that the plant and its root fungal community as a whole, not individually, drove

these changes. For many parts of Alexandra Fiord and other places in the arctic, vegetation development is assumed to be the main pedogenic factor for these soils (Muc et al. 1994). Interestingly, the amount of $NO_3$ decreased with increasing time of exposure; total N and ammonium did not change with time. The decrease in $NO_3$ may be due to: 1) its status as the main nitrogen source for plants at this succession site, but this would be surprising because only some ectomycorrhizal fungi have the ability to reduce nitrate (Paul and Clark 1996); 2) its highly solubility so it may have leached from the soil; 3) High Arctic processes whose rates are slower than those found in alpine systems, so not enough time has transpired to test if the amount of N will increase; or 4) increases in denitrifiers that reduce nitrate to ammonium.

The CEC decreased as the chronosequence and plant diversity increased with time. This trend was found in Norway and was attributed to the establishment of vegetation cover and the development of nutrient cycling (Matthews 1992). This was contrary to the expected rise in CEC due to the increase of organic matter as found with a chronosequence at Glacier Bay, AK (Matthews 1992) and Lyman glacier, WA (Jumpponen et al. 1998). However, our sites did not have relatively high level of accumulation of organic matter.

Models for niche apportionment on *Luzula confusa* fit as expected. The youngest plots fit the geometric series model, which often describes communities in harsh environments (Magurran 1988). The fungal community on *L. confusa* in the control, where glaciation had not occurred during the little Ice Age, fit the log normal model, which often describes more diverse plant communities such as temperate or tropical rainforests (Magurran 1988). *Cassiope tetragona* and *Dryas integrifolia* also fit the expected models, where their communities fit the geometric series model in the youngest plots, and log-normal

for older areas. *Salix arctica* for the most part had a log normal distribution, and *Saxifraga oppositifolia* fit the geometric series model. Perhaps when *S. arctica* establishes in a new area, it requires a more established mycorrhizal fungal community to survive, as with other ectomycorrhizal plants, which are often found in older areas during plant succession (Cázares 1992). *Saxifraga oppositifolia* has been reported to be both ectomycorrhizal and non-ectomycorrhizal; therefore, this plant may be better at adapting to environments where the ectomycorrhizal fungal community is not well-established and may depend more on other fungal root endophytes or no fungal endophytes. This may also indicate that those communities with a log normal distribution have more facilitation between its members; whereas, communities that fit the geometric series model have a few dominant species accounting for most of its resource consumption, which would fit the selection effect theory.

Observing succession following exposure by a receding alpine glacier, Cázares (1992) reported that the first plants were non-mycorrhizal, followed by arbuscular mycorrhizal plants, then ectomycorrhizal plants, and finally ericoid plants, with DSEs found throughout. Our succession follows the same trend except that we did not find any arbuscular mycorrhizae. Arbuscular mycorrhizae are thought to be rare in the arctic (Haselwandter 1987, Bledsoe et al. 1990), although Kohn and Stasovski (1990) found arbuscular mycorrhizae on a fern, *Dryopteris fragrans* (L.) Schott, from Ellesmere Island. Arbuscular mycorrhizae may have been present on *Salix arctica* as found on alpine *Salix* spp. (Cázares 1992, Trowbridge and Jumpponen 2004), but colonization on these alpine willows constituted less than 1% (Trowbridge and Jumpponen 2004) so they could have easily been missed in this study. Interestingly, a shift in mycorrhizal status was found in *Saxifraga oppositifolia*. The communities that had been exposed for 30 years grouped with *Luzula*

*confusa*, which is associated with septate endophytes. Communities exposed for 40 years clustered with *Salix arctica* and *Dryas integrifolia*, both ectomycorrhizal plants that grouped separately from the other host plants.

This site was unique in that the directional, non-replacement plant succession allowed for the root-associated fungal community to be assessed as plant biodiversity increased and host plants were not replaced. This type of succession is an example of facilitation as demonstrated by the change of soil properties as the plant community increases in biodiversity. More plant diversity brought an increase in root-associated fungal diversity because each plant had a relatively unique fungal community. For individual host plants, the fungal richness did not increase with plant diversity except for *Luzula confusa* and *Dryas integrifolia,* and for all communities species evenness remained the same. Zak et al. (2003) found that increases in plant diversity and thus production changed the microbial community in a field experiment. They hypothesized that because plant species differ in their biochemical composition, they can control the composition and function of heterotrophic microbial communities. In our study, we found that the increase of species richness in the plant community may control the composition and function of the root-associated fungal community because most of the fungal communities had low similarity to each other. The increase in plant diversity may have caused a shift in the fungal community composition as Zak et al. (2003) suggested for the microbial community they examined.

Niche differentiation may help explain the diversity of the fungal community as well. Species and species combination are more important in controlling supplies of nutrients than species richness (Hooper and Vitousek 1997, Tilman et al. 1997b). Perhaps, the different fungal members were exploiting different niches and obtaining nutrients at different depths

or areas, which do not interfere with the acquisition by fungal communities found on neighboring hosts. Biodiversity may not be important for maintenance of an ecosystem, but it can allow changes to occur (Loreau et al. 2001).

Directional, non-replacement succession at the edge of a glacier forefront gave a unique opportunity to examine how the root-associated fungal community responded to an increase in plant diversity. The diversity of the root-associated fungal community was higher than expected for most host plants and for the most part, was not as species-poor as its aboveground counterparts. Co-existence was evident above-ground as well and for most of the belowground fungal communities; aboveground because of the increase of plant diversity along the chronosequence, and belowground by the high genotype richness found on most host plants. Perhaps for ectomycorrhizal and ericoid plants, an established fungal community was needed for plants to succeed. These systems may also fit the selection effect model. Plants with fungal communities that fit the geometric series model may be more dependent on fewer fungal partners or have more competitive fungi colonizing their roots. These few dominant species may be important in shaping the fungal community. The possibility that the septate endophytic community was more diverse that previously assumed, and their role in plant establishment may be critical. However, more research in identifying the fungal players would be needed to interpret the richness established by their community fingerprint.

## V. Synthesis of results

The effects of site and temperature on the root-associated fungal community of *Salix arctica* was tested by two methodologies, one based on PCR techniques and the other by isolating fungi from the sterilized root surface. Comparison of fungal genotypes using the two methods was done by matching T-RFLP fragments generated from fungal DNA extracted directly from root tips (T-RFLP – OTC study), to fragments from RFLPs produced from fungal cultures isolated from root tips (culture study) for each of the 18 plots. If a T-RFLP fragment size was within 10% of the RFLP band size, they were considered a putative match between the T-RFLP and RFLP datasets. The 10% threshold was to account for the different algorithms to determine the fragments sizes of the two methods. Matches were counted only if each of the four primer-enzyme combination (i.e. ITS 1 – *Alu*I, ITS 4 – *Alu*I, ITS 1 – *Hin*fI, and ITS 4 – *Hin*fI) matched RFLP fragments for the two restriction enzymes.

Of the possible 150 fungal cultures isolated from *Salix arctica* from all plots, 65 (43%) matched samples from T-RFLP analysis. For the lowland OTC plots, 85% of the fungal cultures were also found in the T-RFLP analysis. Most of these lowland isolates were morphologically identified as *Phialocephala fortinii* or as hyaline septate endophytes. The highest percentage of matches between RFLP bands from cultures and T-RFLPs fragments were from the lowland plots. The percent of cultures with matching T-RFLP fragments for the three sites ranged from 17 to 50%, in which the 17% were found on the dolomitic OTC plots. This low percentage may be due to the small number of isolates found (4) on the dolomitic site, which was the lowest number of isolates found for all the plots.

The total number of genotypes from the T-RFLP – OTC study that had matching cultures characterized by RFLP patterns was 188 (21%). This is out of 894 possible

genotypes, which was the number of genotypes found by the primer-enzyme combination ITS 4 - *Alu*I. This primer-enzyme combination was used to make comparisons between the T-RFLP – OTC and culture studies instead of the other three primer-enzyme combinations because the least number of genotypes were generated from ITS 4 -*Alu*I. Seventy-nine percent of the community from the OTC -T-RFLP study was probably not dark or hyaline septate endophytes isolated from root tips and were likely to be mycorrhizal fungi and pathogens. This difference between culturing and PCR-based techniques has been noted for ericaceous plants where *Sebacina* was the most dominant fungus when amplifying from roots and *Capronia*-like fungi from fungal cultures (Allen et al. 2003).

Although *Phialocephala fortinii* accounted for 31% (58/188) of the RFLP samples that were also present in the T – RFLP community, it only accounted for 6.5% (58/894) of the whole community from the T – RFLP-OTC study. This percentage was lower than expected because *P. fortinii* has been hypothesized to fill the functional void left by arbuscular mycorrhiza (Bledsoe et al. 1990). This low percent may be due in part because fewer root tips were used for isolating fungal cultures in comparison to the number of root tips used for DNA extraction; only five roots from each plant were used for fungal isolation in contrast to approximately 30 root tips per plant used for T-RFLP analysis.

Based on the T-RFLP – OTC study, genotype frequency of the fungal community on *Salix arctica* increased in the warmed plots compared to the ambient plots overall; however, this was only significant on the granitic site when examined for each site. One explanation is that there was insufficient time for the warming to affect the root system. Initially, *S. arctica* uses any excess resources for seed production (Jones et al.1997). The increase in genotype frequency may be the beginning of further changes. The fungal community from the culture

study, which used an additional three plant species, did not change due to the warming treatment. Again, not enough time may have passed for the warming to impact root growth. As with *S. arctica, Dryas integrifolia* and *Saxifraga oppositifolia* initially direct resources to for reproduction (Henry and Molau 1997, Stenström et al. 1997). Changes may not have been found for the fungal community associated with *Cassiope tetragona* because not many endophytes were isolated from its roots. Of the three sites examined, the impacts of warming probably will be observed first on the dolomitic site, where resource limitations are maximal.

Although the sample sizes were different for the two studies, some trends were found. The genotype richness and frequency were different for T-RFLP – OTC and culture studies. For the fungal community in the T-RFLP – OTC study, genotype frequency and richness were comparable between the lowland and granitic sites. In contrast, for the culture study, genotype richness and frequency were comparable between the lowland and dolomitic sites. Genotype frequency was higher on the lowland and granitic sites than on the dolomitic site for the T-RFLP – OTC study, and was highest on the granitic site for the culture study. Although there was a trend where frequency was higher in the warmed plots of the lowland and dolomitic sites, frequency was highest in the control plot of the granitic site.

Although sampling may account for some of the discrepancies between the T-RFLP – OTC and culture studies, other possibilities can contribute to these differences. The root-associated fungal community on *Salix arctica* may be dominated by ectomycorrhizal fungi, which are more difficult to culture, so richness and frequency may be higher when directly amplifying DNA versus isolating fungi from root tips. The reason why frequency and richness were high from the granitic site when isolating fungi from root tips may be because these sites had more root endophytes. Although the soil chemistry of the two sites was

comparable, the granitic site was more arid and had lower plant diversity than the lowland site, so *S. arctica* on the upland site may have been dominated by non-mycorrhizal fungal endophytes. This is supported by the comparison between PCR-RFLP and T-RFLP fragments; of the 65 fungal isolates that had matching T-RFLP fragments, 47.7% were from the granitic site while only 20% were from the lowland plot, suggesting that a higher proportion of the root-associated fungal community on the granitic site were endophytes.

Host plant was a more important determinant of the fungal community in the succession study compared to the culture study. This can be attributed in part to the different spatial and temporal scales between the two studies. The succession study encompased a more restricted area with soils of similar composition, compared to the culture study; with the range of abiotic and soil factors diminished, the effect of host became more apparent. Over the larger environmental range and variation in soil parent materials represented in the culture study, site was more important in determining community structure. Also, since isolating from root tips favors fungi that grow quickly and have less host specificity (Kernaghan et al. 2003), the role of the host plant may have been more difficult to detect in the culture study.

Genotype richness, in general, was higher for the fungal community in the T-RFLP – OTC study in comparison to the fungal community in the succession study. However, the average genotype frequency of the control plot of the succession study, an area which had not been covered by the retreating glacier, was comparable to the lowland and granitic site of the T-RFLP – OTC study, and the earlier successional plots were comparable to the average genotype frequency found on the dolomitic site. Some of the differences in genotype richness between the T-RFLP – OTC and succession studies were correlated with soil

chemistry. Higher amounts of Al, Na, Fe, P, and $NH_4$ were found in the control plot of the succession study compared to the lowland and granitic sites of the warming study (see Table A5.1). The control site from the succession study had lower amounts of Mg and $NO_3$ than the lowland and granitic sites.

By using two methodologies, direct PCR amplification and culturing, to examine the root-associated fungal community, a more comprehensive interpretation was possible because each method favoured different groups of fungi. The succession study provided an added dimension, and gave insight into the development of this arctic oasis.

## VI. Conclusion

Hypotheses revisited.

*$H_0$ 1.1 The root-associated fungal community, based on DNA directly extracted from root tips from* Salix arctica, *will not differ between warmed plots and ambient plots.*

The root-associated fungal community based on DNA directly extracted from root tips did not significantly differ between warmed and ambient plots; therefore, there was not sufficient evidence to reject the null hypothesis. However, although not statistically significant, the cumulative frequency of the genotypes from the root-associated fungal community on *Salix arctica* tended to be higher in the warmed plots compared to ambient plots in the T-RFLP – OTC study. The first five to seven years of passive warming may have contributed mainly to reproduction (Jones et al. 1997), so the immediate effects of warming may not have impacted the root system. However, as warming continues, more carbon may be allocated to growing roots and initiating new lateral roots, increasing root colonization by fungi (Pregitzer et al. 2000). It may be too soon to tell if the reponses of the root-associated fungal community to warming will play a role in plant migration, although migration of southern plants to Alexandria Fiord will likely be difficult as it is surrounded by ice fields and ocean.

*$H_0$ 1.2 The root-associated fungal community, based on DNA directly extracted from root tips from* Salix arctica, *will not differ due to site.*

The root-associated fungal community, based on DNA directly extracted from root tips from *Salix arctica*, varied significantly between sites; therefore, the null hypothesis is

rejected. The dolomitic site had significantly lower genotype frequency and richness than the lowland and granitic sites.

*$H_0$ 1.3 The root-associated fungal community, based on cultures isolated from root tips, will not differ between warmed and ambient plots.*

The root-associated fungal community, based on cultures isolated from root tips did not significantly differ between warmed and ambient plots; therefore the null hypothesis is not rejected.

*$H_0$ 1.4 The root-associated fungal community, based on cultures isolated from root tips, will not differ according to the host plants* Cassiope tetragona, Dryas integrifolia, Salix arctica, *and* Saxifraga oppositifolia.

The root-associated fungal community, based on cultures isolated from root tips, did not differ significantly according to host plants; therefore, the null hypothesis is not rejected.

*$H_0$ 1.5 The root-associated fungal community, based on cultures isolated from root tips, will not differ due to site.*

The root-associated fungal community, based on cultures isolated from root tips, did significantly differ among site; therefore, the null hypothesis is rejected. This is consistent with the results of the T-RFLP study.

*$H_0$ 1.6 The root-associated fungal communities described by the two methods (direct extraction versus culturing) will not differ.*

Although not statistically testable, the root-associated fungal community described by the two methods did differ according to the method used. Not only did the two methods, DNA extraction directly from roots and fungal cultures isolated from roots, distinguish two fungal communities, but these communities appeared to respond to warming differently. The effects of warming on the root-associated fungal community based on fungal cultures isolated from root tips were not as obvious as in the T-RFLP – OTC study. Part of the reason may be because many of the isolates were *P. fortinii*, which accounted for 31% of the fungal cultures but only 6.5% of the genotypes from the T-RFLP – OTC study. Therefore, ectomycorrhizal fungi probably made up a much larger proportion of the fungi in the T-RFLP – OTC study. Based on the morphotyping done prior to T-RFLPs, most of the roots appeared to have at least a mantle.

*$H_0$ 2.1 The root-associated fungal communities will not differ along a chronosequence.*

The root-associated fungal communities did not significantly differ along a chronosequence; therefore, the null hypothesis is not rejected.

*$H_0$ 2.2 The root-associated fungal communities will not differ according to host plant.*

The root-associated fungal communities did not significantly differ according to host plant, but the composition of the root-associated fungal community did differ according to the mycorrhizal status of the host plant during directional, non-replacement succession. This is indicative of facilitation, where the non-mycorrhizal plants and their rhizosphere improve soil conditions for new plants to colonize the land. Also, niche differentiation was evident because each of the plant species in a plot hosted a unique fungal community (although there

was overlap between the two ectomycorrhizal plant host communities, as indicated in the ordinations). Interestingly, host specificity was suggested even with the non-mycorrhizal host plants, *L. confusa* and *P. lapponicum*, as each host plant also had unique communities based on the ordination analysis. Although non-mycorrhizal, these plants were colonized by endophytes.

General conclusions:

The root-associated fungal community was not as simple as expected. The rank abundance curves did not fit the geometric series model which typically characterizes arctic plant communities. The root-associated fungal communities in the T-RFLP – OTC study fit the log normal model and are as diverse as root-associated fungal communities found in temperate forests. Further analysis with Tokeshi's models elucidated a possible mechanism due to an increased number of lateral roots being colonized and how these niches are filled. This may help explain the patchiness of fungal species found on root tips, where 1-2 dominant types are generally found in any one area (Horton and Bruns 2001). These patches of fungi may outcompete other fungi and dominate a certain niche, which is partly supported by our finding that some dominant genotypes remained dominant in warmed plots even though cumulative frequency increased. In this case, observing changes in frequency of individual genotypes was important in elucidating how the community reacted to a disturbance, as suggested by previous researchers (Tilman et al. 1997a, Hooper and Vitousek 1997).

Site was the main variable that differentiated the root-associated fungal community. This was seen from with both DNA directly extracted from roots and by isolating fungal

cultures from roots. The dolomitic site had the lowest genotype richness compared to the other two sites according to both methods. Soil chemistry was a contributing factor. In the two warming studies, differences in soil chemistry correlated with lower genotype frequency and richness on the dolomitic site. The differences in soil chemistry may also help explain why genotype richness was higher from the T-RFLP – OTC study than the control plot of the succession study. According to the ordination analyses, the community based on fungal cultures had higher variability, but this was probably because there were fewer samples. However, when the root-associated fungal community was examined at a smaller scale, such as in the succession study, then the host plant was a bigger factor in defining these fungal communities.

It remains an open question whether endophytes in these arctic communities replace AM fungi, as proposed by Bledsoe et al. (1990). Endophytes were certainly very common inhabitants of plants at these high arctic sites, but it is unknown if they are fulfilling functional roles similar to AM fungi in other systems.

As with previous fungal community studies based on RFLP analysis (Gardes and Bruns 1996a, Dahlberg et al. 1997, Jonsson et al. 1999), sporocarps were not indicative of which fungi were colonizing the roots. None of the genotypes of fungi found on roots matched any of the sporocarps based on either T-RFLP and RFLP analyses.

T-RFLP was a helpful tool in assessing the root-associated fungal community. Although there were some difficulties, such as interpreting four datasets derived from a common rDNA fragment, the possible presence of pseudo-fragments, and different estimates of diversity depending upon which restriction enzymes and primers were used, we were able to use this technique to assess changes in the genotype cumulative frequency and diversity.

Other advantages of this technique were that it allowed comparisons of T-RFs with RFLPs, and it allowed detection of multiple fungi on single root tips.

This thesis has examined how global climate change may affect the root-associated fungal community. Further studies are needed. Changes may be only beginning, as indicated by the still limited change in genotype frequency. Genotype richness may change as warming progresses, and perhaps more effects of warming may be observed as soil properties and moisture change with increased warming. As with most community-level studies on the root-associated fungal community, the functions of these fungi are not known. Gene expression techniques using genes involved in particular mycorrhizal functions would be helpful, such as genes involved in uptake of ammonium, nitrate or phosphorus.

Further study on the dark- and hyaline-septate fungi is needed. This area is understudied, and assumptions about this group of fungi may be inaccurate. Because they are ubiquitous, more diverse, and more host-specific than previously thought, their function in the rhizosphere may be more important than currently recognized.

## VII. Literature cited


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Table A2.1 2-way, nested ANOVA Table for effects of site, treatment, and treatment replicate on soil properties

| Source of variation | Effect (fixed/ random) | Degrees of freedom | Sum of squares | | Mean square | | F | | p-value | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Exch Mg | Exch Mn | Exch Mg | Exch Mn | Exch Mg | Exch Mn | Exch Mg | Exch Mn |
| Intercept | Fixed | 1 | 161.502 | 0.004 | 161.502 | 0.004 | 1094.729 | 113.311 | <0.001 | <0.001 |
| Site | Fixed | 2 | 7.654 | 0.002 | 3.827 | <0.001 | 7.808 | 7.257 | 0.013 | 0.016 |
| Treatment | Fixed | 1 | 0.052 | <0.001 | 0.052 | <0.001 | 0.351 | 0.218 | 0.586 | 0.665 |
| Site* Treatment | Fixed | 2 | 0.701 | <0.001 | 0.350 | <0.001 | 0.715 | 0.018 | 0.518 | 0.982 |
| Replicate (treatment) | Random | 4 | 0.590 | <0.001 | 0.148 | <0.001 | 0.301 | 0.272 | 0.869 | 0.888 |
| Error | | 8 | 3.921 | <0.001 | 0.490 | <0.001 | | | | |

Table A2.1 2-way, nested ANOVA Table for effects of site, treatment, and treatment replicate on soil properties (cont'd)

| Source of variation | Effect (fixed/ random) | Degrees of freedom | Sum of squares | | Mean square | | F | | p-value | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | pH | C:N ratio | pH | C:N ratio | pH | C:N ratio | pH | C:N ratio |
| Intercept | Fixed | 1 | 770.936 | 39454.08 | 770.936 | 39454.08 | 9578.161 | 59.822 | <0.001 | 0.002 |
| Site | Fixed | 2 | 22.653 | 40275.61 | 11.327 | 20137.80 | 81.752 | 28.828 | <0.001 | <0.001 |
| Treatment | Fixed | 1 | 0.062 | 479.70 | 0.062 | 479.70 | 0.776 | 0.727 | 0.428 | 0.442 |
| Site*Treatment | Fixed | 2 | 0.100 | 858.60 | 0.050 | 429.30 | 0.360 | 0.614 | 0.708 | 0.565 |
| Replicate (treatment) | Random | 4 | 0.322 | 2638.09 | 0.081 | 659.52 | 0.581 | 0.944 | 0.685 | 0.486 |
| Error | | 8 | 1.108 | 5588.41 | 0.139 | 698.55 | | | | |

Table 2.1 2-way, nested ANOVA Table for effects of site, treatment, and treatment replicate on soil properties (cont'd)

| Source of variation | Effect (fixed/ random) | Degree of Free-dom | Sum of squares | | | Mean square | | | F | | | p-value | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Exch Al | Exch Na | CEC | Exch Al | Exch Na | CEC | Exch Al | Exch Na | CEC | Exch Al | Exch Na | CEC |
| Intercept | Fixed | 1 | 0.013 | 0.055 | 1173.54 | 0.013 | 0.055 | 1173.54 | 9.861 | 120.11 | 714.93 | 0.034 | <0.001 | <0.001 |
| Site | Fixed | 2 | 0.007 | 0.003 | 44.052 | 0.003 | 0.001 | 22.026 | 1.765 | 2.445 | 7.869 | 0.231 | 0.148 | 0.013 |
| Treatment | Fixed | 1 | 0.003 | <0.001 | 3.846 | 0.003 | <0.001 | 3.846 | 1.938 | 1.086 | 2.343 | 0.236 | 0.356 | 0.201 |
| Site* Treatment | Fixed | 2 | 0.002 | <0.001 | 5.401 | 0.001 | <0.001 | 2.701 | 0.571 | 0.426 | 0.965 | 0.587 | 0.667 | 0.421 |
| Replicate (treatment) | Random | 4 | 0.005 | 0.002 | 6.566 | 0.001 | <0.001 | 1.641 | 0.697 | 0.824 | 0.586 | 0.615 | 0.545 | 0.681 |
| Error | | 8 | 0.015 | 0.004 | 22.394 | 0.002 | <0.001 | 2.799 | | | | | | |

Table A2.1-2-way, nested ANOVA Table for effects of site, treatment, and treatment replicate on soil properties (cont'd)

| Source of variation | Effect (fixed/ random) | Degrees of Freedom | Sum of squares | | | Mean square | | | F | | | p-value | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Exch Ca | Exch Fe | Exch K | Exch Ca | Exch Fe | Exch K | Exch Ca | Exch Fe | Exch K | Exch Ca | Exch Fe | Exch K |
| Intercept | Fixed | 1 | 425.853 | <0.001 | 0.252 | 425.853 | <0.001 | 0.252 | 523.172 | 5.329 | 411.127 | <0.001 | 0.082 | <0.001 |
| Site | Fixed | 2 | 31.1634 | <0.001 | 0.039 | 15.582 | <0.001 | 0.020 | 14.208 | 1.428 | 95.784 | 0.002 | 0.295 | <0.001 |
| Treatment | Fixed | 1 | 2.701 | <0.001 | <0.001 | 2.701 | <0.001 | <0.001 | 3.318 | 0.648 | 0.304 | 0.143 | 0.466 | 0.611 |
| Site* Treatment | Fixed | 2 | 2.621 | <0.001 | 0.001 | 1.310 | <0.001 | <0.001 | 1.1950 | 1.058 | 3.508 | 0.352 | 0.391 | 0.081 |
| Replicate (treatment) | Random | 4 | 3.256 | <0.001 | 0.002 | 0.814 | <0.001 | <0.001 | 0.742 | 2.006 | 2.992 | 0.589 | 0.187 | 0.088 |
| Error | | 8 | 8.773 | <0.001 | 0.002 | 1.097 | <0.001 | <0.001 | | | | | | |

Table A2.1 2-way, nested ANOVA Table for effects of site, treatment, and treatment replicate on soil properties (cont'd)

| Source of variation | Effect (fixed/ random) | Degrees of freedom | Sum of squares | | Mean square | | F | | p-value | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | $NO_3$ | $NH_4$ | $NO_3$ | $NH_4$ | $NO_3$ | $NH_4$ | $NO_3$ | $NH_4$ |
| Intercept | Fixed | 1 | 15444.62 | 87.252 | 15444.62 | 87.252 | 91.257 | 37.932 | <0.001 | <0.001 |
| Site | Fixed | 2 | 3963.52 | 53.954 | 1981.76 | 26.977 | 9.482 | 7.601 | 0.008 | 0.014 |
| Treatment | Fixed | 1 | 138.67 | 6.468 | 138.67 | 6.468 | 0.819 | 2.812 | 0.417 | 0.169 |
| Site * Treatment | Fixed | 2 | 109.86 | 9.138 | 54.93 | 4.569 | 0.263 | 1.287 | 0.775 | 0.328 |
| Replicate (treatment) | Random | 4 | 676.97 | 9.201 | 169.24 | 2.300 | 0.810 | 0.648 | 0.552 | 0.6439 |
| Error | | 8 | 1672.02 | 28.394 | 209.00 | 3.549 | | | | |

Table A2.1 2-way, nested ANOVA Table for effects of site, treatment, and treatment replicate on soil properties (cont'd)

| Source of variation | Effect (fixed/ random) | Degrees of freedom | Sum of squares | Mean square | F | p-value |
|---|---|---|---|---|---|---|
| | | | Avail P | Avail P | Avail P | Avail P |
| Intercept | Fixed | 1 | 68.562 | 68.562 | 130.269 | <0.001 |
| Site | Fixed | 2 | 37.229 | 18.615 | 61.496 | <0.001 |
| Treatment | Fixed | 1 | 0.276 | 0.276 | 0.524 | 0.509 |
| Site * Treatment | Fixed | 2 | 0.166 | 0.083 | 0.274 | 0.767 |
| Replicate (treatment) | Random | 4 | 2.105 | 0.526 | 1.739 | 0.234 |
| Error | | 8 | 2.42156 | 0.30269 | | |

Table A2.2 2-way ANOVA table for the effects of site, treatment, and treatment replicate on genotype richness.

| Source of variation | Effect (fixed/ random) | Degrees of freedom | Sum of squares | | Mean square | | F | | p-value | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | ITS1-*HinfI* | ITS4-*HinfI* | ITS1-*HinfI* | ITS4-*HinfI* | ITS1-*HinfI* | ITS4-*HinfI* | ITS1-*HinfI* | ITS4-*HinfI* |
| Intercept | Fixed | 1 | 8756.056 | 6086.722 | 8756.056 | 6086.722 | 2717.397 | 319.420 | <0.001 | <0.001 |
| Site | Fixed | 2 | 561.444 | 320.111 | 280.722 | 160.056 | 15.991 | 25.052 | 0.002 | <0.001 |
| Treatment | Fixed | 1 | 16.056 | 1.389 | 16.056 | 1.389 | 4.983 | 0.073 | 0.089 | 0.801 |
| Site * Treatment | Fixed | 2 | 24.111 | 3.444 | 12.056 | 1.722 | 0.687 | 0.270 | 0.531 | 0.770 |
| Replicate (treatment) | Random | 4 | 12.889 | 76.222 | 3.222 | 19.056 | 0.184 | 2.983 | 0.940 | 0.0881 |
| Error | | 8 | 140.444 | 51.111 | 17.556 | 6.389 | | | | |

Table A2.2 2-way ANOVA table for the effects of site, treatment, and treatment replicate on genotype richness (cont'd)

| Source of variation | Effect (fixed/ random) | Degrees of freedom | Sum of squares | | Mean square | | F | | p-value | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | ITS1-*AluI* | ITS4-*AluI* | ITS1-*AluI* | ITS4-*AluI* | ITS1-*AluI* | ITS4-*AluI* | ITS1-*AluI* | ITS4-*AluI* |
| Intercept | Fixed | 1 | 10368.00 | 7160.056 | 10368.00 | 7160.056 | 1829.647 | 1006.883 | <0.001 | <0.001 |
| Site | Fixed | 2 | 730.33 | 602.778 | 365.17 | 301.389 | 5.084 | 20.055 | 0.038 | <0.001 |
| Treatment | Fixed | 1 | 242.00 | 40.500 | 242.00 | 40.500 | 42.706 | 5.695 | 0.003 | 0.075 |
| Site * Treatment | Fixed | 2 | 16.33 | 37.000 | 8.17 | 18.500 | 0.114 | 1.231 | 0.893 | 0.342 |
| Replicate (treatment) | Random | 4 | 22.67 | 28.444 | 5.67 | 7.111 | 0.079 | 0.473 | 0.987 | 0.755 |
| Error | | 8 | 574.67 | 120.222 | 71.83 | 15.028 | | | | |

Table A2.3 2-way ANOVA table for the effects of site, treatment, and treatment replicate on genotype evenness

| Source of variation | Effect (fixed/ random) | Degrees of freedom | Sum of squares | | Mean square | | F | | p-value | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | ITS1-*Hin*fI | ITS4-*Hin*fI | ITS1-*Hin*fI | ITS4-*Hin*fI | ITS1-*Hin*fI | ITS4-*Hin*fI | ITS1-*Hin*fI | ITS4-*Hin*fI |
| Intercept | Fixed | 1 | 15.198 | 14.240 | 15.198 | 14.240 | 18484.57 | 4635.083 | <0.001 | <0.001 |
| Site | Fixed | 2 | 0.004 | 0.018 | 0.002 | 0.009 | 3.01 | 7.965 | 0.106 | 0.012 |
| Treatment | Fixed | 1 | <0.001 | 0.002 | <0.001 | 0.002 | 0.11 | 0.523 | 0.759 | 0.510 |
| Site * Treatment | Fixed | 2 | 0.001 | <0.001 | <0.001 | <0.001 | 1.02 | 0.243 | 0.402 | 0.790 |
| Replicate (treatment) | Random | 4 | 0.003 | 0.012 | <0.001 | 0.003 | 1.22 | 2.738 | 0.373 | 0.105 |
| Error | | 8 | 0.005 | 0.009 | <0.001 | 0.001 | | | | |

Table A2.3 2-way ANOVA table for the effects of site, treatment, and treatment replicate on genotype evenness (cont'd)

| Source of variation | Effect (fixed/ random) | Degrees of freedom | Sum of squares | | Mean square | | F | | p-value | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | ITS1-*Alu*I | ITS4-*Alu*I | ITS1-*Alu*I | ITS4-*Alu*I | ITS1-*Alu*I | ITS4- *Alu*I | ITS1-*Alu*I | ITS4-*Alu*I |
| Intercept | Fixed | 1 | 13.090 | 15.327 | 13.090 | 15.327 | 305.448 | 23783.80 | <0.001 | <0.001 |
| Site | Fixed | 2 | 0.103 | 0.010 | 0.051 | 0.005 | 0.766 | 5.97 | 0.496 | 0.026 |
| Treatment | Fixed | 1 | 0.155 | <0.001 | 0.155 | <0.001 | 3.615 | 0.08 | 0.130 | 0.794 |
| Site * Treatment | Fixed | 2 | 0.095 | <0.001 | 0.048 | <0.001 | 0.711 | 0.08 | 0.520 | 0.925 |
| Replicate (treatment) | Random | 4 | 0.171 | 0.003 | 0.043 | <0.001 | 0.639 | 0.76 | 0.649 | 0.582 |
| Error | | 8 | 0.536 | 0.007 | 0.067 | <0.001 | | | | |

Table A2.4 Relative abundance of genotypes from control to OTC for each primer-enzyme combination. Numbers in bold indicate a 50% change in relative abundance from the control to OTC for each site. The three most dominant genotypes in each plot are italicized.

| ITS 1-*Hinf* I | Site | | | | | |
|---|---|---|---|---|---|---|
| | Lowland | | Dolomitic | | Granitic | |
| Genotype | Control (%) | OTC (%) | Control (%) | OTC (%) | Control (%) | OTC (%) |
| 82 | 4.85 | 3.85 | **0.971** | **0** | **2.94** | **0** |
| 110 | **0.49** | **0** | 0 | 0 | **0.59** | **2.74** |
| 139 | 3.88 | 3.85 | *7.77* | 3.95 | **2.35** | ***8.22*** |
| 150 | 0 | 0 | 0 | 0 | **0** | **4.57** |
| 166 | 0 | 0 | 5.83 | 3.15 | 0 | 0 |
| 210 | *5.83* | *8.55* | 0 | 0 | **0** | **6.39** |
| 238 | **0.49** | **0** | 3.88 | 3.94 | 0 | 0 |
| 248 | **2.43** | **0.86** | **0.97** | **0** | **0.59** | **0.91** |
| 252 | **2.14** | **0.49** | **1.94** | **3.94** | **2.35** | **4.57** |
| 253 | 2.91 | 1.71 | 4.85 | 3.94 | **4.12** | **0.91** |
| 256 | 2.43 | 1.71 | **3.88** | ***7.09*** | **2.35** | **0** |
| 271 | 0 | 0 | **0.97** | **0** | **0** | **2.74** |
| 274 | 2.43 | 2.56 | *15.53* | *20.47* | **1.18** | ***7.31*** |
| 277 | **3.88** | ***9.4*** | ***6.8*** | ***14.17*** | *10.59* | 5.94 |
| 280 | 2.91 | 1.71 | **0** | **1.57** | **1.18** | ***8.22*** |
| 283 | **2.43** | **4.27** | **1.94** | **0.79** | 3.53 | 1.83 |
| 286 | 0.97 | 0.427 | 3.88 | 4.72 | **5.29** | **0** |
| 289 | **0.49** | **1.28** | **0** | **2.36** | ***10.59*** | **0.46** |
| 292 | ***12.62*** | ***23.08*** | 0.97 | 0.79 | **4.12** | **1.37** |
| 307 | **4.37** | **0** | 0 | 0 | **0.59** | **0.91** |
| 311 | 2.43 | 2.14 | **1.94** | **0** | 2.94 | 1.83 |
| 314 | 1.94 | 1.28 | **0.97** | **0** | 2.94 | 1.83 |
| 317 | ***16.51*** | **3.85** | 0 | 0 | 2.35 | 2.28 |
| 321 | **0.48** | **2.99** | **0** | **1.58** | 2.35 | 1.37 |
| 331 | 2.42 | 1.28 | **3.88** | **6.3** | 0 | 0 |
| 344 | **1.94** | **0.85** | 0 | 0 | **4.71** | **0.46** |
| 347 | 0.97 | 0.86 | 0 | 0 | **0** | **0.46** |
| 356 | 0 | 1.71 | 0.97 | 0.79 | *11.18* | *7.31* |
| 358 | **0** | **0.43** | **5.83** | **2.36** | 0.59 | 0.46 |
| 363 | **0.49** | **0** | 2.91 | 1.57 | **2.94** | **0.46** |
| 401 | 0.49 | 0.43 | **5.83** | **0** | 0 | 0 |

Table A2.4 Relative abundance of genotypes. Numbers in bold indicate a 50% change in relative abundance from the control to OTC for each site (cont'd). The three most dominant genotypes in each plot are italicized

| ITS4-*Hin*fI | Site | | | | | |
|---|---|---|---|---|---|---|
| | Lowland | | Dolomitic | | Granitic | |
| Genotype | Control (%) | OTC (%) | Control (%) | OTC (%) | Control (%) | OTC (%) |
| 82 | **0.99** | **2.88** | 0 | 0 | **5.30** | **0** |
| 91 | 0 | 0 | 0 | 0 | **3.79** | **1.44** |
| 97 | 0.99 | 0.82 | **2.30** | ***6.84*** | 0 | 0 |
| 112 | *8.42* | 5.76 | 0 | 0 | **3.03** | **0.72** |
| 178 | **0.50** | **1.65** | **0** | **2.56** | **4.55** | **1.44** |
| 182 | 2.97 | 2.06 | 8.05 | *8.55* | 3.03 | 2.88 |
| 198 | **4.46** | **2.06** | 1.15 | 1.71 | 0 | 0 |
| 215 | **0** | **2.06** | **0** | **5.13** | ***12.88*** | **1.44** |
| 218 | **0** | **0.41** | 5.75 | 4.27 | **0** | **0.72** |
| 240 | 7.92 | *10.70* | **2.30** | **0** | **0** | **10.07** |
| 262 | 1.48 | 1.23 | 0 | 0 | *9.85* | *12.23* |
| 265 | *14.85* | *18.93* | **1.15** | **6.84** | 0 | 0 |
| 268 | *11.88* | *14.81* | ***9.20*** | ***20.51*** | *7.58* | 5.76 |
| 271 | **0** | **2.47** | **0** | **1.71** | 1.52 | 2.88 |
| 276 | 0.99 | 0.41 | 6.90 | 3.42 | **0** | **0.72** |
| 282 | 0.50 | 0.41 | 0 | 0 | **0** | **2.88** |
| 305 | 1.98 | 2.06 | 0 | 0 | **0** | **3.60** |
| 308 | **2.47** | **0** | 0 | 0 | **0.76** | **5.04** |
| 315 | **0.99** | **3.29** | 0 | 0 | 0 | 0 |
| 318 | 2.97 | 1.65 | 0 | 0 | **0** | **0.72** |
| 325 | 2.47 | 2.47 | 0 | 0 | **3.06** | **5.76** |
| 329 | **0** | **0.41** | **0** | **0.85** | **6.06** | **0** |
| 335 | 0 | 0 | ***9.20*** | **2.56** | 3.03 | 2.88 |
| 338 | 1.49 | 2.88 | 3.45 | 3.42 | **5.30** | ***12.23*** |
| 341 | 4.95 | 6.17 | ***10.34*** | **4.27** | ***7.58*** | ***12.23*** |
| 344 | **2.97** | **0.82** | 3.45 | 3.41 | 0 | 0 |
| 356 | **3.96** | **0.41** | **1.15** | **0** | **0.76** | **0** |
| 382 | **2.97** | **0.82** | 0 | 0 | 0 | 0 |

Table A2.4 Relative abundance of genotypes. Numbers in bold indicate a 50% change in relative abundance from the control to OTC for each site (cont'd). The three most dominant genotypes in each plot are italicized

| ITS1-*AluI* | Site | | | | | |
|---|---|---|---|---|---|---|
| | Lowland | | Dolomitic | | Granitic | |
| Genotype | Control (%) | OTC (%) | Control (%) | OTC (%) | Control (%) | OTC (%) |
| 70 | *11.89* | *14.09* | 0 | 0 | **1.75** | **0.45** |
| 77 | 3.24 | 3.64 | 1.49 | 1.74 | **0.58** | **3.13** |
| 109 | 1.62 | 2.27 | 0 | 0 | 1.17 | 1.34 |
| 115 | 0.54 | 0.91 | **0** | **0.87** | 7.02 | 6.25 |
| 127 | 2.70 | 3.18 | 0 | 0 | 0 | 0 |
| 135 | 4.32 | 2.27 | 0 | 0 | **1.17** | **0** |
| 148 | *8.11* | *5.46* | 0 | 0 | **1.75** | **0** |
| 169 | 1.08 | 1.82 | ***13.43*** | **4.35** | *8.77* | *9.38* |
| 171 | **0.54** | **1.82** | 0 | 0 | ***8.19*** | **0.45** |
| 355 | **0** | **1.36** | **2.99** | ***6.96*** | **3.51** | **1.33** |
| 358 | 0.54 | 0.46 | **0** | ***7.82*** | **1.75** | **0.45** |
| 361 | **2.70** | **1.36** | **2.99** | ***8.70*** | **1.17** | **0.45** |
| 373 | 0 | 0 | **1.49** | **2.61** | 2.34 | 2.68 |
| 386 | 3.24 | 2.27 | 0 | 0 | **0** | **1.34** |
| 391 | 0 | 0 | **0** | **0.87** | **0** | **2.68** |
| 402 | 0 | 0 | *4.48* | 5.22 | 0 | 0 |
| 416 | *18.38* | *14.09* | 0 | 0 | **0.58** | **3.13** |
| 426 | **1.08** | **2.73** | **1.49** | **0** | **0** | **0.45** |
| 437 | 0 | 0 | **0** | **0.87** | **4.09** | **0.89** |
| 441 | 0 | 0 | **0** | **0.87** | **3.51** | **0.89** |
| 446 | 3.24 | 2.27 | ***14.93*** | **5.22** | **1.17** | **5.80** |
| 449 | 3.78 | 2.27 | **1.49** | **0** | **1.17** | **4.01** |
| 462 | **1.08** | **2.27** | 0 | 0 | **0** | **0.45** |
| 510 | **0.54** | **0** | **0** | **0.87** | **0** | **2.68** |
| 530 | 3.24 | 4.09 | 0 | 0 | *8.77* | *8.04* |
| 533 | 0.54 | 0.91 | **0** | **0.87** | *8.19* | *9.38* |
| 536 | **0.54** | **1.82** | **0** | **0.87** | 4.09 | 5.36 |
| 539 | 0 | 0 | **0** | **5.22** | 0.59 | 0.45 |
| 559 | **0.54** | **0** | 0 | 0 | **4.09** | **0** |

Table A2.4 Relative abundance of genotypes. Numbers in bold indicate a 50% change in relative abundance from the control to OTC for each site (cont'd). The three most dominant genotypes in each plot are italicized

| ITS 4-*Alu*I | Site | | | | | |
|---|---|---|---|---|---|---|
| | Lowland | | Dolomitic | | Granitic | |
| Genotype | Control (%) | OTC (%) | Control (%) | OTC (%) | Control (%) | OTC (%) |
| 70 | **0.60** | **4.07** | 0 | 0 | 0 | 0 |
| 73 | ***10.84*** | ***5.43*** | 4.23 | 2.86 | 1.96 | 1.67 |
| 88 | 3.01 | 2.26 | **0** | **0.95** | 0.65 | 0.56 |
| 94 | 4.22 | 4.52 | 0 | 0 | **1.31** | **3.89** |
| 97 | **1.20** | **2.71** | 0 | 0 | 0.65 | 0.56 |
| 115 | 0 | 0 | 0 | 0 | **3.27** | **0.56** |
| 130 | **1.81** | ***7.24*** | 0 | 0 | **0.65** | **3.89** |
| 147 | **0.60** | **1.36** | 0 | 0 | **0** | **5.56** |
| 155 | 0.60 | 0.45 | **0** | **4.76** | **0** | **0.56** |
| 170 | **0.60** | **2.26** | **2.82** | **4.76** | **1.31** | **0** |
| 173 | **0.60** | ***5.43*** | 7.04 | 7.62 | **0** | **6.67** |
| 176 | 0 | 0 | **2.82** | **4.76** | 0 | 0 |
| 184 | **4.22** | ***9.05*** | 5.63 | 6.67 | **0** | **2.22** |
| 187 | **1.20** | **0** | *14.08* | *17.14* | **0** | **2.22** |
| 190 | ***6.63*** | **1.81** | *9.86* | *9.52* | **5.23** | ***12.78*** |
| 193 | **3.61** | ***5.43*** | ***18.31*** | **3.81** | ***20.92*** | ***9.44*** |
| 230 | **6.02** | **0.45** | 0 | 0 | 0 | 0 |
| 239 | **3.01** | **0.45** | 0 | 0 | **0.65** | **0** |
| 246 | **0** | ***5.43*** | 0 | 0 | 0 | 0 |
| 380 | *6.63* | 4.52 | **1.41** | **0** | **1.31** | **0** |
| 403 | **5.42** | **2.72** | **1.41** | **0** | 0 | 0 |
| 442 | **0** | **2.71** | **2.82** | ***9.52*** | 0 | 0 |
| 466 | **0** | **3.62** | 0 | 0 | **0** | **5.56** |
| 483 | **1.81** | **3.62** | 0 | 0 | **2.61** | **0** |
| 486 | 5.42 | 4.07 | 0 | 0 | **1.31** | **0.56** |
| 530 | 3.61 | 3.17 | **1.41** | **0** | 5.88 | 8.33 |
| 533 | 1.81 | 2.26 | 0 | 0 | *11.11* | *13.33* |
| 536 | **1.81** | **0.91** | **0** | **0.95** | *6.54* | 7.78 |
| 559 | 0 | 0 | 0 | 0 | **5.23** | **0** |
| 640 | 0 | 0 | 0 | 0 | **0.65** | **2.78** |

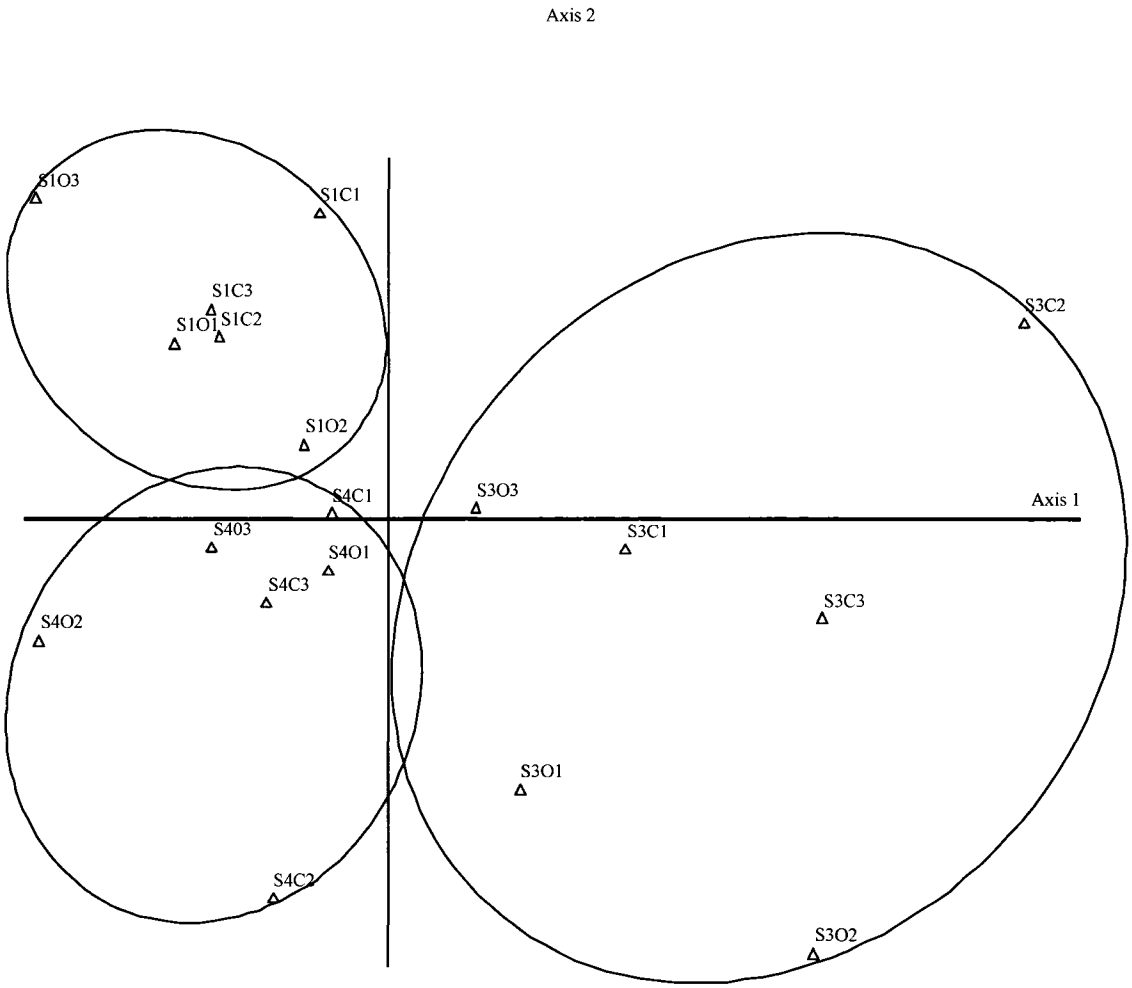
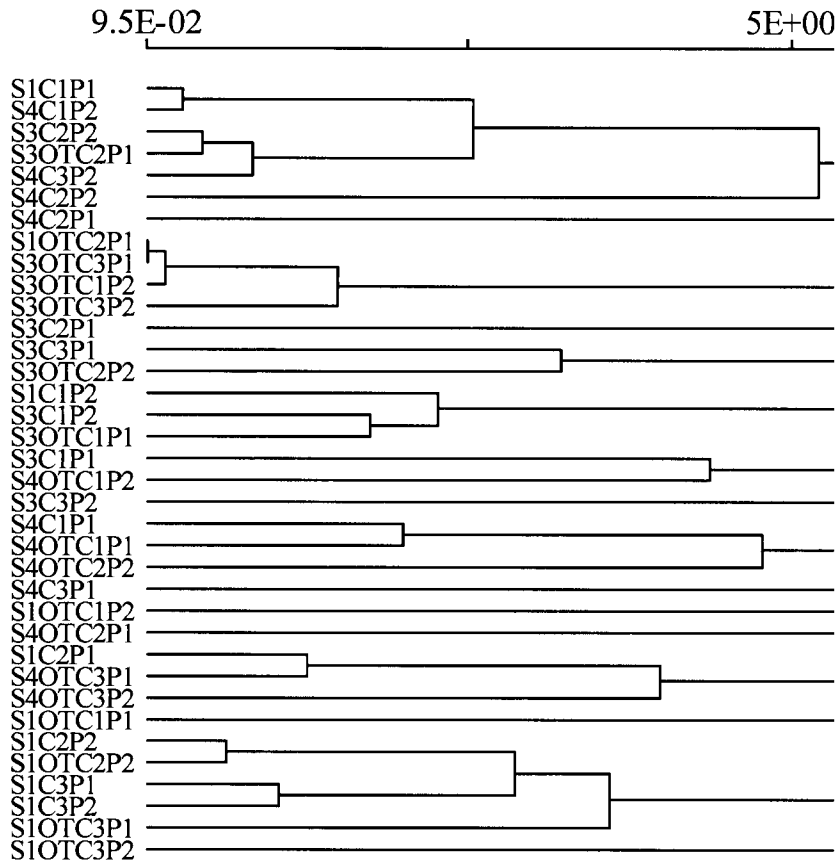



Fig. A2.1 NMS for site and warming treatment effect. Rotated at 75°. Coding is as follows: first two characters represent site, S1 (lowland), S3 (highland dolomitic), S4 (highland granitic); the last two characters signify the treatment and the replicate of that treatment, e.g. C1 would be replicate one of control, O3 would be replicate three of OTC. Site is clustered together with the lowland and highland granitic sites clustering closer together than the lowland and highland dolomitic sites.

(a) ITS 1-*Hin*fI




(b) ITS 4-*Hin*fI

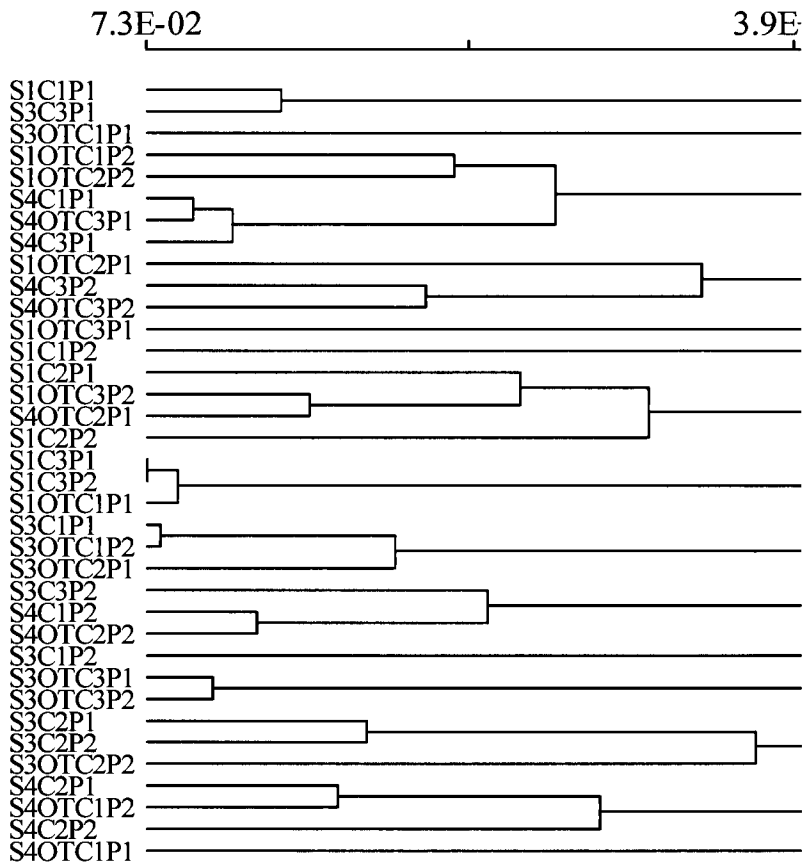
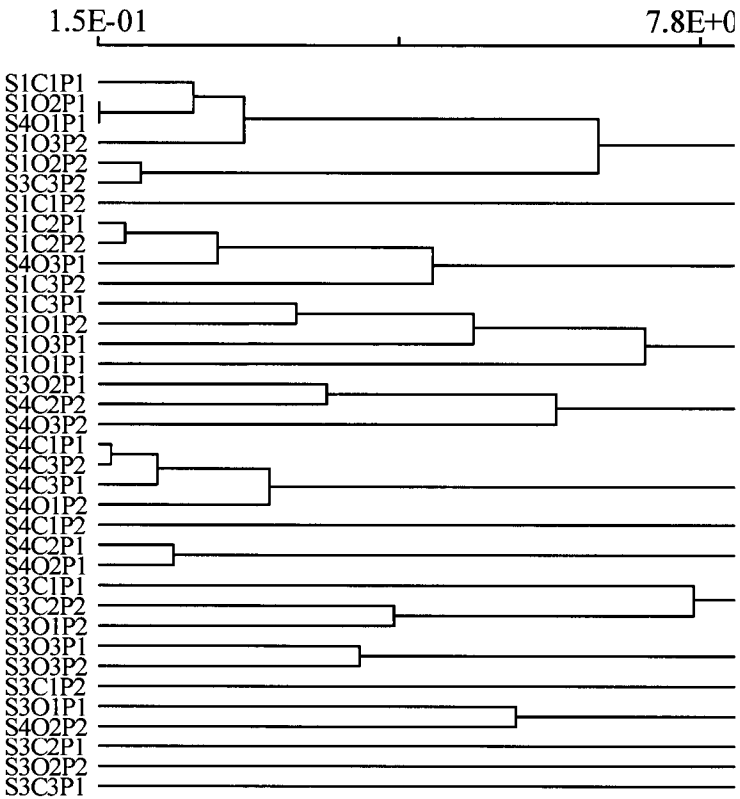



Fig. A2.2 Cluster analysis of plots, treatments and plant specimen based on frequency of genotypes. S1 = lowland, S3= dolomitic, and S4 = granitic. C and OTC represent control or warmed plots, and P1, P2 distinguishes plant specimen replication.

(c) ITS 1-*Alu*I




(d) ITS4-*Alu*I

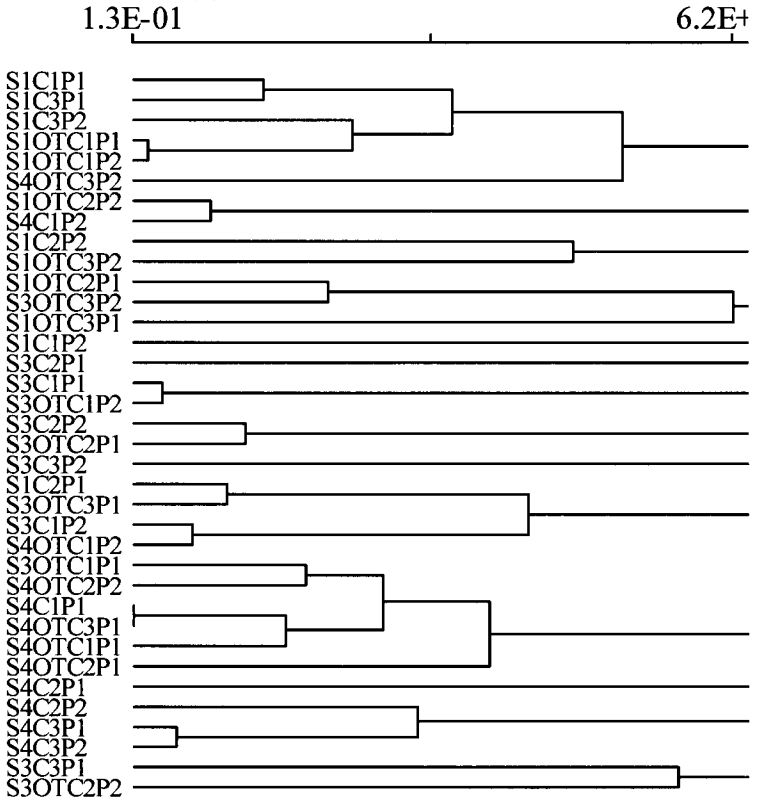



Fig. A2.2 (cont'd) Cluster analysis of plots, treatments and plant specimen based on frequency of genotypes.

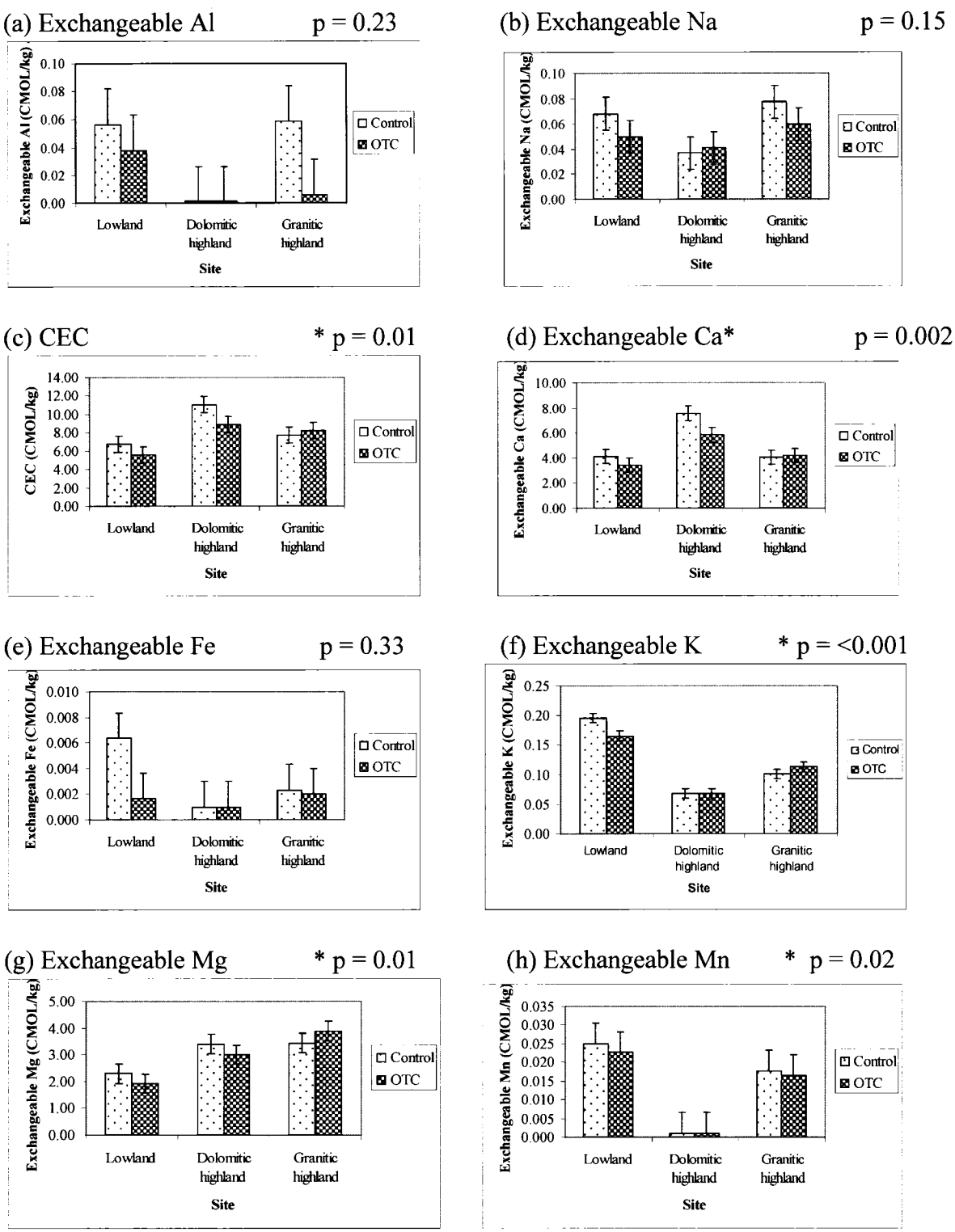



Fig. A2.3 Least square means for soil properties. Error bar is one standard error. *Significant for site effect, p-value for site given along with soil property name.

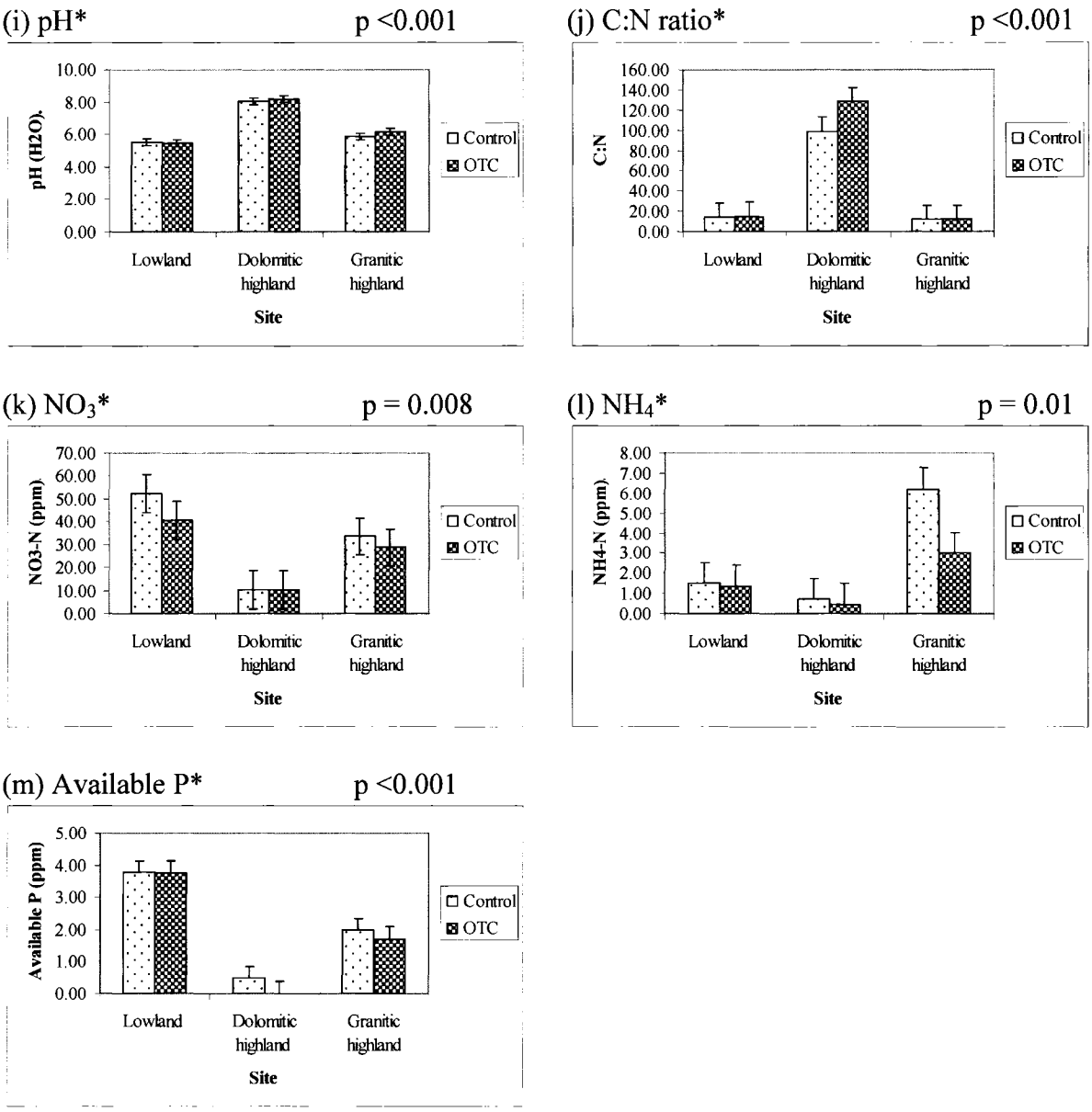
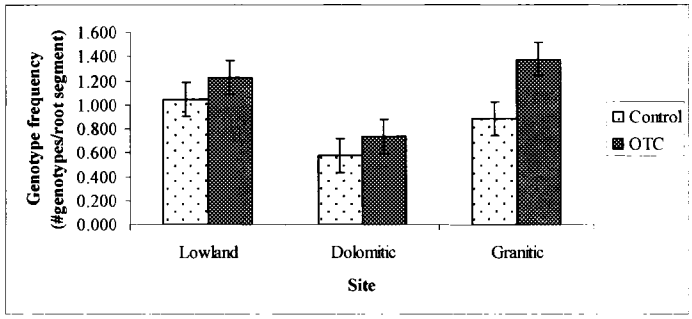
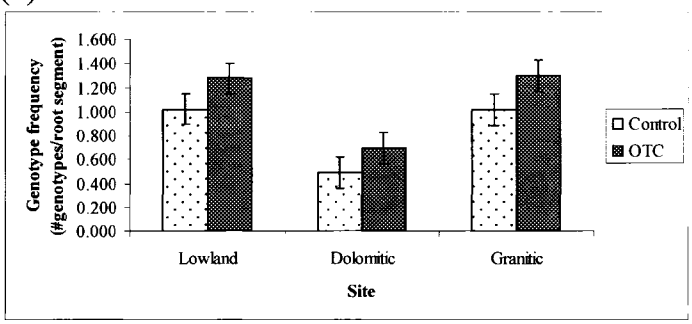



Fig. A2.3 (cont'd). Least square means for soil properties. Error bar is one standard error. *Significant site effect, p-value for site given along with soil property name.

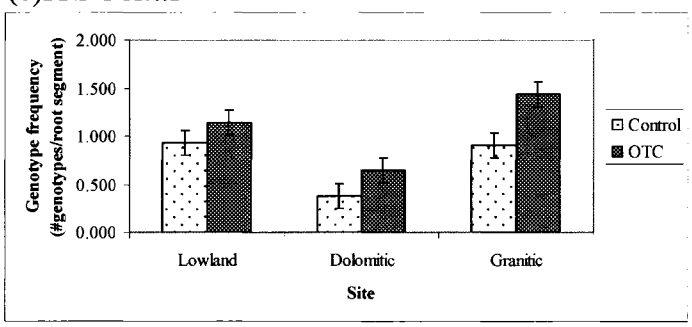
(a) ITS 1-*Hin*fI




(b)ITS 4-*Hin*fI




(c)ITS 1-*Alu*I




(d)ITS 4-*Alu*I

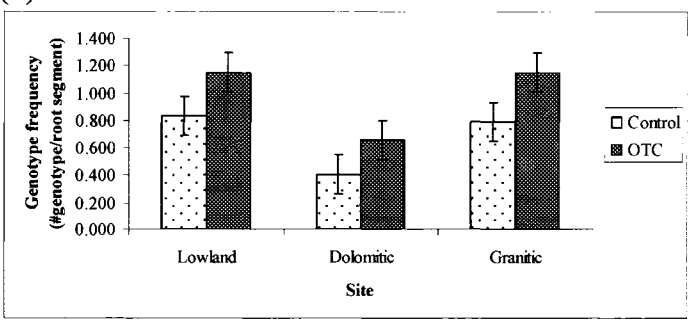
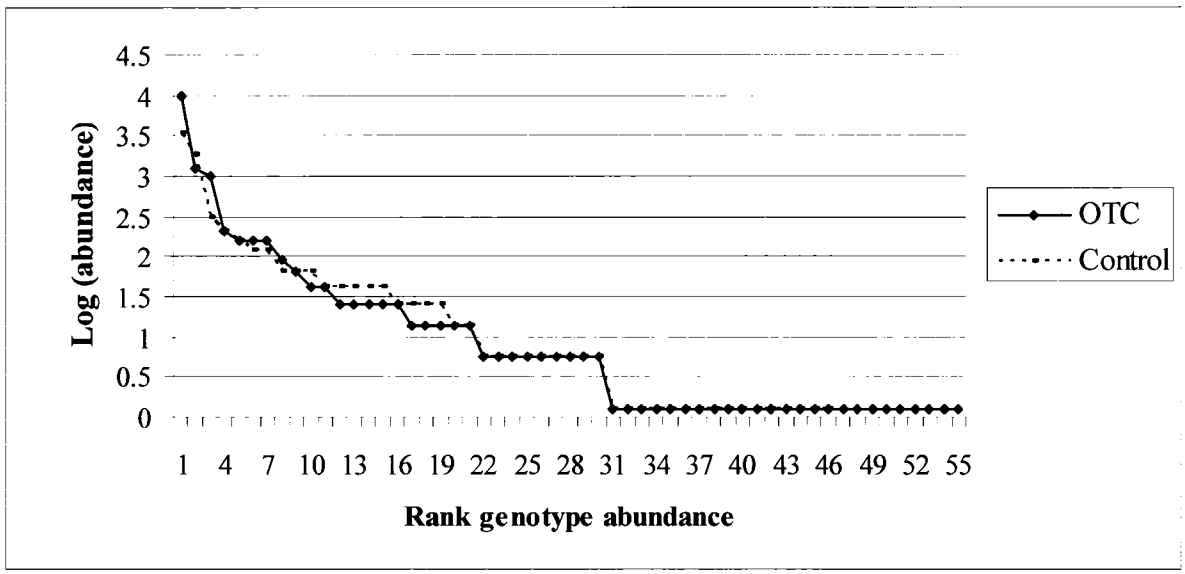



Fig. A2.4 Least square means for genotype frequency for each treatment per plot. Error bar represents one standard error.

(a) ITS 1-*Hin*fI




(b)ITS 4-*Hin*fI

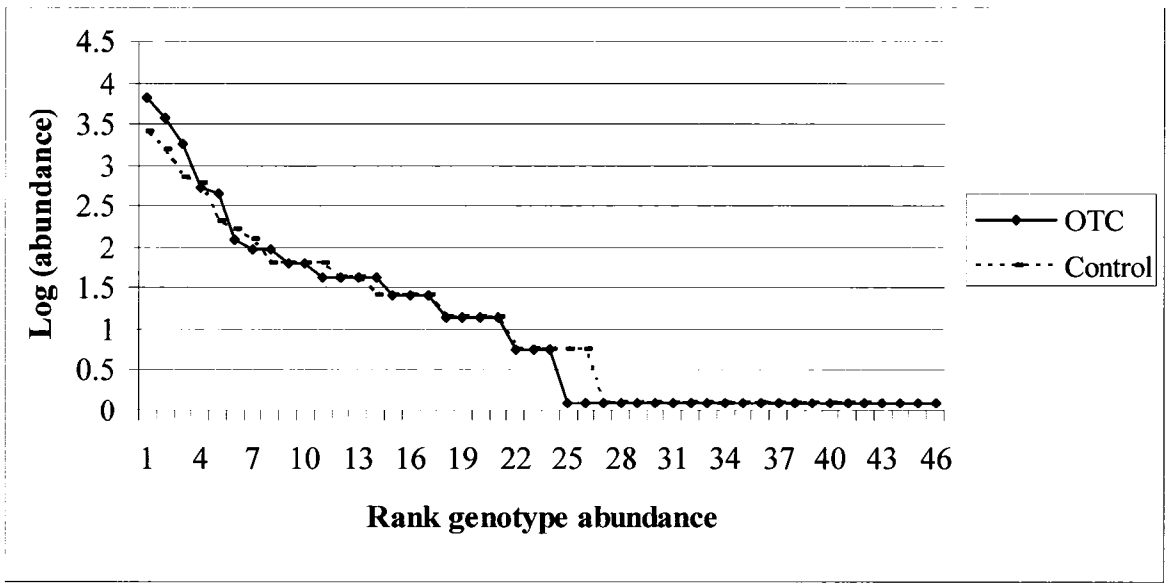
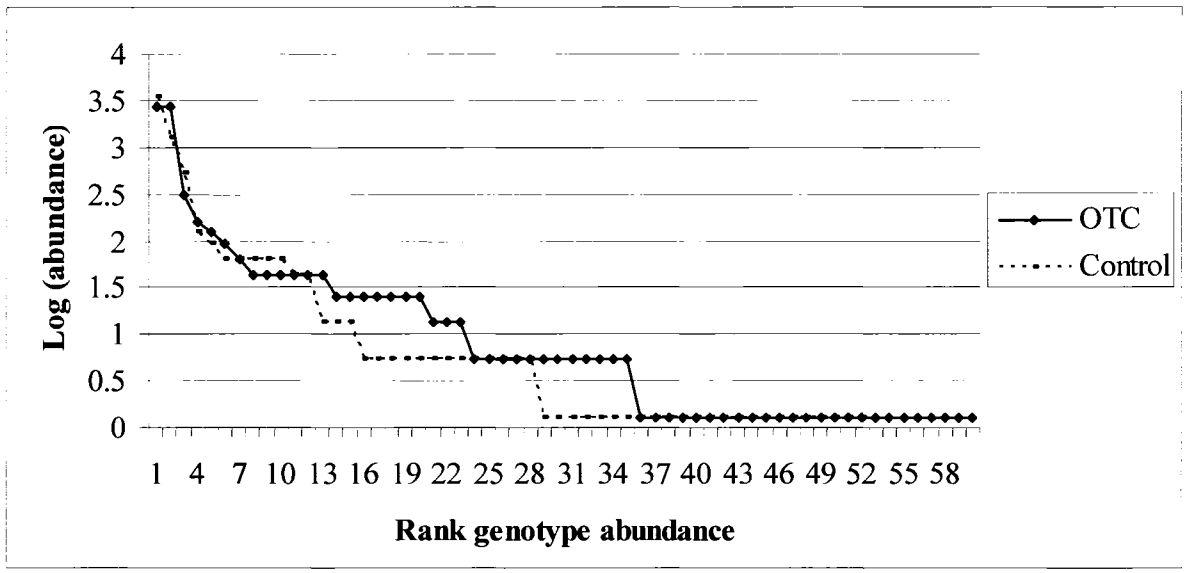



Fig. A2.5 Rank abundance curves for lowland sites.

(c)ITS 1-*Alu*I




(d)ITS 4-*Alu*I

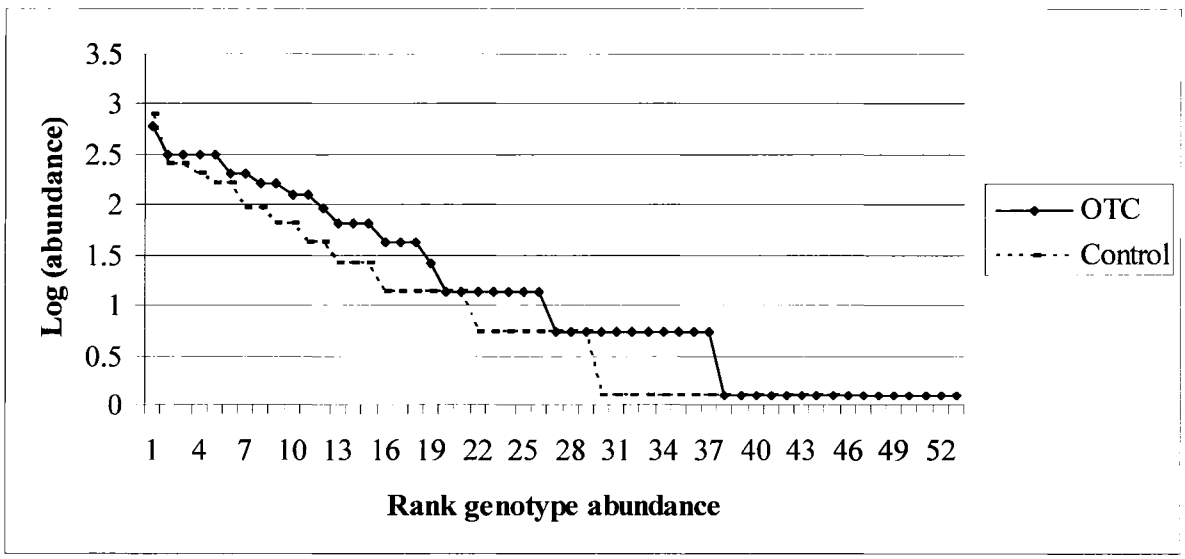
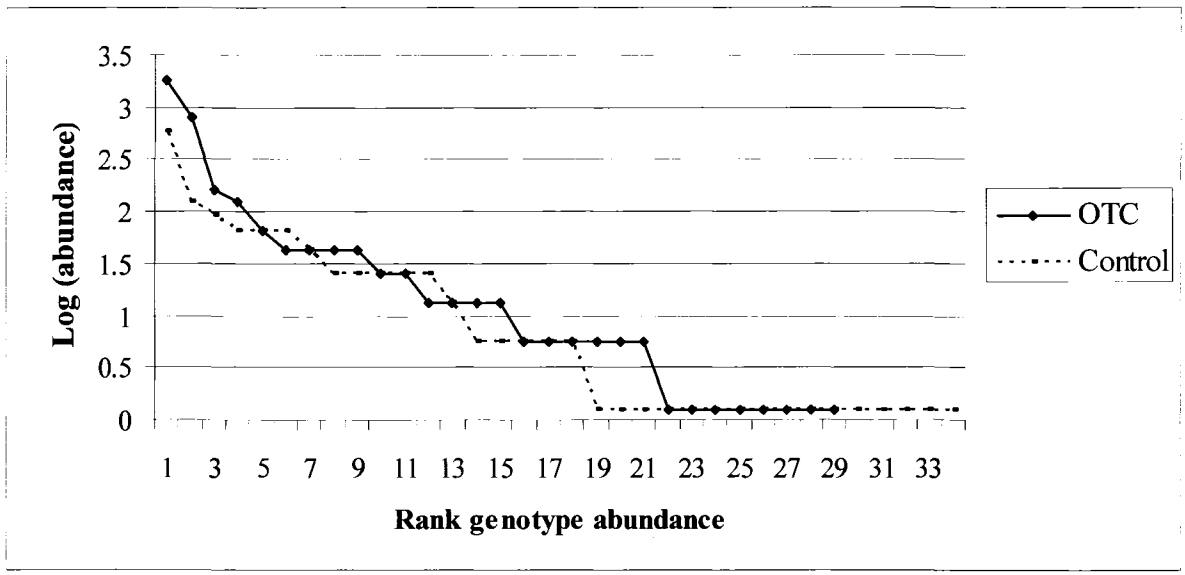



Fig. A2.5 (cont'd) Rank abundance curves. Relative abundance is log scaled.

(a) ITS 1-*Hin*fI




(b)ITS 4-*Hin*fI

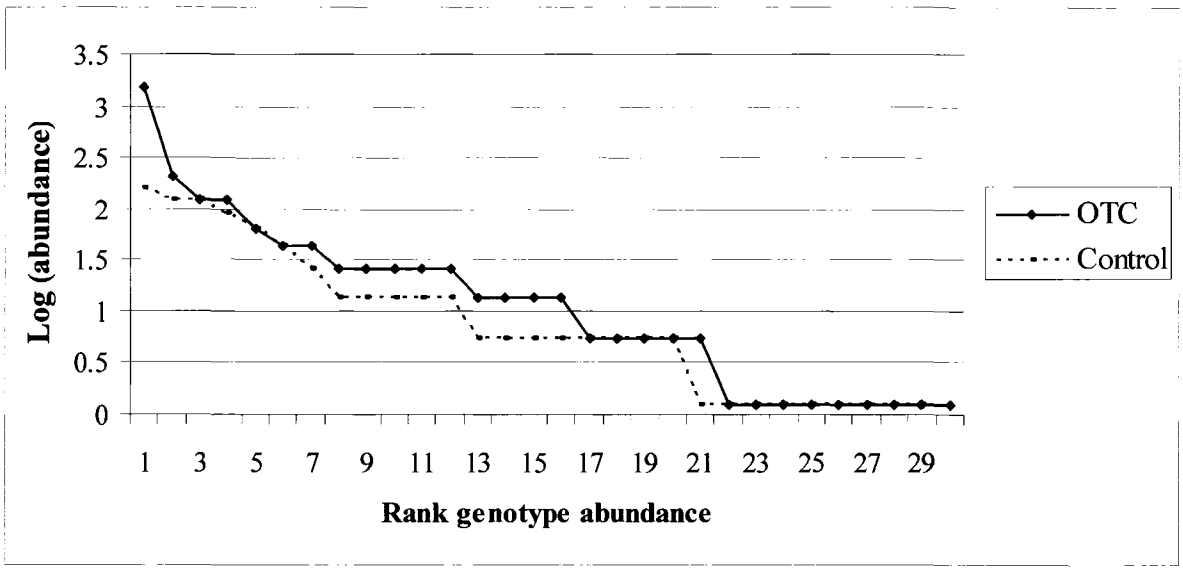
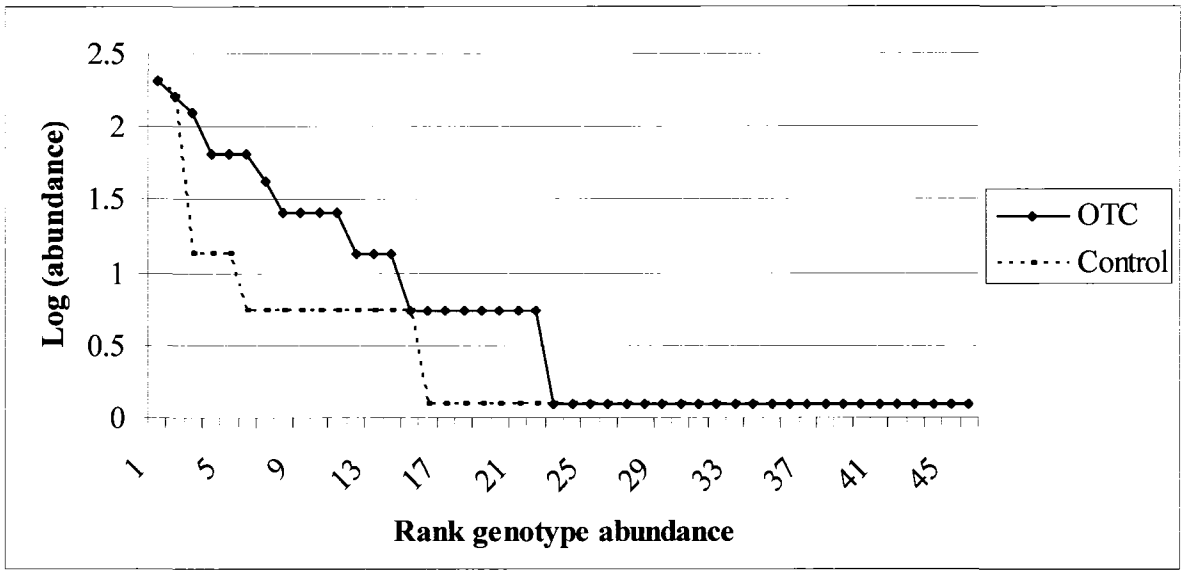



Figure A2.6 Rank abundance curve for highland dolomitic site. Relative abundance is log scaled.

(c)ITS 1-*Alu*I




(d)ITS 4-*Alu*I

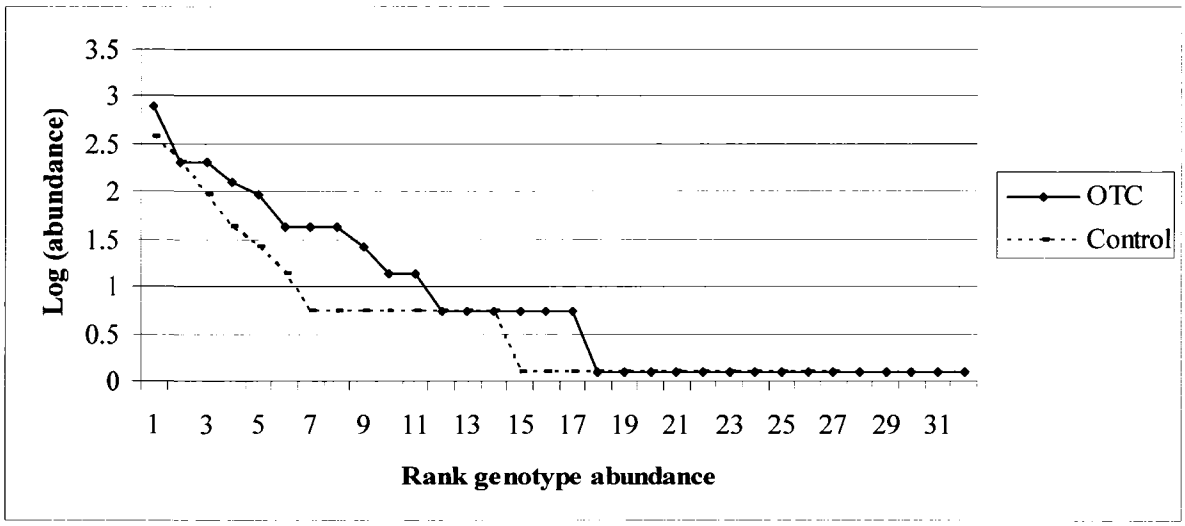
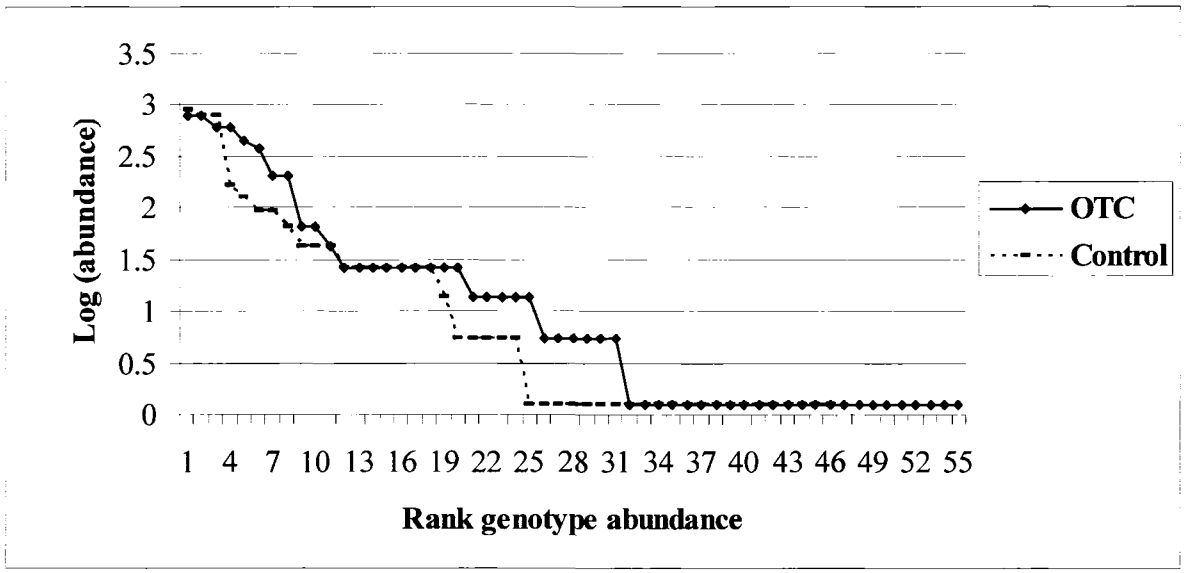



Fig. A2.6 (cont'd) Rank abundance curve for highland dolomitic site. Relative abundance is log scaled.

(a) ITS 1-*Hin*fI




(b)ITS 4-*Hin*fI

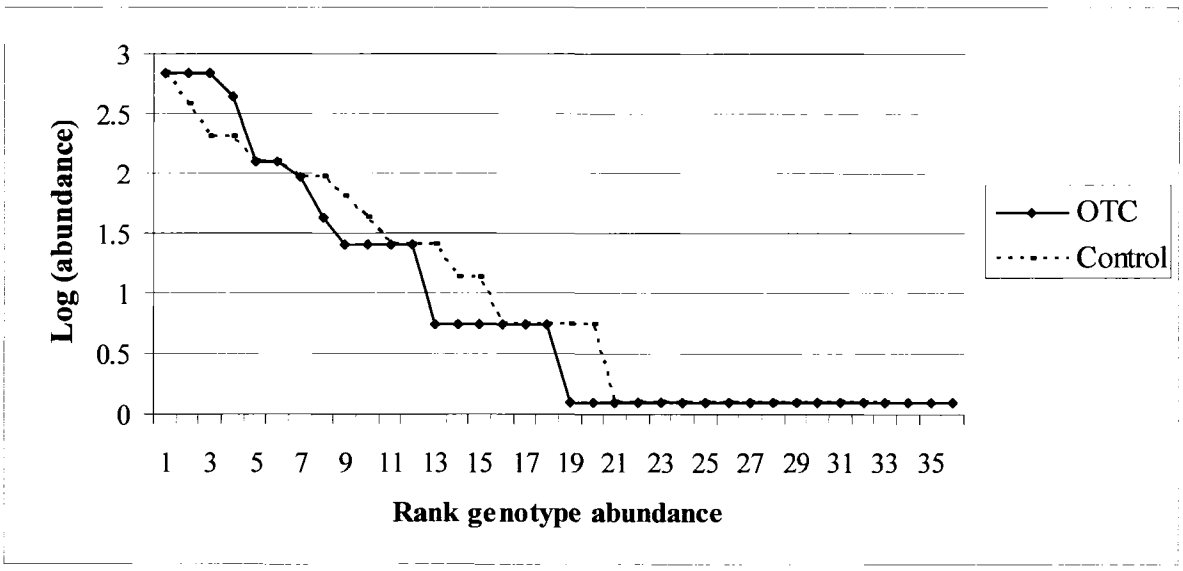
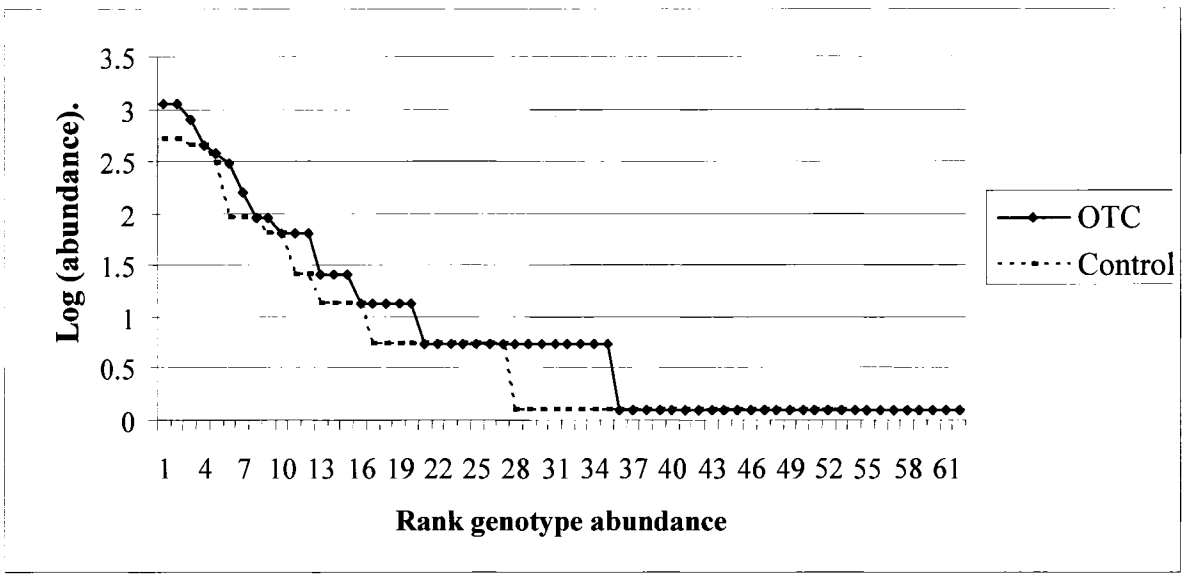



Fig. A2.7 Rank abundance curves for highland granitic site. Relative abundance is log scaled.

(c)ITS 1-*Alu*I




(d)ITS 4-*Alu*I

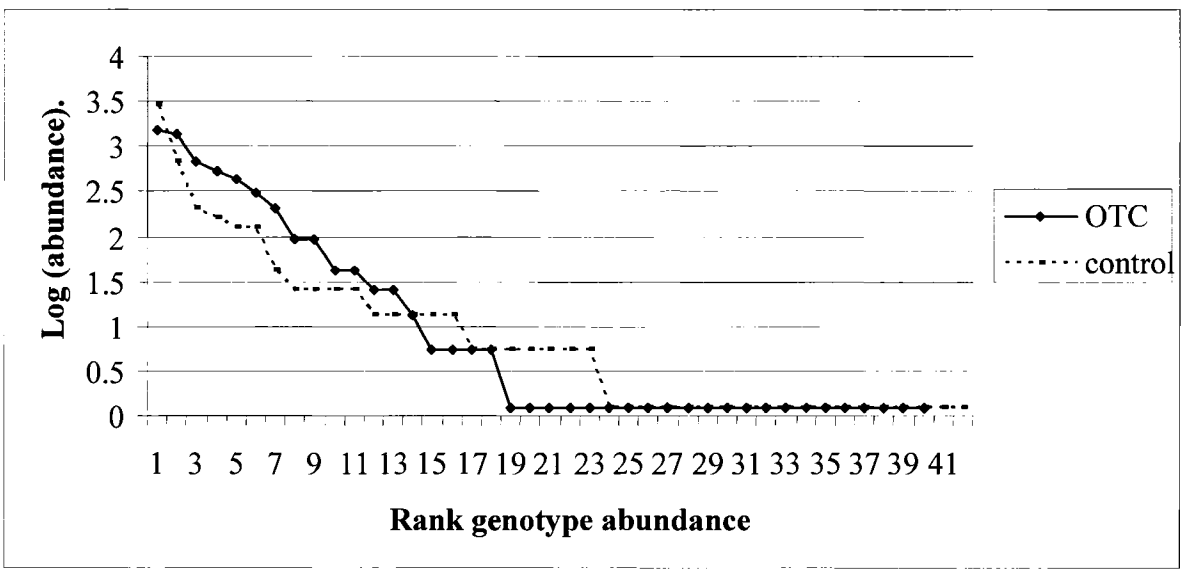
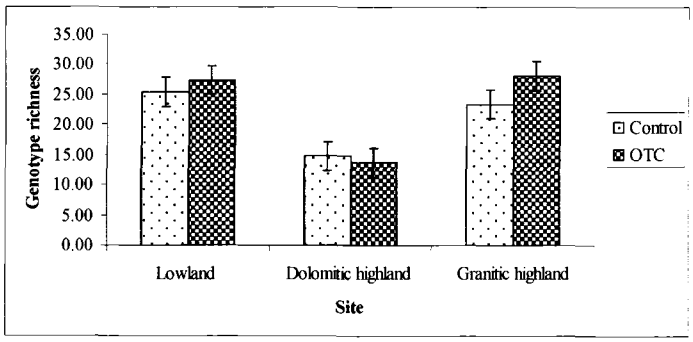
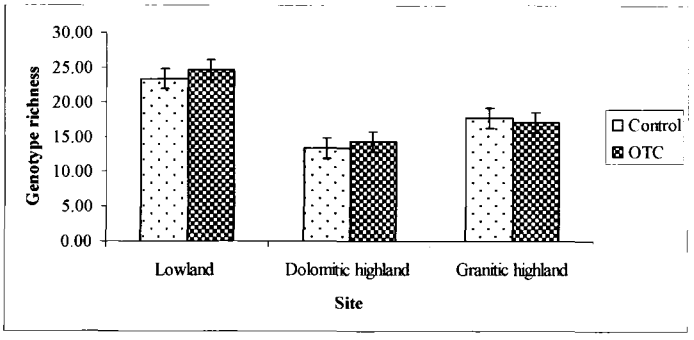



Fig. A2.7 (cont'd) Rank abundance curves for highland granitic site. Relative abundance is log scaled.

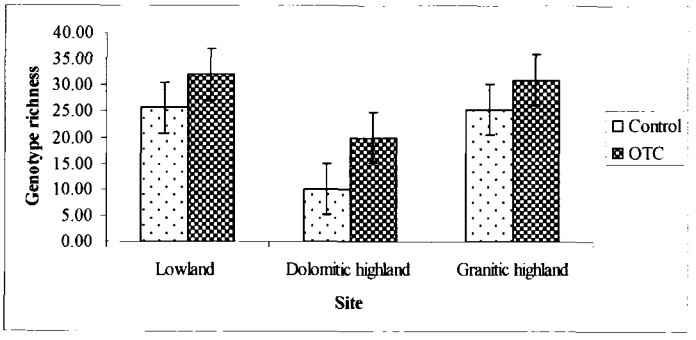
(a) ITS 1-*Hin*fI




(b)ITS 4-*Hin*fI




(c)ITS 1-*Alu*I




(d)ITS 4-*Alu*I

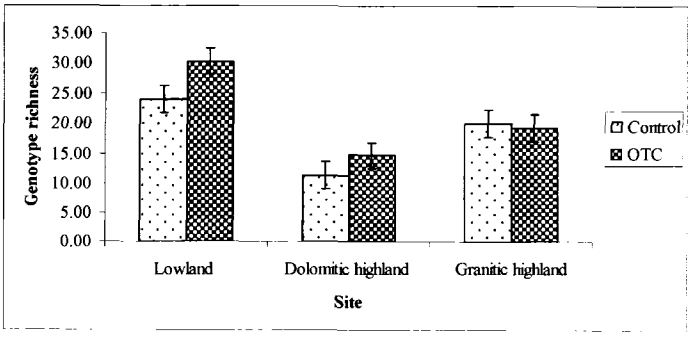
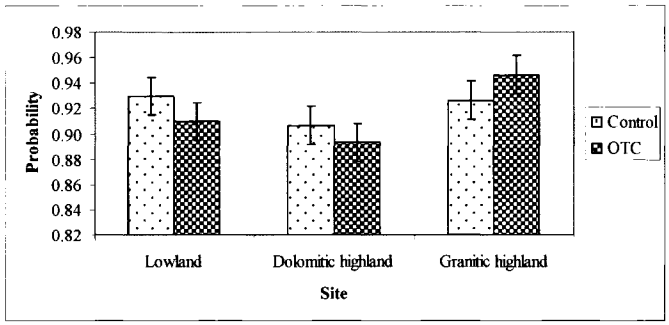
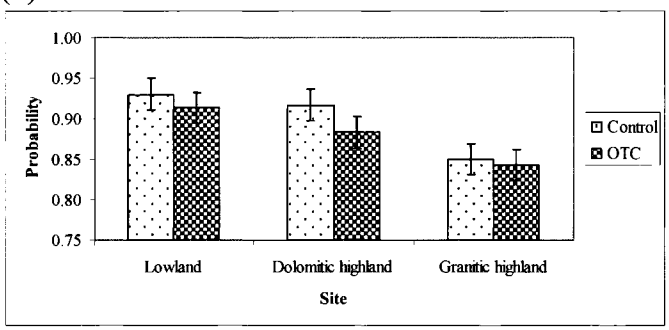



Fig. A2.8. Least square means of genotype richness for each treatment per site. Error bar represents one standard error.

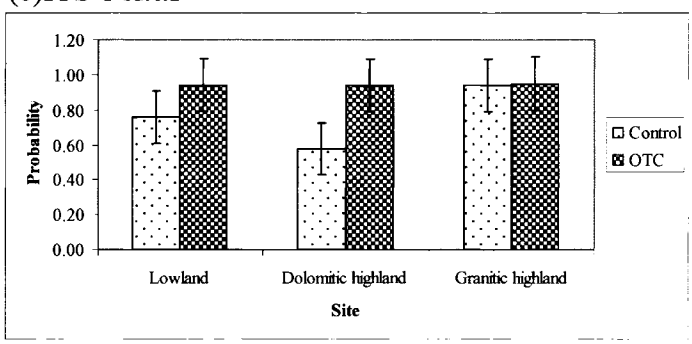
(a) ITS 1-*Hin*fI




(b)ITS 4-*Hin*fI




(c)ITS 1-*Alu*I




(d)ITS 4-*Alu*I

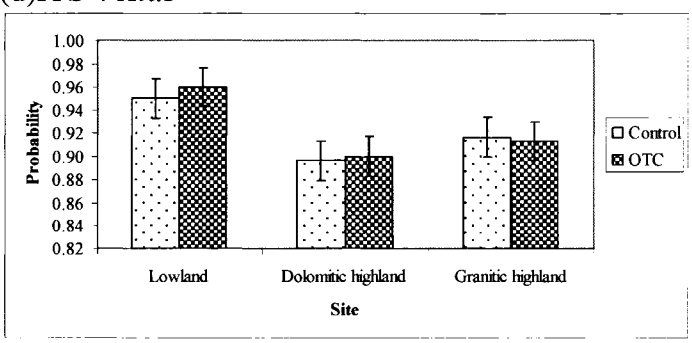



Fig. A2.9. Least square means of genotype evenness measured by Hurlbert's PIE index. Error bar represents one standard error.

## VIII. Appendix for chapter 3

Table A3.1 Comparison of identification (based on molecular techniques) with corresponding morphological identification - *Phialocephala*. Identifications by sequencing and morphology indicate samples are likely *Phialocephala fortinii*

| **ID based on RFLP and sequence analyses: *Phialocephala fortinii*** | **ID based on morphology** |
|---|---|
| RFLP type $1_{Pf}$ | |
| 2-S1-C1-Drin 3.2 | dark sterile |
| 2-C1-S1-Saar 2.3 | *Phialocephala fortinii* |
| 2-S1-OTC1-Saar 2.1 | dark sterile |
| 2-S1-OTC1-Saar 2.2 | NA |
| RFLP type $2_{Pf}$ | |
| 1-S1-OTC1-Drin 1.1 | *Phialocephala fortinii* |
| 1-S1-OTC2-Saop 5.5 | *Phialocephala fortinii* |
| 1-S1-OTC3-Drin 1.3 | NA |
| 1-S4-OTC1-Saop 4.1 | *Phialocephala fortinii* |
| 2-S1-C2-Drin 1.1 | *Phialocephala fortinii* |
| 2-S1-C2-Saar 1.1 | *Phialocephala fortinii* |
| 2-S1-OTC1-Saar 1.1 | *Phialocephala fortinii* |
| 2-S1-OTC1-Saar 1.2 | *Phialocephala fortinii* |
| 2-S1-OTC1-Saar 3.1 | *Phialocephala fortinii* |
| 2-S1-OTC1-Saar 4.1 | *Phialocephala fortinii* |
| 2-S1-OTC2-Drin 2.1 | pigmented warty/ dark sterile |
| 2-S1-OTC2-Saar 2.2 | *Phialocephala fortinii* |
| 2-S4-C3-Saar 4.6 | *Phialocephala fortinii* |
| RFLP type $3_{Pf}$ | |
| 1-S1-C1-Drin 2.1 | *Phialocephala fortinii* |
| 1-S1-C1-Saar 4.4 | *Phialocephala fortinii* |
| 1-S1-OTC3-Cate 4.1A | *Phialocephala fortinii* |
| 1-S1-OTC3-Cate 4.3 | NA |
| 1-S4-C1-Cate 3.8 | *Phialocephala fortinii* |
| 1-S4-C1-Cate 5.7 | *Phialocephala fortinii* |
| 1-S4-C1-Cate 5.8 | NA |
| 1-S4-C2-Saop 4.2 | *Phialocephala fortinii* |
| 1-S4-OTC1-Saop 4.2 | *Phialocephala fortinii* |
| 2-S4-C1-Saar 2.5 | *Phialocephala fortinii* |

Table A3.1 Comparison of identification (based on molecular techniques) with corresponding morphological identification - *Phialocephala*. Identifications by sequencing and morphology indicate samples are likely *Phialocephala fortinii* (cont'd)

| **ID based on RFLP and sequence analyses: *Phialocephala fortinii*** | **ID based on morphology** |
|---|---|
| 2-S4-Saar 5.18 | *Phialocephala fortinii* |
| 2-S4-C1-Saar 5.4 | *Phialocephala fortinii* |
| 2-S4-C1-Saar 5.9 | *Phialocephala fortinii* |
| 2-S4-C2-Saop 4.1a | NA |
| 1-S4-C3-Saar 3.4 | *Phialocephala fortinii* |
| 2-S4-CTL-Cate 2.2 | NA |
| 2-S4-OTC3-Saop 1.3 | dark sterile |
| 2-S4-OTC3-Saop 5.3 | *Phialocephala fortinii* |
| RFLP type $4_{Pf}$ | |
| 1-S1-C2-Drin 2.2 | *Phialocephala fortinii* |
| 1-S1-C3-Cate 5.3 | NA |
| 1-S1-OTC2-Saar 3.2 | *Phialocephala fortinii* |
| 1-S1-OTC2-Saar 4.11 | *Phialocephala fortinii* |
| 1-S1-0TC2-Saar 4.6 | *Phialocephala fortinii* |
| 1-S1-OTC2-Saop 5.5a | NA |
| 1-S1-OTC3-Cate 5.1 | *Phialocephala fortinii* |
| RFLP type $5_{Pf}$ | |
| 1-S1-C3-Saar 1.3 | NA |
| 1-S1-C3-Saar 4.2 | *Phialocephala fortinii* |
| 1-S1-OTC1-Saar 2.1 | NA |
| 1-S1-OTC3-Cate 3.1 | *Phialocephala fortinii* |
| 2-S1-OTC3-Saar 5.1 | *Phialocephala fortinii* |
| 3-S1-C3-Saar 4.2 | NA |
| RFLP type $6_{Pf}$ | |
| 1-S1-OTC1-Saar 5.1 | dark septate sterile |
| 1-S4-C1-Saop2.3 | NA |
| 2-S4-C1-Saar 1.2 | dark sterile |
| 2-S4-C1-Saar4.2 | *Phialocephala fortinii* |

Table A3.2 Comparison of identification (based on molecular techniques) with corresponding morphological identification – Helotiales. Sequence analysis indicated that members belonged to the Helotiales

| **ID based on RFLP and sequence analyses: Helotiales** | **ID based on morphology** |
|---|---|
| RFLP Type $1_H$ | |
| 1-S1-C2-Cate 1.4 | NA |
| 1-S4-Ctl-Cate 4.2 | dark monolioid |
| 1-S4-Ctl-Cate 4.5 | NA |
| 2-S4-C1-Saar 1.1a | NA |
| 2-S4-C3-Saop 3.2 | NA |
| RFLP Type $2_H$ | |
| 1-S4-OTC1-Saop 5.9 | inconclusive |
| 1-S4-C2-Saar 2.7 | inconclusive |
| RFLP Type $3_H$ | |
| 1-S1-C2-Drin5.2 | dark monolioid/ mixed |
| 1-S4-OTC2-Saop2.1 | NA |
| 2-S1-OTC2-Saar2.1 | NA |
| 2-S4-C3-Saop3.5 | hyaline sterile |
| 2-S4-OTC2-Saar2 | NA |
| 2-S4-OTC3-Saar5.2 | mixed/ hyaline sterile |
| 3-S1-C3-Saar1.1 | mixed |
| RFLP Type $4_H$ | |
| 1-S4-OTC2-Saop 4.2 | inconclusive |
| 2-S3-C2-Saop 5.1 | NA |
| 2-S3-OTC1-Drin 1.2 | dark sterile |
| 2-S3-OTC1-Drin 1.4 | NA |
| RFLP Type $5_H$ | |
| 1-S4-C2-Saar 2.1 | hyaline conidia/mixed |
| 1-S4-C2-Saar 2.2 | NA |
| RFLP Type $6_H$ | |
| 1-S4-C1-Cate 3.7 | NA |
| 1-S4-C1-Saop 1.11 | hyaline sterile |

Table A3.2 Comparison of identification (based on molecular techniques) with corresponding morphological identification - Helotiales. Sequence analysis indicated that members belonged to the Helotiales (cont'd)

| **ID based on RFLP and sequence analyses: Helotiales** | **ID based on morphology** |
|---|---|
| RFLP Type $7_H$ | |
| 1-S1-C3-Drin 2.4 | dark septate monolioid |
| 1-S1-OTC3-Cate-4.2 | NA |
| 1-S4-C1-Saop 5.4 | NA |
| 1-S4-C2-Saar 5.1 | NA |
| 1-S4-C3-Saop 5.1 | dark septate monolioid |
| 1-S4-OTC1-Saar 4.5 | dark sterile/ mixed |
| 1-S4-OTC1-Saop 3.7 | NA |
| 1-S4-OTC1-Saop 4.4 | NA |
| 1-S4-OTC2-Saar 2.1 | *Phialocephala fortinii* |
| 1-S4-OTC2-Saop 4.1 | dark monolioid |
| 1-S4-OTC2-Saop 4.3 | *Phialocephala fortinii* |
| 2-S1-OTC2-Drin 1.1 | *Phialocephala fortinii* |
| 2-S4-C1-Saar 5.20 | dark septate monolioid |
| 2-S4-C2-Saar 4.9 | *Phialocephala fortinii* |
| 2-S4-C2-Saop 3.2 | hyaline sterile |
| 2-S4-C2-Saop 3.3 | inconclusive |
| 2-S4-C3-Saop 1.1 | dark septate monolioid |
| 2-S4-C3-Saop 3.1 | *Phialocephala fortinii* |
| 2-S4-OTC1-Saar 1.10 | dark sterile |
| 2-S4-OTC1-Saar 2.1 | dark septate monolioid |

Table A3.3 Comparison of identification (based on molecular techniques) with corresponding morphological identification - unknown. Identifications by sequencing were inconclusive.

| **ID based on RFLP and sequence analyses: Unknowns** | **ID based on morphology** |
|---|---|
| 1-S1-C3-Saop 5.4 | Ascomycete |
| 1-S3-C3-Saop 3.3 | brown |
| 2-S3-C3-Saar 4.1 | brown |
| 2-S4-C3-Saop1.5 | brown |
| 1-S4-OTC2-Saar 4.1 | *Cadophora* |
| 1-S1-C3-Drin 1.3 | dark monolioid |
| 1-S3-C-Drin 1.1 | dark monolioid |
| 2-S1-C2-Saar 3.1 | dark monolioid |
| 2-S3-C2-Saop 5.7 | dark monolioid |
| 2S4-C1-Saar 1.16 | dark monolioid |
| 2-S4-OTC1-Saar 4.4 | dark monolioid |
| 2-S1-OTC2-Saop 2.1 | dark septate, few monolioids |
| 1-S4-OTC1-Saop 3.3 | dark some monolioid |
| 1-S1-C3-Drin 4.1 | dark sterile |
| 1-S1-OTC1-Saar5.1 | dark sterile |
| 1-S1-OTC3-Saop 2.2 | dark sterile |
| 1-S3-C3-Saop5.2 | dark sterile |
| 1-S4-C1-Saop 2.3 | dark sterile |
| 1-S4-Ctl-Cate 2.7 | dark sterile |
| 2-S1-OTC2-Saar 1.1 | dark sterile |
| 2-S3-C3-Drin 4.2 | dark sterile |
| 2-S3-C3-Saop4.2 | dark sterile |
| 2-S3-OTC1-Saar 1.8 | dark sterile |
| 2-S3-OTC3-Drin 4.1 | dark sterile |
| 2-S4-C2-Saar1.2 | dark sterile |
| 1-S4-C2-Saar 3.2 | *Geomyces* |
| 1-S4-CTL Cate 2.1 | *Geomyces* |

Table A3.3 Comparison of identification (based on molecular techniques) with corresponding morphological identification - unknown. Identifications by sequencing were inconclusive (cont'd)

| **ID based on RFLP and sequence analyses: Unknowns** | **ID based on morphology** |
|---|---|
| 1-S4-OTC1-Saar 4.11 | *Geomyces* |
| 1-S4-OTC1-Saar 4.4 | *Geomyces* |
| 1-S4-OTC2-Saop 3.1 | *Geomyces* |
| 2-S4-C2-Saop 4.4B | *Geomyces* |
| 2-S4-C2-Saop 4.8 | *Geomyces* |
| 1-S3-OTC3-Drin 3.3 | Hyaline arthroconidia |
| 2-S4-C3-Saar 2.1 | hyaline brown; swollen cells |
| 1-S4-OTC3-Saop 4.3 | hyaline conidia, monolioid |
| 2-S4-C3-Saar 1.5 | hyaline hyphae, dark spored |
| 3-S1-C2-Cate-4.1 | hyaline monolioid |
| 1-S1-C3-Cate3.1 | hyaline spherical, ovoid conidia |
| 1-S1-C1-Drin 2.3 | hyaline sterile |
| 1-S1-C1-Drin 2.5 | hyaline sterile |
| 1-S1-OTC3-Drin -3.2 | hyaline sterile |
| 1-S1-OTC3-Saar 3.1 | hyaline sterile |
| 1-S1-OTC3-Saar 3.2 | hyaline sterile |
| 1-S1-OTC3-Saop 4.1 | hyaline sterile |
| 1-S3-C2-Drin 2.1 | hyaline sterile |
| 1-S3-C-Drin 2.1 | hyaline sterile |
| 1-S3-OTC2-Drin 2.2B | hyaline sterile |
| 1-S3-OTC3-Saar 1.2 | hyaline sterile |
| 1-S4-C1-Cate 4.1 | hyaline sterile |
| 1-S4-C1-Saar 3.2 | hyaline sterile |
| 1-S4-C1-Saop 1.11 | hyaline sterile |
| 1-S4-C2-Saar 4.2 | hyaline sterile |
| 1-S4-C2-Saar 4.3 | hyaline sterile |
| 1-S4-Ctl-Cate 4.7 | hyaline sterile |
| 1-S4-OTC1-Saar 3.10 | hyaline sterile |
| 1-S4-OTC2-Saop 4.4 | hyaline sterile |
| 1-S4-OTC3-Saop 4.4 | hyaline sterile |

Table A3.3 Comparison of identification (based on molecular techniques) with corresponding morphological identification. Identifications by sequencing were inconclusive (cont'd)

| **ID based on RFLP and sequence analyses: Unknowns** | **ID based on morphology** |
|---|---|
| 1-S4-OTC3-Saop 4.6 | hyaline sterile |
| 1-S4-OTC3-Saop 4.9 | hyaline sterile |
| 2-S3-C3-Drin 3.3 | hyaline sterile |
| 2-S3-C3-Saop 2.1 | hyaline sterile |
| 2-S3-OTC3-Saar 1.3 | hyaline sterile |
| 2-S4-C1-Saar 3.20 | hyaline sterile |
| 2-S4-C3-Saar 1.3 | hyaline sterile |
| 2-S4-C3-Saop 1.7 | hyaline sterile |
| 2-S4-C3-Saop1.2 | hyaline sterile |
| 2-S4-C3-Saop3.5 | hyaline sterile |
| 2-S4-OTC2-Saop 1.3 | hyaline sterile |
| 2-S4-OTC2-Saop 3.2 | hyaline sterile |
| 2-S4-OTC3-Saar 2.1 | hyaline sterile |
| 3-S1-C2-Cate 2.1 | hyaline sterile |
| 3-S1-OTC3-Drin 1.2 | hyaline sterile |
| 2-S3-OTC3-Saop 4.2 | hyaline sterile/ hyaline monolioid |
| 2-S4-OTC3-Saar 2.4 | hyaline sterile/ hyaline monolioid |
| 1-S1-C2-Cate 4.1 | inconclusive |
| 1-S1-C3-Cate 3.2 | inconclusive |
| 1-S1-C3-Drin 2.2 | inconclusive |
| 1-S1-C3-Drin 4.2 | inconclusive |
| 1-S1-C3-Saop 2.1 | inconclusive |
| 1-S3-C1-Saar 3.4 | inconclusive |
| 1-S3-C1-Saop 2.2 | inconclusive |
| 1-S3-C2-Drin 5.5 | inconclusive |
| 1-S3-C3-Drin 3.1 | inconclusive |
| 1-S3-C3-Saop 1.8 | inconclusive |
| 1-S3-C3-Saop 4.4 | inconclusive |
| 1-S3-OTC1-Saar 3.8 | inconclusive |
| 1-S4-C1-Cate 4.3 | inconclusive |
| 1-S4-C1-Saop 1.7 | inconclusive |
| 1-S4-C1-Saop 5.6 | inconclusive |
| 1-S4-C2-Saar 1.1 | inconclusive |
| 1-S4-OTC1-Saar 4.7 | inconclusive |

Table A3.3 Comparison of identification (based on molecular techniques) with corresponding morphological identification - unknowns. Identifications by sequencing were inconclusive (cont'd)

| **ID based on RFLP and sequence analyses: Unknowns** | **ID based on morphology** |
|---|---|
| 1-S4-OTC1-Saop 1.1 | inconclusive |
| 1-S4-OTC1-Saop 1.7 | inconclusive |
| 1-S4-OTC1-Saop 3.4 | inconclusive |
| 1-S4-OTC1-Saop 5.5 | inconclusive |
| 1-S4-OTC3-Saop 4.5 | inconclusive |
| 2-S1-C1-Drin 4.5 | inconclusive |
| 2-S1-C1-Saop 3.3 | inconclusive |
| 2-S1-C1-Saop 4.4 | inconclusive |
| 2-S4-C1-Saar 1.12 | inconclusive |
| 2-S4-C1-Saar 1.5 | inconclusive |
| 2-S4-C1-Saar 1.6 | inconclusive |
| 2-S1-OTC1-Saop 2.4 | light brown sterile |
| 1-S4-C2-Saop 2.3 | lightly pigmented sterile |
| 1-S1-C3-Saop 1.2 | *Monodictys* |
| 1-S1-C1-Cate2.1 | NA |
| 1-S1-C1-Cate2.3 | NA |
| 1-S1-C1-Saop 5.1 | NA |
| 1-S1-C2-Cate 4.2 | NA |
| 1-S1-C3-Cate 4.1 | NA |
| 1-S1-C3-Cate 5.1 | NA |
| 1-S1-OTC1-Saar 1.3 | NA |
| 1-S1-OTC1-Saop 5.2 | NA |
| 1-S1-OTC2-Drin 1.1 | NA |
| 1-S1-OTC2-Drin 4.1 | NA |
| 1-S1-OTC2-Saop 5.4 | NA |
| 1-S1-OTC3-Cate 5.2 | NA |
| 1-S1-OTC3-Cate4.2 | NA |
| 1-S1-OTC3-Cate 5.2 | NA |
| 1-S1-OTC3-Drin 2.1 | NA |
| 1-S1-OTC3-Saar 1.2 | NA |
| 1-S3-C1-Drin 4.2 | NA |
| 1-S3-C3-Drin 1.3 | NA |

Table A3.3 Comparison of identification (based on molecular techniques) with corresponding morphological identification. Identifications by sequencing were inconclusive (cont'd)

| **ID based on RFLP and sequence analyses: Unknowns** | **ID based on morphology** |
|---|---|
| 1-S3-C3-Saop 2.1 | NA |
| 1-S3-C3-Saop 2.3 | NA |
| 1-S3-C3-Saop 4.1 | NA |
| 1-S3-C3-Saop 4.2 | NA |
| 1-S3-C3-Saop 4.3 | NA |
| 1-S3-C3-Saop 5.3 | NA |
| 1-S3-C-Drin 1.2 | NA |
| 1-S3-OTC1-Drin 2.1 | NA |
| 1-S3-OTC3-Drin 2.2 | NA |
| 1-S3-OTC3-Saop 2 | NA |
| 1-S3-OTC3-Saop 2.1 | NA |
| 1-S3-OTC3-Saop 4.5 | NA |
| 1-S4-C1-Cate 3.7 | NA |
| 1-S4-C1-Cate 5.2 | NA |
| 1-S4-C2-Saar 2.2 | NA |
| 1-S4-C2-Saar 2.8 | NA |
| 1-S4-C2-Saop 5.1 | NA |
| 1-S4-CTL-Cate 1.6 | NA |
| 1-S4-CTL-Cate 2.5 | NA |
| 1-S4-CTL-Cate 2.6 | NA |
| 1-S4-Ctl-Cate 3.3 | NA |
| 1-S4-CTL-Cate 3.5 | NA |
| 1-S4-CTL-Cate 5.1 | NA |
| 1-S4-OTC1-Saar 4.15 | NA |
| 1-S4-OTC1-Saop 1.5 | NA |
| 1-S4-OTC1-Saop 5.2 | NA |
| 1-S4-OTC3-Saop 5.2 | NA |
| 2-S1-C1-Drin 4.1 | NA |
| 2-S1-C1-Drin 4.2 | NA |
| 2-S1-C1-Saar 2.1 | NA |
| 2-S1-C1-Saar-3.1 | NA |
| 2-S1-C1-Saop 5.2 | NA |

Table A3.3 Comparison of identification (based on molecular techniques) with corresponding morphological identification - unknowns. Identifications by sequencing were inconclusive (cont'd)

| **ID based on RFLP and sequence analyses: Unknowns** | **ID based on morphology** |
|---|---|
| 2-S1-C1-Saop 5.3 | NA |
| 2-S1-C1-Saop 5.5 | NA |
| 2-S1-C2-Drin 2.2 | NA |
| 2-S1-C2-Drin 3.2 | NA |
| 2-S1-C2-Saar 2.1 | NA |
| 2-S3-C1-Drin 3.1 | NA |
| 2-S3-C1-Saar 2.1 | NA |
| 2-S3-C1-Saar 3.1 | NA |
| 2-S3-C1-Saar 5.1 | NA |
| 2-S3-C2-Drin 5.2 | NA |
| 2-S3-C2-Drin 1.1 | NA |
| 2-S3-C2-Drin 1.2 | NA |
| 2-S3-C2-Drin 1.4 | NA |
| 2-S3-C2-Drin 5.2 | NA |
| 2-S3-C2-Saar 5.2 | NA |
| 2-S3-C3-Drin 3.1 | NA |
| 2-S3-C3-Saar 1 | NA |
| 2-S3-C3-Saar 2.5 | NA |
| 2-S3-C3-Saar 3.5 | NA |
| 2-S3-C3-Saop 1.1 | NA |
| 2-S3-C3-Saop 3.3 | NA |
| 2-S3-C3-Saop 5.2 | NA |
| 2-S3-OTC1-Saop 3.5 | NA |
| 2-S3-OTC2-Saar 3.5 | NA |
| 2-S3-OTC2-Saar 3.7 | NA |
| 2-S3-OTC3-Saar 3.2 | NA |
| 2-S3-OTC3-Saar 4.1 | NA |
| 2-S3-OTC3-Saar 4.3 | NA |
| 2-S3-OTC3-Saop 4.1 | NA |
| 2-S3-OTC3-Saop 4.4 | NA |
| 2-S3-OTC3-Saop 4.5 | NA |
| 2-S4-C1-Saar 1.11 | NA |
| 2-S4-C1-Saar 1.14 | NA |
| 2-S4-C1-Saar 1.3 | NA |

Table A3.3 Comparison of identification (based on molecular techniques) with corresponding morphological identification. Identifications by sequencing were inconclusive (cont'd)

| **ID based on RFLP and sequence analyses: Unknowns** | **ID based on morphology** |
|---|---|
| 2-S4-C1-Saar 2.4 | NA |
| 2-S4-C2-Saar 2.4 | NA |
| 2-S4-C2-Saop 4.1B | NA |
| 2-S4-C3-Saar 4.3 | NA |
| 2-S4-C3-Saar 4.4 | NA |
| 2-S4-C3-Saop 2.3 | NA |
| 2-S4-C3-Saop 2.4 | NA |
| 2-S4-OTC2-Saar 1.2 | NA |
| 2-S4-OTC3-Saar 1.2 | NA |
| 2-S4-OTC3-Saop 5.6 | NA |
| 3-S1-C2-Cate1.3 | NA |
| 3-S1-OTC3-Drin 1.1 | NA |
| 3-S1-OTC3-Drin 5.2 | NA |
| 1-S4-OTC1-Saar 4.12 | *Penicillium* |
| 1-S1-C1-Drin 4.1 | *Phialocephala fortinii* |
| 1-S1-OTC2-Saar 5.5 | *Phialocephala fortinii* |
| 1-S1-OTC3-Drin 5.3 | *Phialocephala fortinii* |
| 1-S3-OTC3-Saop 4.3 | *Phialocephala fortinii* |
| 1-S4-OTC1-Saop 4.3 | *Phialocephala fortinii* |
| 1-S4-OTC3-Saop 4.1 | *Phialocephala fortinii* |
| 2-S1-OTC1-Saop 2.3 | *Phialocephala fortinii* |
| 2-S1-OTC2-Saar 3.3 | *Phialocephala fortinii* |
| 2-S3-OTC3-Saar 4.2 | *Phialocephala fortinii* |
| 2-S4-C1-Saar 5.1 | *Phialocephala fortinii* |
| 2-S4-OTC3-Saar 3.4 | *Phialocephala fortinii* |
| 2-S3-OTC1-Drin 4.5 | *Sebacina* |
| 2-S1-C1-Saop 5.6 | *Trichocladium* |
| 2-S4-OTC1-Saar 1.5 | *Trichoderma sporulosum* |
| 2-S4-OTC1-Saar 2.5 | *Trichoderma sporulosum* |
| 2-S3-OTC3-Drin 3.1 | white |
| 2-S4-C3-Saar 2.3 | white |
| 2-S4-OTC2-Saar 4.3 | white |

Table A3.4 Comparison of identification (based on molecular techniques) with corresponding morphological identification – *Hymenoscyphus*. Samples showed affiliation to *Hymenoscyphus* according to sequence and RFLP analyses

| **ID based on RFLP and sequence analyses: *Hymenoscyphus*** | **ID based on morphology** |
|---|---|
| RFLP type $1_{Hy}$ | |
| 1-S4-C1-Saop 2.11 | dark spherical conidia |
| 1-S4-OTC1-Saar 3.5 | dark sterile |
| RFLP type $2_{Hy}$ | |
| 1-S1-C3-Saar 1.2 | intercalary chlamydospore fried egg |
| 1-S1-OTC1-Drin 2.3 | black type |
| 1-S1-OTC1-Drin 4.1 | inconclusive |
| 1-S1-OTC2-Drin 3.1 | *Leptodontidium* |
| 1-S4-CTL-Cate 2.4 | black type |
| 1-S4-OTC2-Saar 4.6 | inconclusive |
| 2-S1-C1-Saop 3.2 | inconclusive |
| 2-S1-C3-Drin 3.2 | NA |
| 2-S4-C2-Saar 4.10 | inconclusive |
| 3-S1-C3-Saar 2.1 | NA |
| RFLP type $3_{Hy}$ | |
| 1-S4-OTC1-Saop 5.7 | inconclusive |
| 2-S4-OTC1-Saar 1.9 | inconclusive |
| RFLP type $4_{Hy}$ | |
| 1-S1-C1-Drin 2.2 | *Phialocephala fortinii* |
| 1-S1-OTC3-Cate 4.2a | NA |
| 1-S4-C1-Saar 4.6 | dark sterile |
| 2-S4-C1-Saar 1.16 | dark monolioid |
| 2-S1-C3-Drin 3.2 | NA |

Table A3.5 Comparison of identification (based on molecular techniques) with corresponding morphological identification – *Mycosphaerella.* Samples showed affiliation to *Mycosphaerella* according to sequence and RFLP analyses

| **ID based on RFLP and sequence analyses: *Mycosphaerella*** | **ID based on morphology** |
|---|---|
| RFLP type $1_M$ | |
| 1-S1-C2-Cate 5.3 | NA |
| 1-S3-OTC1-Drin 4.4 | inconclusive |
| 1-S3-OTC1-Saop 3.4 | white |
| 1-S3-OTC1-Saop 3.6 | inconclusive |
| 2-S3-C3-Saop 1.2 | white |
| 2-S4-C2-Saar 5.7 | inconclusive |
| 2-S4-C2-Saop 2 | NA |
| 2-S4-C2-Saop 2.2 | *Staphylotrichum* |
| 2-S4-C2-Saop 2.4 | inconclusive |
| RFLP type $2_M$ | |
| 1-S3-C1-Saop 4.19 | dark sterile |
| 1-S4-OTC3-Saop 2.1 | NA |
| RFLP type $3_M$ | |
| 1-S1-C3-Saop 3.3 | inconclusive |
| 1-S3-C Drin 4.9 | inconclusive |
| 1-S4-C1-Saar 4.3 | inconclusive |
| 1-S4-C2-Saar 3.3 | *Phialocephala fortinii* |
| 1-S4-OTC1-Saar 4.6 | inconclusive |
| 2-S4-C2-Saop 44A | dark septate |
| 2-S4-C2-Saop 4.9 | inconclusive |
| 2-S4-OTC1-Saar 4.1 | NA |
| RFLP type $4_M$ | |
| 2-S3-OTC1-Saar 1.9 | NA |
| 1 D/G-Saar 3.3 | dark sterile |
| RFLP type $5_M$ | |
| 2-S3-C3-Saar 1.7 | NA |
| 2-S3-C3-Saar 1.8 | NA |
| 2-S3-C3-Saar 3.1 | NA |
| 2-S3-OTC1-Saar 4.8 | NA |
| 2-S3-OTC1-Saar 5.1 | NA |
| 2-S3-OTC1-Saar 5.5 | NA |
| 1-D/G-Saar 3.4 | dark sterile |

Table A3.6 Comparison of identification (based on molecular techniques) with corresponding morphological identification – Dothideales. Samples showed affiliation to the Dothideales according to sequence and RFLP analyses

| **ID based on RFLP and sequence analyses: Dothideales** | **ID based on morphology** |
|---|---|
| 1-S1-C3-Saop 4.1 | NA |
| 1-S1-OTC1-Saop 1.2 | NA |
| 1-S1-OTC1-Saop 2.1 | inconclusive |
| 1-S1-OTC2-Saop 2.2 | *Phialocephala fortinii* |
| 1-S1-OTC2-Saop 5.3 | *Phialocephala fortinii* |
| 1-S4-OTC1-Saop 3.9 | hyaline *Phialocephala fortinii* -like |
| 2-S1-C1-Saop 1.6 | dark sterile |
| 2-S1-OTC2-Saop 3.3 | *Acremonium* |
| 2-S1-OTC2-Saop 5.1 | black type |

Table A3.7 Comparison of identification (based on molecular techniques) with corresponding morphological identification - *Phoma*. Samples showed affiliation to Phoma according to sequence and RFLP analyses

| **ID based on RFLP and sequence analyses: *Phoma*** | **ID based on morphology** |
|---|---|
| RFLP type $1_P$ | |
| 1-S3-OTC2-Drin 2.2A | NA |
| 1-S4-C1-Saop 4.7 | NA |
| 1-S4-C2-Saop 2.2 | inconclusive |
| 1-S4-OTC2-Saop 1.1B | *Monodictys* |
| 1-S4-OTC2-Saop 2.2 | *Monodictys* |
| 1-S4-OTC3-Saop 3.9 | NA |
| 1-S4-OTC3-Saop 4.2 | *Trichocladium* |
| 2-S3-OTC3-Saar 2.3 | hyaline sterile |
| 2-S3-OTC3-Saar 3.3 | dark sterile |
| RFLP type $2_P$ | |
| 1-S1-OTC2-Saop 3.2 | inconclusive |
| 1-S1-OTC3-Saop 3.2 | *Monodictys* |
| 1-S3-C1-Saop 4.2 | inconclusive |

Table A3.8 Comparison of identification (based on molecular techniques) with corresponding morphological identification - *Cryptosporiopsis*. Samples showed affiliation to Cryptosporiopsis according to sequence and RFLP analyses

| **ID based on RFLP and sequence analyses: *Cryptosporiopsis*** | **ID based on morphology** |
|---|---|
| RFLP type $1_L$ | |
| 1-S1-C3-Saop 3.2 | dark sterile |
| 1-S4-C1-Cate 2.2 | NA |
| 1-S4-C1-Cate 2.4 | NA |
| 1-S4-C2-Saar 3.4 | *Cryptosporiopsis radicola* |
| 1-S4-C2-Saop 3.2 | brown |
| 1-S4-C2-Saop 3.3 | inconclusive |
| 1-S4-C2-Saop 3.4 | inconclusive |
| 1-S4-C2-Saop 4.5 | inconclusive |
| 1-S4-C3-Saar 3.1 | inconclusive |
| 1-S4-C3-Saar 5.1 | dark sterile monolioid |
| 1-S4-C3-Saop 3.6 | hyaline sterile |
| 1-S4-CTL-Cate 1.2 | hyaline sterile |
| 1-S4-OTC2-Saop 3.2 | inconclusive |
| 1-S4-OTC3-Saop 3.1 | hyaline sterile |
| 1-S4-OTC3-Saop 4.11 | inconclusive |
| 1-S4-CTL Cate 3.9 | *Cryptosporiopsis radicola* |
| 2-S1-C1-Saop 4.1 | inconclusive |
| 2-S3-OTC3-Drin 2.2 | hyaline sterile |
| 2-S4-C3-Saar 3.3 | white |
| 2-S4-CTL-Cate 3.6 | white |
| RFLP type $2_L$ | |
| 1-S3-C2-Drin 2.2 | *Cryptosporiopsis radicola* |
| 1-S3-C2-Drin 5.6 | NA |

Table A3.9 Comparison of identification (based on molecular techniques) with corresponding morphological identification - *Cadophora*. Samples showed affiliation to *Cadophora* according to sequence and RFLP analyses

| **ID based on RFLP and sequence analyses: *Cadophora*** | **ID based on morphology** |
|---|---|
| RFLP Type $1_{Ca}$ | |
| 2-S3-C3-Saop 2.2 | dark sterile |
| 1-S4-OTC1-Saop 4.5 | NA |
| RFLP Type $2_{Ca}$ | |
| 1-S1-C1-Saar 3.6 | *Phialocephala fortinii* |
| 1-S1-OTC1-Drin 4.2 | *Phialocephala fortinii* |
| 1-S1-OTC3-Cate 4.1 | inconclusive |
| RFLP Type $3_{Ca}$ | |
| 1-S3-OTC2-Drin 2.4 | inconclusive |
| 1-S4-C2-Saar 2.10 | hyaline sterile |
| 1-S4-Ctl Cate 2.1A | *Phialocephala fortinii* |
| 2-S4-Ctl Cate 3.1 | inconclusive |
| 2-S4-Ctl Cate 3.3 | NA |

Table A3.10 Comparison of identification (based on molecular techniques) with corresponding morphological identification - *Colispora*. Samples showed affiliation to Colispora according to sequence and RFLP analyses

| **ID based on RFLP and sequence analyses: *Colispora*** | **ID based on morphology** |
|---|---|
| 1-S3-C-Drin 5.2 | NA |
| 1-S4-OTC3-Saop 5.4 | NA |

Table A3.11 Comparison of identification (based on molecular techniques) with corresponding morphological identification - Agaricales. Samples showed affiliation to the Agaricales according to sequence and RFLP analyses

| **ID based on RFLP and sequence analyses: unknown Agaricales** | **ID based on morphology** |
|---|---|
| 1-S1-C1-Drin 1.6 | hyaline sterile |
| 1-S4-C1-Saar 4.9 | hyaline sterile |
| 2-S4-C1-Saar 3.11 | hyaline sterile |
| 2-S4-C2-Saop 4.3 | hyaline sterile |

Table A3.12 Comparison of identification (based on molecular techniques) with corresponding morphological identification -*Ceratobasidium*. Samples showed affiliation to *Ceratobasidium* according to sequence and RFLP analyses

| **ID based on RFLP and sequence analyses: *Ceratobasidium*** | **ID based on morphology** |
|---|---|
| 1-S1-OTC2-Saar 2.1 | hyaline sterile |
| 1-S1-OTC2-Saar 2.10 | hyaline sterile |
| 1-S3-C2-Saar 1.3 | hyaline sterile |
| 1-S3-C2-Saar 2.3 | hyaline sterile |
| 1-S3-C2-Saar 5.3 | hyaline sterile |
| 1-S3-OTC2-Saop 2.1 | hyaline sterile |
| 1-S4-C1-Cate 5.4 | hyaline sterile |
| 2-S1-C2-Saop 2.2 | inconclusive |
| 2-S1-OTC2-Drin 3.1 | hyaline sterile |

## IX. Appendix for chapter 4

Table A4.1 Genotype richness for host plants at chronosequence zone. Number preceded by 'Y' represent the earliest time of exposure from the glacier. <Y59 is the control, or refugia plot that was not covered by the glacier.

| *Luzula confusa* Plot | species richness | | | |
|---|---|---|---|---|
| | ITS1-*Hin*fI | ITS4-*Hin*fI | ITS1-*Alu*I | ITS4-*Alu*I |
| Y90 | 16 | 7 | 22 | 11 |
| Y80 | 18 | 6 | 22 | 10 |
| Y70 | 14 | 9 | 16 | 8 |
| Y60 | 19 | 12 | 30 | 18 |
| control | 37 | 14 | 39 | 24 |
| *Papaver lapponicum* Plot | species richness | | | |
| | ITS1-*Hin*fI | ITS4-*Hin*fI | ITS1-*Alu*I | ITS4-*Alu*I |
| Y90 | 11 | NA | 10 | 4 |
| Y80 | 17 | 9 | 27 | 11 |
| Y70 | 22 | 9 | 28 | 13 |
| Y60 | 13 | NA | 21 | 11 |
| control | 11 | 5 | 14 | 5 |
| *Salix arctica* Plot | species richness | | | |
| | ITS1-*Hin*fI | ITS4-*Hin*fI | ITS1-*Alu*I | ITS4-*Alu*I |
| Y80 | 12 | 12 | 20 | 16 |
| Y70 | 13 | 6 | 14 | 6 |
| Y60 | 22 | 14 | 18 | 15 |
| control | 23 | 16 | 24 | 14 |
| *Saxifraga oppositifolia* Plot | species richness | | | |
| | ITS1-*Hin*fI | ITS4-*Hin*fI | ITS1-*Alu*I | ITS4-*Alu*I |
| Y70 | 14 | 6 | 9 | 9 |
| Y60 | 11 | 8 | 20 | 14 |
| *Cassiope tetragona* Plot | species richness | | | |
| | ITS1-*Hin*fI | ITS4-*Hin*fI | ITS1-*Alu*I | ITS4-*Alu*I |
| Y60 | 6 | 3 | 14 | 8 |
| control | 18 | 14 | 33 | 18 |
| *Dryas integrifolia* Plot | species richness | | | |
| | ITS1-*Hin*fI | ITS4-*Hin*fI | ITS1-*Alu*I | ITS4-*Alu*I |
| Y60 | 13 | 5 | 12 | 12 |
| control | 15 | 11 | 14 | 14 |

Table A4.2 Sorensen's Quantitative Index between host plants and chronosequence zones. 1-h = ITS 1-HinfI; 4-h=ITS 4-HinfI; 1-a=ITS 1-AluI; 4-a=ITS 4-AluI. Plot <Y59 is the control. Number preceded by 'Y' represent the earliest time of exposure from the front of the receding glacier. <Y59 is the control, or refugia plot that was not covered by the glacier.

| | | Y90 Pala | Y80 Luco | Y80 Pala | Y80 Saar | Y70 Luco | Y70 Pala | Y70 Saar | Y70 Saop | Y60 Cate | Y60 Drin | Y60 Luco | Y60 Pala | Y60 Saar | Y60 Saop | <Y59 Cate | <Y59 Drin | <Y59 Luco | <Y59 Pala | <Y59 Saar |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Y90 Luco | 1-h | 0.25 | 0.47 | 0.26 | 0.24 | 0.38 | 0.18 | 0.06 | 0.11 | 0.12 | 0.05 | 0.37 | 0.28 | 0.2 | 0.14 | 0.18 | 0.11 | 0.2 | 0.19 | 0.38 |
| | 4-h | 0 | 0.55 | 0.42 | 0.24 | 0.38 | 0.21 | 0.27 | 0.38 | 0.17 | 0.23 | 0.31 | 0.2 | 0.19 | 0.24 | 0.23 | 0.08 | 0.39 | 0 | 0.24 |
| | 1-a | 0.12 | 0.36 | 026 | 0.22 | 0.35 | 0.22 | 0.11 | 0.13 | 0.03 | 0.17 | 0.24 | 0.1 | 0.2 | 0.13 | 0.04 | 0.14 | 0.33 | 0.04 | 0.19 |
| | 4-a | 0 | 0.39 | 0.26 | 0.18 | 0.32 | 0.07 | 0 | 0.24 | 0.05 | 0.09 | 0.35 | 0.1 | 0.07 | 0.29 | 0.12 | 0.12 | 0.39 | 0.04 | 0.07 |
| Y80 Luco | 1-h | | | 0.2 | 0.14 | 0.33 | 0.28 | 0.11 | 0.23 | 0.16 | 0.17 | 0.44 | 0.13 | 0.27 | 0.3 | 0.21 | 0.37 | 0.45 | 0.06 | 0.32 |
| | 4-h | | | 0.26 | 0.1 | 0.46 | 0.17 | 0.19 | 0.26 | 0.09 | 0.16 | 0.36 | 0.1 | 0.2 | 0.19 | 0.17 | 0.07 | 0.48 | 0.05 | 0.18 |
| | 1-a | | | 0.24 | 0.13 | 0.4 | 0.18 | 0.11 | 0.06 | 0.11 | 0.07 | 0.43 | 0.22 | 0.1 | 0.14 | 0.15 | 0.09 | 0.48 | 0.19 | 0.15 |
| | 4-a | | | 0.11 | 0.06 | 0.4 | 0.17 | 0.04 | 0.13 | 0.08 | 0.14 | 0.37 | 0.04 | 0.21 | 0.11 | 0.02 | 0.11 | 0.38 | 0.08 | 0.16 |
| Y70 Luco | 1-h | | | | | | 0.13 | 0.08 | 0.04 | 0.09 | 0.06 | 0.62 | 0.14 | 0.14 | 0.1 | 0.2 | 0.04 | 0.43 | 0.1 | 0.2 |
| | 4-h | | | | | | 0.05 | 0.18 | 0.16 | 0.17 | 0.13 | 0.61 | 0.18 | 0.16 | 0.14 | 0.18 | 0.12 | 0.62 | 0.15 | 0.23 |
| | 1-a | | | | | | 0.15 | 0.15 | 0.06 | 0.07 | 0.2 | 0.52 | 0.18 | 0.18 | 0.19 | 0.17 | 0.07 | 0.53 | 0.11 | 0.24 |
| | 4-a | | | | | | 0.05 | 0.17 | 0 | 0.06 | 0.18 | 0.61 | 0.15 | 0.19 | 0.14 | 0.07 | 0.05 | 0.48 | 0.06 | 0.2 |
| Y60 Luco | 1-h | | | | | | | | | | | | 0.11 | 0.19 | 0.15 | 0.21 | 0.15 | 0.42 | 0.1 | 0.33 |
| | 4-h | | | | | | | | | | | | 0.15 | 0.23 | 0.2 | 0.2 | 0.1 | 0.46 | 0.11 | 0.27 |
| | 1-a | | | | | | | | | | | | 0.26 | 0.19 | 0.19 | 0.14 | 0.12 | 0.47 | 0.18 | 0.2 |
| | 4-a | | | | | | | | | | | | 0.15 | 0.25 | 0.34 | 0.16 | 0.08 | 0.48 | 0.06 | 0.22 |
| Y59 Luco | 1-h | | | | | | | | | | | | | | | | | | 0.06 | 0.25 |
| | 4-h | | | | | | | | | | | | | | | | | | 0.17 | 0.3 |
| | 1-a | | | | | | | | | | | | | | | | | | 0.16 | 0.24 |
| | 4-a | | | | | | | | | | | | | | | | | | 0.02 | 0.19 |

Table A4.2 Sorensen's Index, 1-h = ITS 1-*Hin*fI; 4-h=ITS 4-*Hin*fI; 1-a=ITS 1-*Alu*I; 4-a=ITS 4-*Alu*I (cont'd). Number preceded by 'Y' represent the earliest time of exposure from the front of the receding glacier. <Y59 is the control, or refugia plot that was not covered by the glacier.

| | | Y80 Luco | Y80 Pala | Y80 Saar | Y70 Luco | Y70 Pala | Y70 Saar | Y70 Saop | Y60 Cate | Y60 Drin | Y60 Luco | Y60 Pala | Y60 Saar | Y60 Saop | <Y59 Cate | <Y59 Drin | <Y59 Luco | <Y59 Pala | <Y59 Saar |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Y90 Pala | 1-h | 0.13 | 0.47 | 0.06 | 0.06 | 0.41 | 0.13 | 0.03 | 0.18 | 0.03 | 0.08 | 0.58 | 0.12 | 0.04 | 0.19 | 0 | 0.08 | 0.27 | 0.13 |
| | 4-h | 0 | 0 | 0.06 | 0.03 | 0.1 | 0 | 0 | 0 | 0 | 0.02 | 0.18 | 0.05 | 0 | 0.05 | 0 | 0.03 | 0.2 | 0 |
| | 1-a | 0.23 | 0.15 | 0.05 | 0.12 | 0.23 | 0 | 0 | 0.03 | 0 | 0.14 | 0.46 | 0.07 | 0 | 0.08 | 0.11 | 0.18 | 0.4 | 0.02 |
| | 4-a | 0 | 0.18 | 0.05 | 0 | 0.29 | 0.06 | 0 | 0.07 | 0 | 0.02 | 0.34 | 0 | 0 | 0 | 0 | 0.03 | 0.1 | 0.05 |
| Y80 Pala | 1-h | | | 0.12 | 0.14 | 0.5 | 0.13 | 0.15 | 0.14 | 0.08 | 0.11 | 0.51 | 0.17 | 0.13 | 0.25 | 0.08 | 0.19 | 0.3 | 0.2 |
| | 4-h | | | 0.26 | 0.3 | 0.17 | 0.15 | 0.24 | 0.26 | 0.16 | 0.23 | 0.31 | 0.14 | 0.33 | 0.17 | 0.22 | 0.28 | 0.3 | 0.19 |
| | 1-a | | | 0.13 | 0.25 | 0.39 | 0.08 | 0.07 | 0.1 | 0.05 | 0.22 | 0.37 | 0.04 | 0.24 | 0.18 | 0.1 | 0.31 | 0.3 | 0.1 |
| | 4-a | | | 0.11 | 0.23 | 0.24 | 0 | 0.11 | 0.15 | 0 | 0.28 | 0.36 | 0.04 | 0.13 | 0.1 | 0.13 | 0.16 | 0.1 | 0.08 |
| Y70 Pala | 1-h | | | | | | 0.2 | 0.17 | 0.14 | 0.23 | 0.19 | 0.49 | 0.25 | 0.3 | 0.24 | 0.2 | 0.24 | 0.3 | 0.25 |
| | 4-h | | | | | | 0.1 | 0.35 | 0.13 | 0.08 | 0.04 | 0.15 | 0.14 | 0.19 | 0.14 | 0.17 | 0.1 | 0.1 | 0.22 |
| | 1-a | | | | | | 0.15 | 0.09 | 0.09 | 0.15 | 0.16 | 0.37 | 0.1 | 0.29 | 0.13 | 0.07 | 0.21 | 0.3 | 0.22 |
| | 4-a | | | | | | 0.19 | 0.18 | 0.22 | 0.03 | 0.17 | 0.35 | 0.25 | 0.23 | 0.05 | 0.08 | 0.13 | 0.4 | 0.3 |
| Y60 Pala | 1-h | | | | | | | | | | | | 0.15 | 0.1 | 0.22 | 0.11 | 0.1 | 0.3 | 0.18 |
| | 4-h | | | | | | | | | | | | 0.2 | 0.36 | 0.28 | 0.21 | 0.17 | 0.2 | 0.26 |
| | 1-a | | | | | | | | | | | | 0.07 | 0.11 | 0.16 | 0.07 | 0.21 | 0.6 | 0.04 |
| | 4-a | | | | | | | | | | | | 0.08 | 0.25 | 0.13 | 0.05 | 0.15 | 0.3 | 0.17 |
| <Y59 Pala | 1-h | | | | | | | | | | | | | | | | | | 0.18 |
| | 4-h | | | | | | | | | | | | | | | | | | 0.18 |
| | 1-a | | | | | | | | | | | | | | | | | | 0.02 |
| | 4-a | | | | | | | | | | | | | | | | | | 0.08 |

Table A4.2 Sorensen's Quantitative Index, 1-h = ITS 1-*Hin*fI; 4-h=ITS 4-*Hin*fI; 1-a=ITS 1-*Alu*I; 4-a=ITS 4-*Alu*I (cont'd). Number preceded by 'Y' represent the earliest time of exposure from the glacier. <Y59 is the control, or refugia plot that was not covered by the glacier.

| | | Y70 Luco | Y70 Pala | Y70 Saar | Y70 Saop | Y60 Cate | Y60 Drin | Y60 Luco | Y60 Pala | Y60 Saar | Y60 Saop | <Y59 Cate | <Y59 Drin | <Y59 Luco | <Y59 Pala | <Y59 Saar |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Y80 Saar | 1-h | 0.14 | 0.17 | 0.38 | 0.06 | 0.17 | 0.18 | 0.12 | 0.1 | 0.61 | 0.19 | 0.15 | 0.09 | 0.14 | 0.09 | 0.3 |
| | 4-h | 0.16 | 0.17 | 0.3 | 0.13 | 0.09 | 0.16 | 0.19 | 0.26 | 0.39 | 0.19 | 0.25 | 0.31 | 0.17 | 0.16 | 0.3 |
| | 1-a | 0.2 | 0.13 | 0.12 | 0 | 0.19 | 0.16 | 0.14 | 0.14 | 0.19 | 0.23 | 0.23 | 0.16 | 0.18 | 0.04 | 0.15 |
| | 4-a | 0.17 | 0.13 | 0.33 | 0.17 | 0.08 | 0.11 | 0.27 | 0.16 | 0.45 | 0.36 | 0.18 | 0.11 | 0.23 | 0.12 | 0.29 |
| Y70 Saar | 1-h | | | | 0.09 | 0.19 | 0.19 | 0.05 | 0.14 | 0.35 | 0.17 | 0.12 | 0.1 | 0.16 | 0.06 | 0.28 |
| | 4-h | | | | 0.46 | 0.11 | 0.47 | 0.25 | 0.25 | 0.53 | 0.17 | 0.38 | 0.19 | 0.35 | 0.06 | 0.53 |
| | 1-a | | | | 0.07 | 0 | 0.25 | 0.25 | 0.13 | 0.4 | 0.19 | 0.07 | 0.13 | 0.12 | 0 | 0.28 |
| | 4-a | | | | 0 | 0.25 | 0.25 | 0.2 | 0.1 | 0.48 | 0.3 | 0.1 | 0.09 | 0.14 | 0 | 0.44 |
| Y60 Saar | 1-h | | | | | | | | | | 0.38 | 0.21 | 0.17 | 0.25 | 0.12 | 0.35 |
| | 4-h | | | | | | | | | | 0.15 | 0.37 | 0.17 | 0.21 | 0.08 | 0.56 |
| | 1-a | | | | | | | | | | 0.21 | 0.07 | 0.25 | 0.23 | 0.03 | 0.42 |
| | 4-a | | | | | | | | | | 0.31 | 0.11 | 0.27 | 0.16 | 0.07 | 0.48 |

Table A4.2 Sorensen's Quantitative Index, 1-h = ITS 1-*Hin*fI; 4-h=ITS 4-*Hin*fI; 1-a=ITS 1-*Alu*I; 4-a=ITS 4-*Alu*I (cont'd). Number preceded by 'Y' represent the earliest time of exposure from the glacier. <Y59 is the control, or refugia plot that was not covered by the glacier.

| | | Y60 Cate | Y60 Drin | Y60 Luco | Y60 Pala | Y60 Saar | Y60 Saop | <Y59 Cate | <Y59 Drin | <Y59 Luco | <Y59 Pala | <Y59 Saar |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Y70 Saop | 1-h | 0.1 | 0.18 | 0.1 | 0.05 | 0.14 | 0.34 | 0.13 | 0.33 | 0.25 | 0.03 | 0.16 |
| | 4-h | 0.13 | 0.42 | 0.19 | 0.16 | 0.39 | 0.34 | 0.25 | 0.13 | 0.18 | 0.08 | 0.45 |
| | 1-a | 0 | 0.05 | 0.04 | 0.02 | 0.04 | 0.09 | 0.02 | 0 | 0.04 | 0 | 0.03 |
| | 4-a | 0.18 | 0 | 0.17 | 0.06 | 0.12 | 0.36 | 0.08 | 0 | 0.1 | 0.06 | 0.04 |
| Y60 Saop | 1-h | | | | | | | 0.13 | 0.24 | 0.16 | 0.08 | 0.2 |
| | 4-h | | | | | | | 0.15 | 0.14 | 0.16 | 0.19 | 0.32 |
| | 1-a | | | | | | | 0.17 | 0.09 | 0.25 | 0.06 | 0.34 |
| | 4-a | | | | | | | 0.17 | 0.13 | 0.26 | 0.05 | 0.26 |

Table A4.2 Sorensen's Quantitative Index, 1-h = ITS 1-HinfI; 4-h=ITS 4-HinfI; 1-a=ITS 1-AluI; 4-a=ITS 4-AluI (cont'd). Number preceded by 'Y' represent the earliest time of exposure from the glacier. <Y59 is the control, or refugia plot that was not covered by the glacier.

| | | Y60 Drin | Y60 Luco | Y60 Pala | Y60 Saar | Y60 Saop | <Y59 Cate | <Y59 Drin | <Y59 Luco | <Y59 Pala | <Y59 Saar |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Y60 Cate | 1-h | 0.1 | 0.08 | 0.17 | 0.21 | 0.19 | 0.37 | 0.11 | 0.17 | 0.03 | 0.25 |
| | 4-h | 0.09 | 0.14 | 0.35 | 0.07 | 0.22 | 0.18 | 0.09 | 0.11 | 0 | 0.12 |
| | 1-a | 0.03 | 0.1 | 0.15 | 0.03 | 0.18 | 0.29 | 0.07 | 0.09 | 0.08 | 0.04 |
| | 4-a | 0.03 | 0.19 | 0.18 | 0.19 | 0.25 | 0.21 | 0.05 | 0.15 | 0 | 0.21 |
| <Y59 Cate | 1-h | | | | | | | 0.21 | 0.3 | 0.11 | 0.2 |
| | 4-h | | | | | | | 0.2 | 0.17 | 0.04 | 0.26 |
| | 1-a | | | | | | | 0.23 | 0.21 | 0.08 | 0.14 |
| | 4-a | | | | | | | 0.07 | 0.28 | 0.03 | 0.11 |

Table A4.2 Sorensen's Quantitative Index, 1-h = ITS 1-*Hin*fI; 4-h=ITS 4-*Hin*fI; 1-a=ITS 1-*Alu*I; 4-a=ITS 4-*Alu*I (cont'd). Number preceded by 'Y' represent the earliest time of exposure from the glacier. <Y59 is the control, or refugia plot that was not covered by the glacier.

| | | Y60 Luco | Y60 Pala | Y60 Saar | Y60 Saop | <Y59 Cate | <Y59 Drin | <Y59 Luco | <Y59 Pala | <Y59 Saar |
|---|---|---|---|---|---|---|---|---|---|---|
| Y60 Drin | 1-h | 0.12 | 0.13 | 0.27 | 0.39 | 0.09 | 0.13 | 0.17 | 0.06 | 0.16 |
| | 4-h | 0.24 | 0.1 | 0.47 | 0.13 | 0.25 | 0.16 | 0.17 | 0 | 0.38 |
| | 1-a | 0.12 | 0.07 | 0.38 | 0.15 | 0.1 | 0.1 | 0.24 | 0 | 0.25 |
| | 4-a | 0.1 | 0.07 | 0.32 | 0.19 | 0.06 | 0.18 | 0.12 | 0 | 0.22 |
| <Y59 Drin | 1-h | | | | | | | 0.23 | 0.03 | 0.21 |
| | 4-h | | | | | | | 0.2 | 0.16 | 0.18 |
| | 1-a | | | | | | | 0.15 | 0.02 | 0.11 |
| | 4-a | | | | | | | 0.16 | 0 | 0.25 |

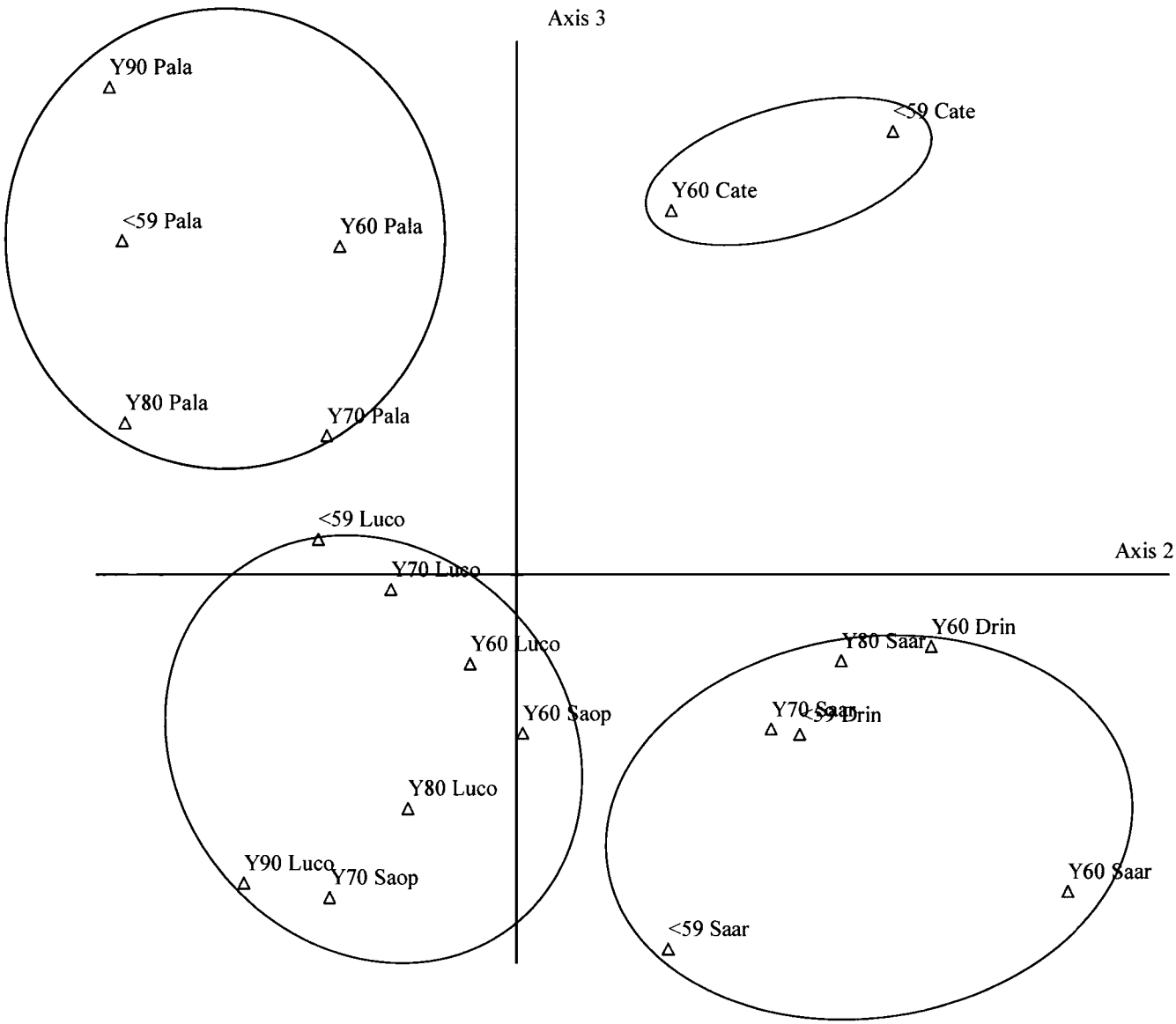
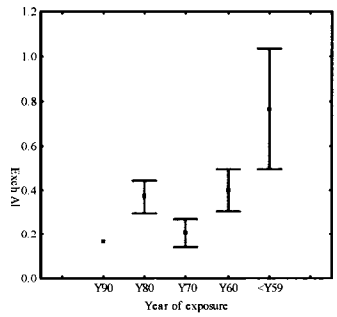
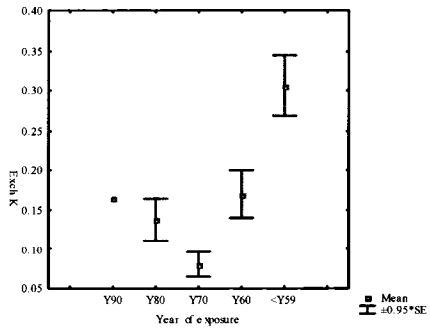



Fig. A4.1 NMS of host plant and chronosequence zones. Rotated at 315°. Number preceded by 'Y' represent the earliest time of exposure from the glacier. <Y59 is the control, or refugia plot that was not covered by the glacier. Plants are: Cate=*Cassiope tetragona*, Drin = *Dryas integrifolia*, Luco=*Luzula confusa*, Pala=*Papaver lapponicum*, Saar=*Salix arctica*, Saop=*Saxifraga oppositifolia.*

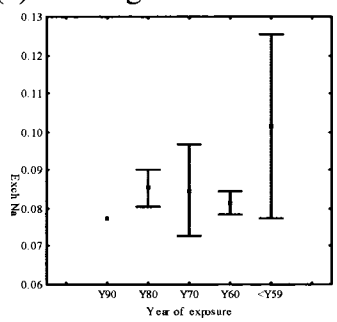
(a) Exchangeable Al



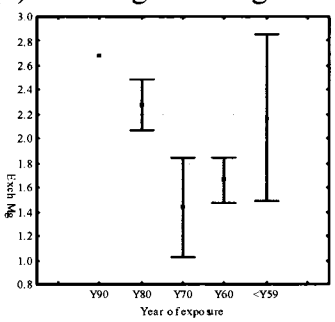

(b) Exchangeable K



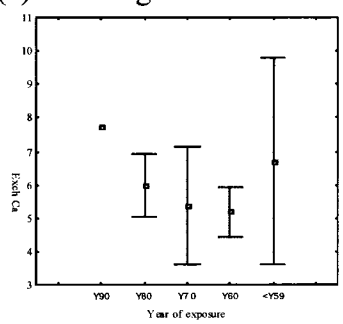

(c)Exchangeable Na



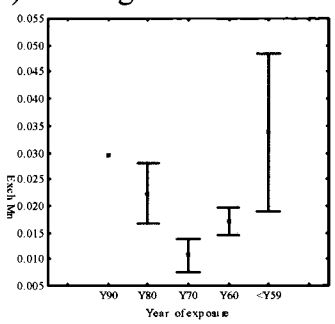

(d) Exchangeable Mg



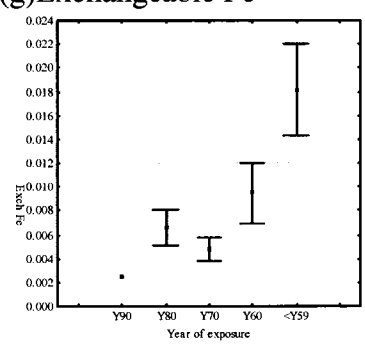

(e) Exchangeable Ca




(f)Exchangeable Mn




(g)Exchangeable Fe




(h)pH

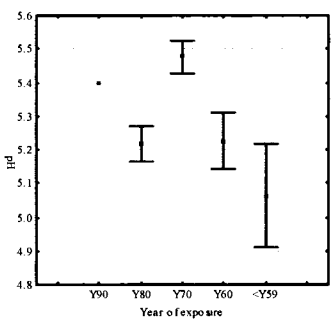
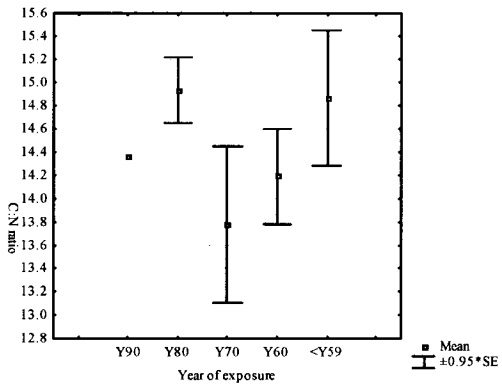
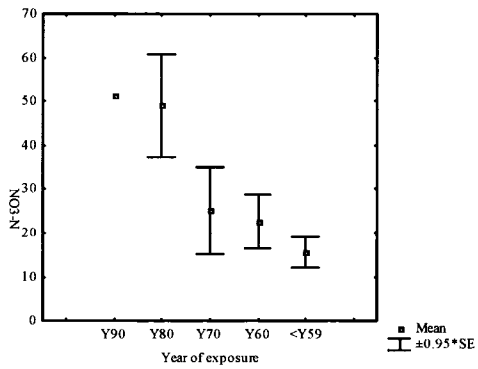



Fig A4.2 LS means of soil property for each chronosequence zone. Error bars designate 1 SE. Number preceded by 'Y' represent the earliest time of exposure from the glacier. <Y59 is the control, or refugia plot that was not covered by the glacier.

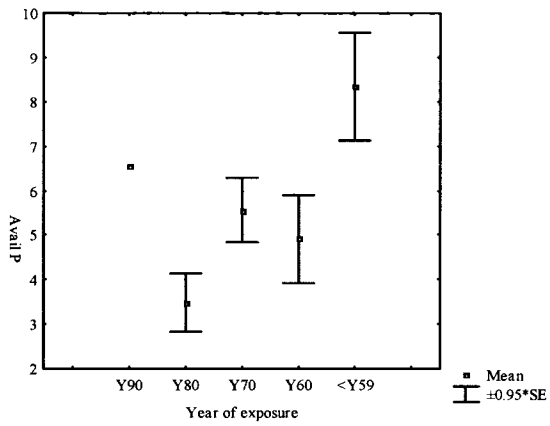
(i) C:N ratio



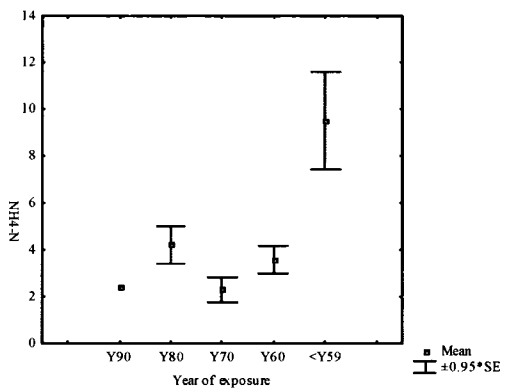

(j) $NO_3$-N



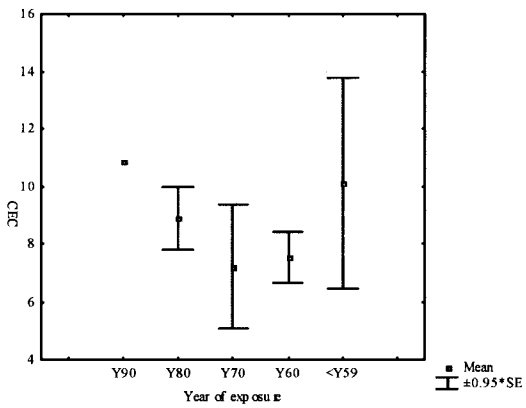

(k) Available P




(l) $NH_4$-N




(m) CEC




(n) Total N

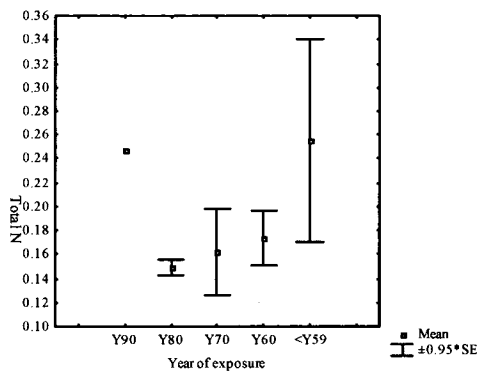



Fig A4.2 LS means of soil property for each chronosequence zone (cont'd). Number preceded by 'Y' represent the earliest time of exposure from the glacier. <Y59 is the control, or refugia plot that was not covered by the glacier.

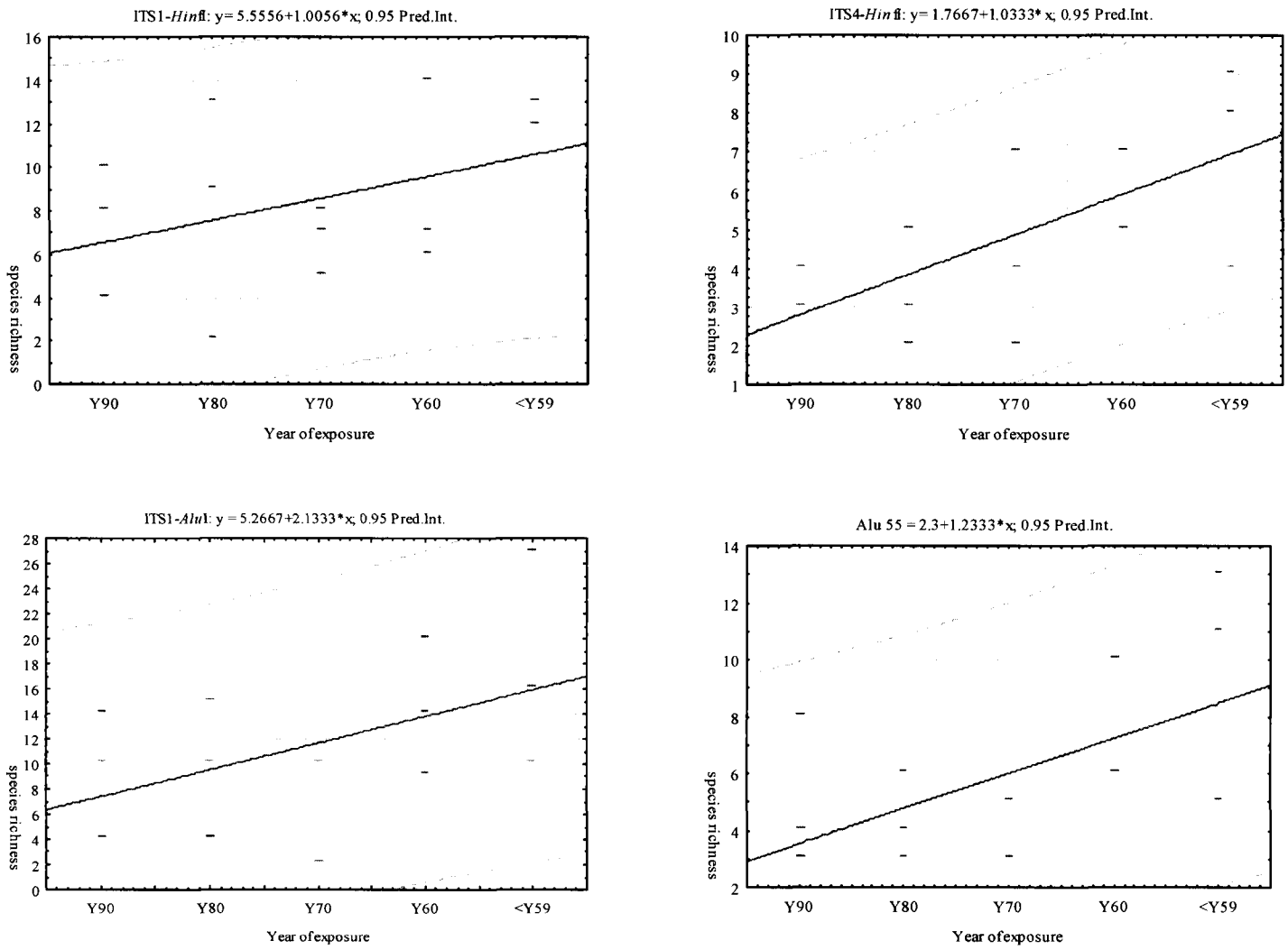



Fig. A4.3 Simple regression for genotype richness for *Luzula confusa*. Number preceded by 'Y' represent the earliest time of exposure from the glacier. <Y59 is the control, or refugia plot that was not covered by the glacier.

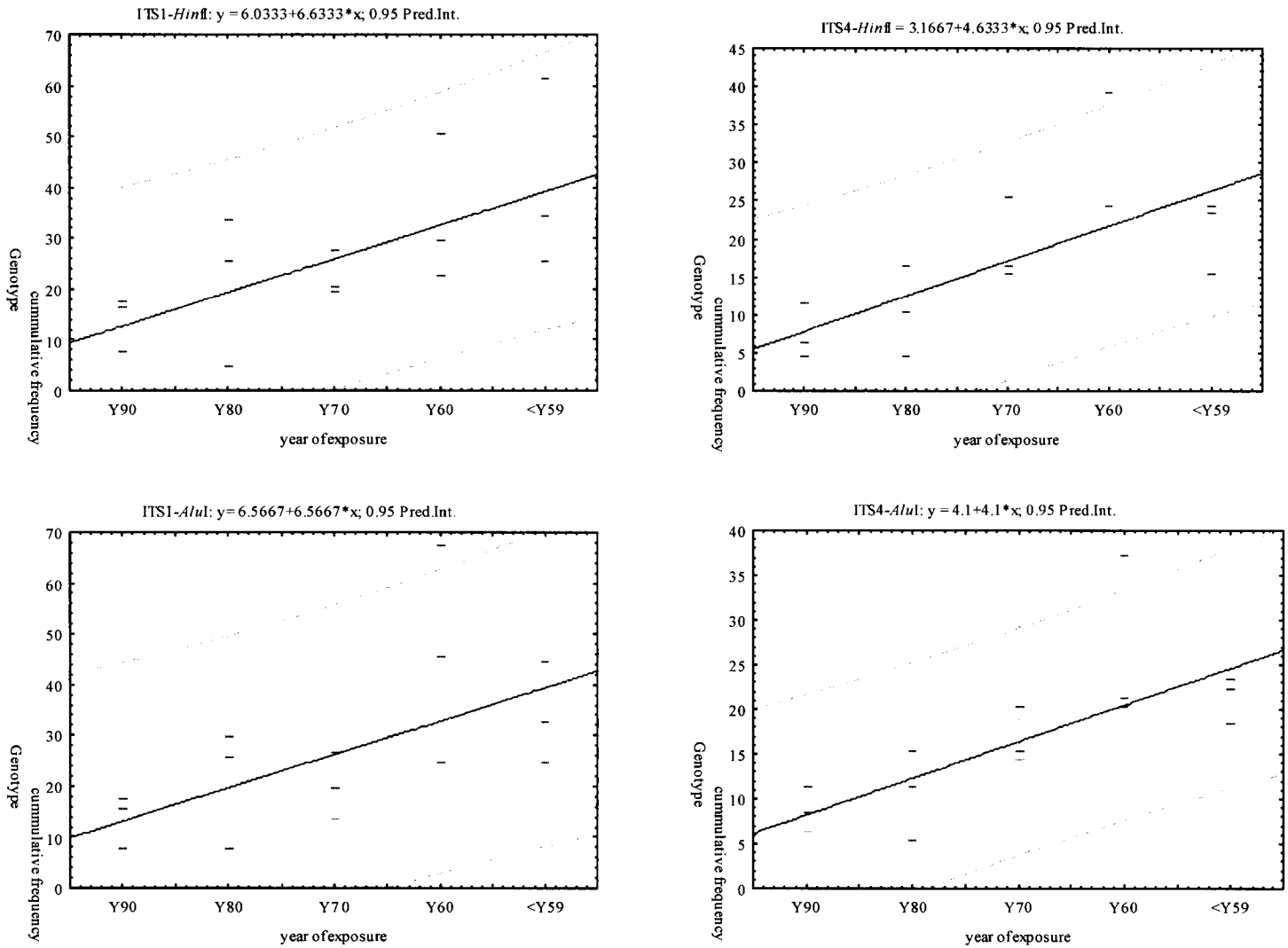
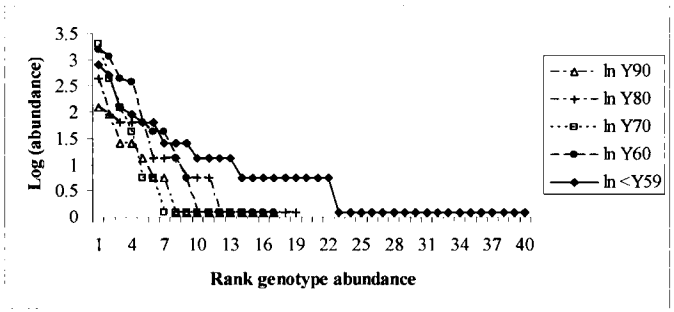
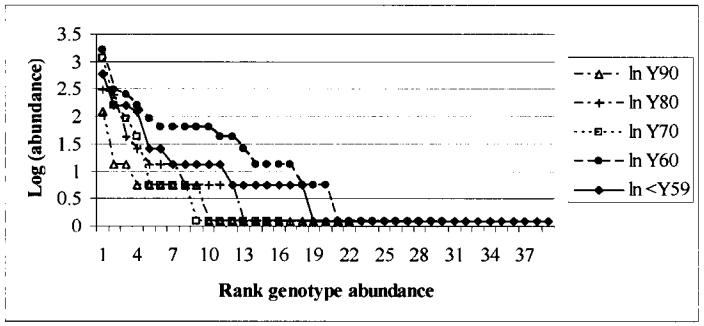



Fig. A4.4 Simple regression of genotype cumulative frequency of *Luzula confusa*. Number preceded by 'Y' represent the earliest time of exposure from the glacier. <Y59 is the control, or refugia plot that was not covered by the glacier.

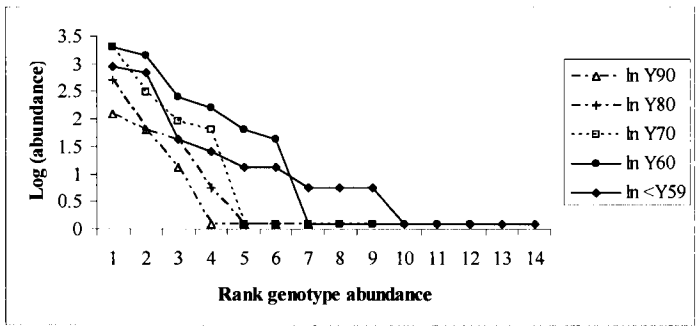
(a) ITS 1-*Hin*fI




(b) ITS 1-*Alu*I




(c) ITS 4-*Hin*fI




(d) ITS 4-*Alu*I

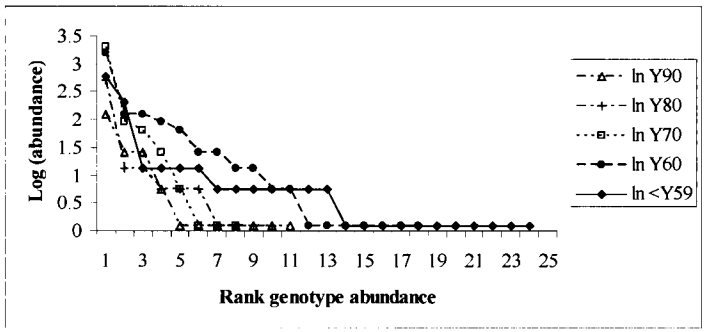
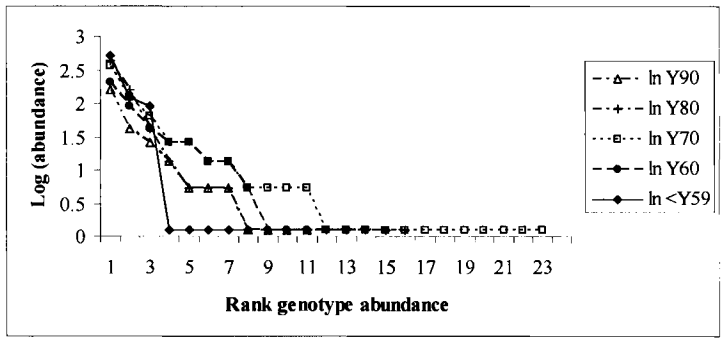
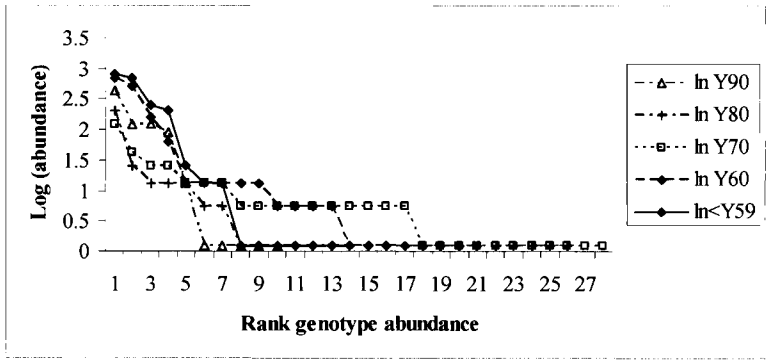



Fig. A4.5 Rank abundance curve for each chronosequence zone for *Luzula confusa.*

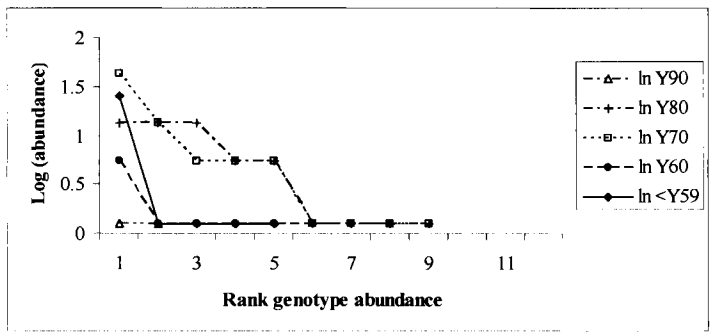
(a) ITS 1-*Hin*fI




(b) ITS 1-*Alu*I




(c) ITS 4-*Hin*fI




(d) ITS 4-*Alu*I

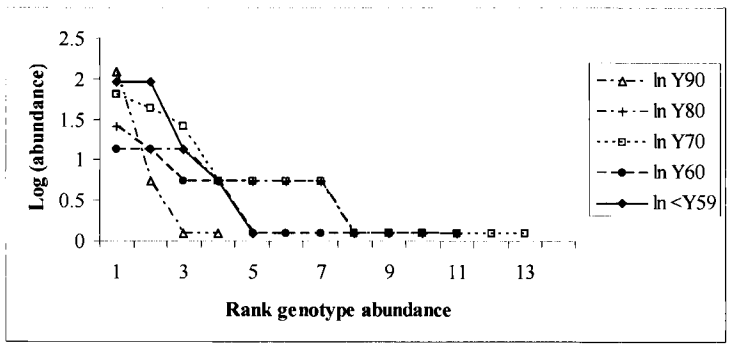
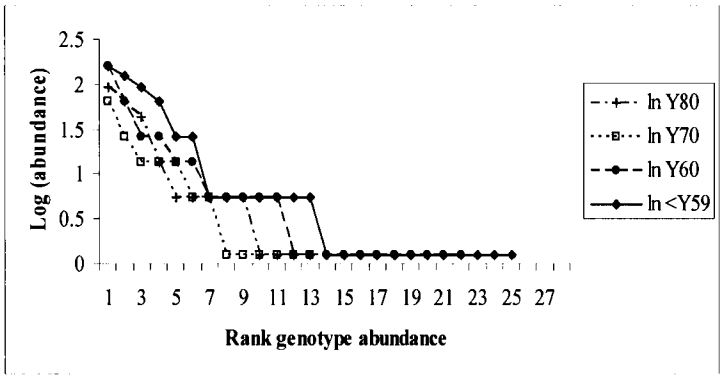
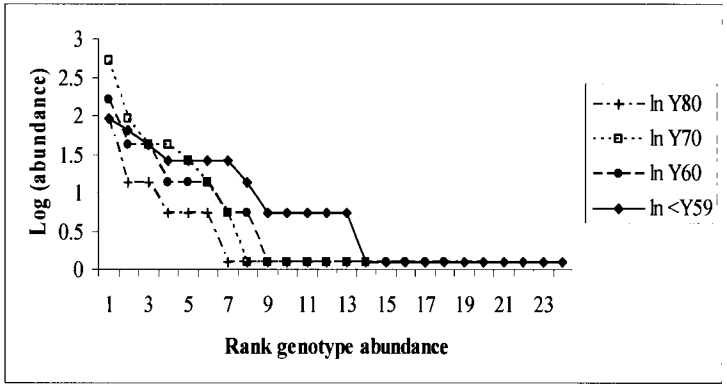



Fig. A4.6 Rank abundance curve for each chronosequence zone for *Papaver lapponicum.*.

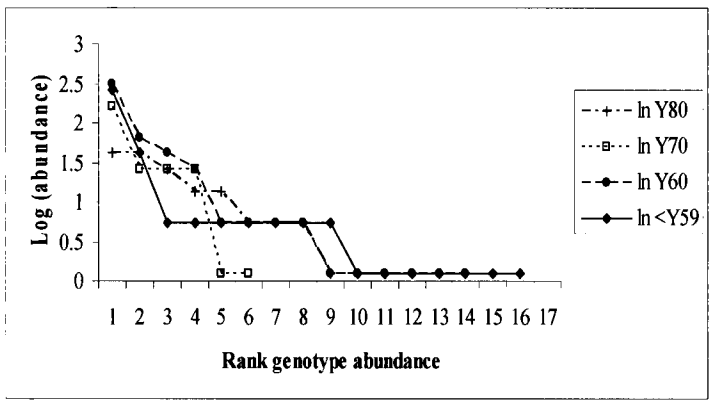
(a) ITS 1-*Hin*fI




(b) ITS 1-*Alu*I




(c) ITS 4-*Hin*fI




(d) ITS 4-*Alu*I

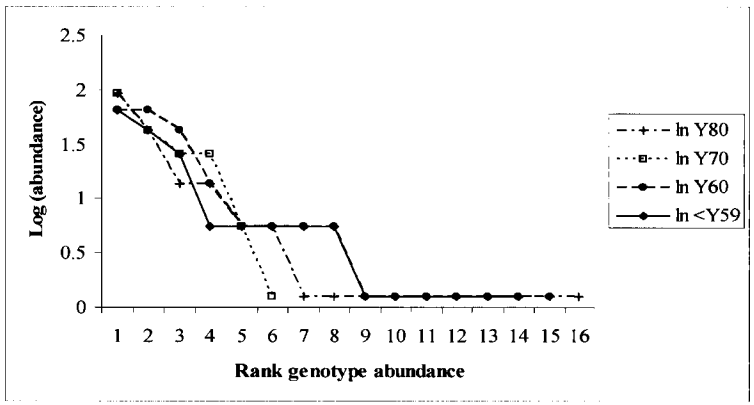
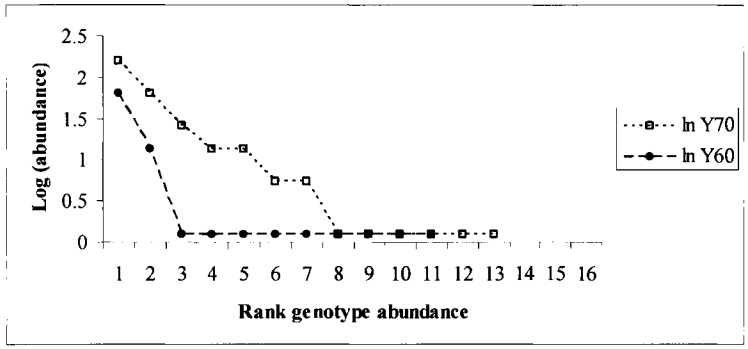
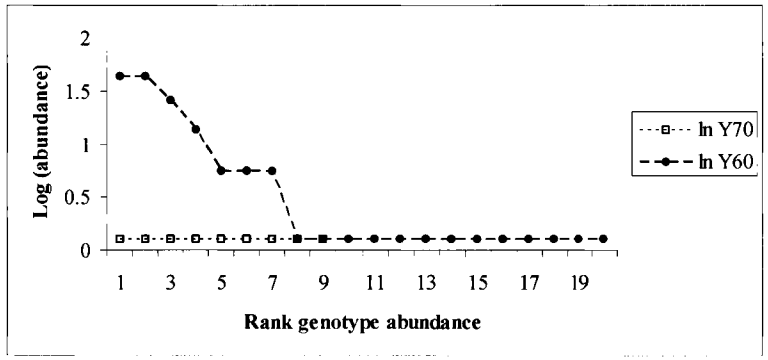
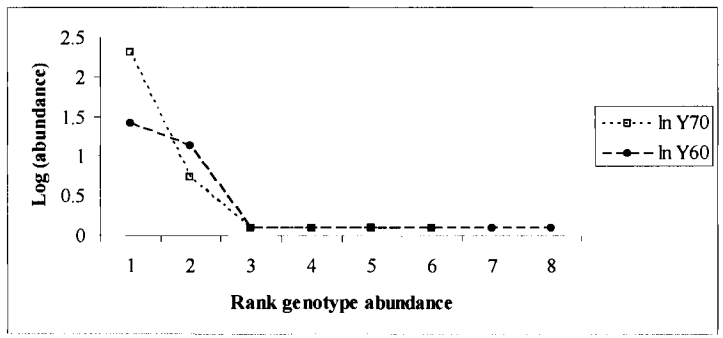



Fig. A4.7 Rank abundance curve for each chronosequence zone for *Salix arctica*..
(a) ITS 1-*Hin*fI (b) ITS 1-*Alu*I




(c) ITS 4-*Hin*fI




(d) ITS 4-*Alu*I

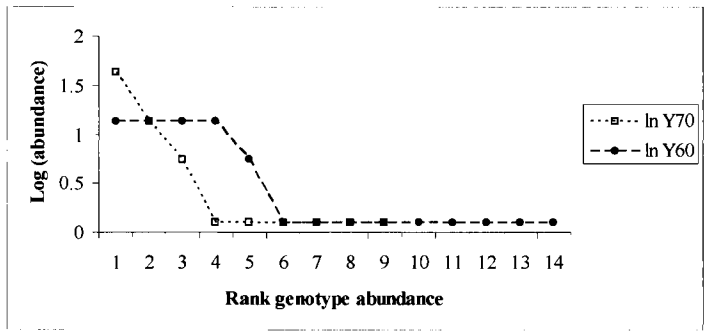
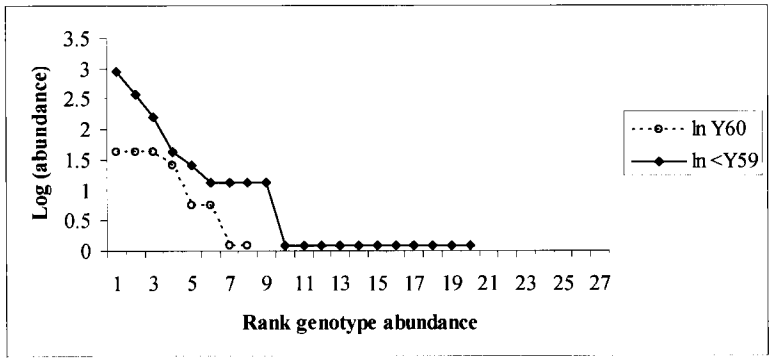
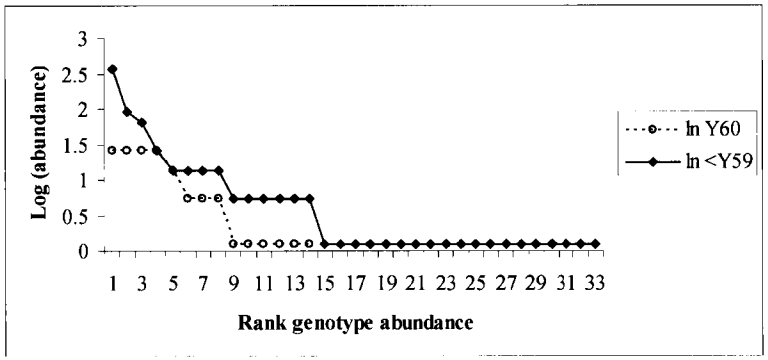



Fig. A4.8 Rank abundance curve for each chronosequence zone for *Saxifraga oppositifolia*

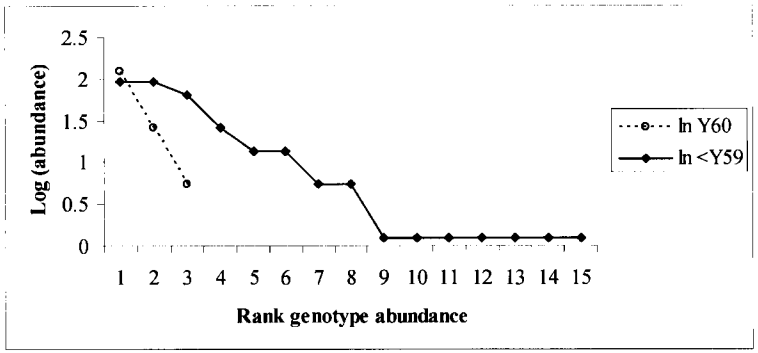
(a) ITS 1-HinfI




(b) ITS 1-*Alu*I




(c) ITS 4-*Hin*fI




(d) ITS 4-*Alu*I

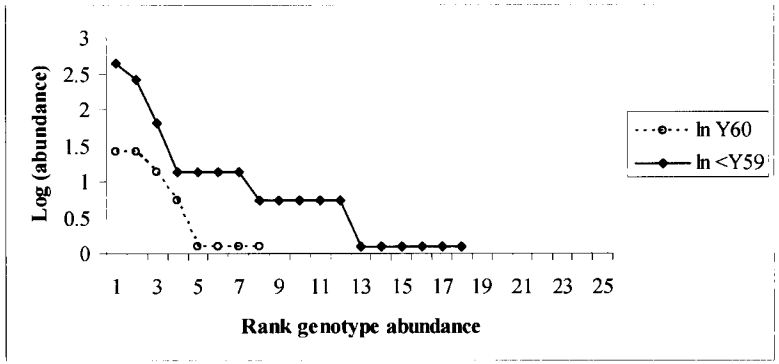
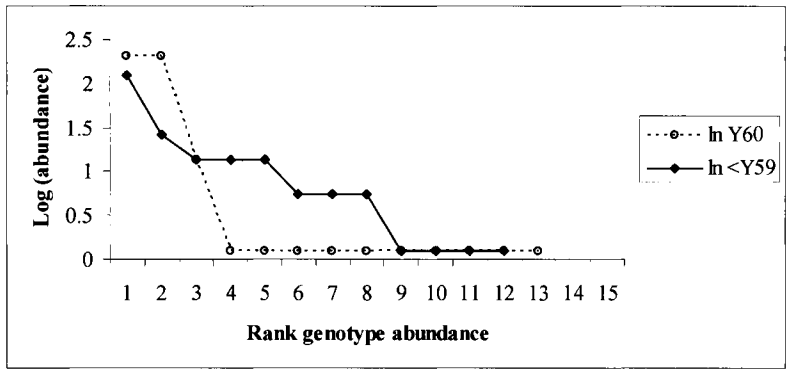
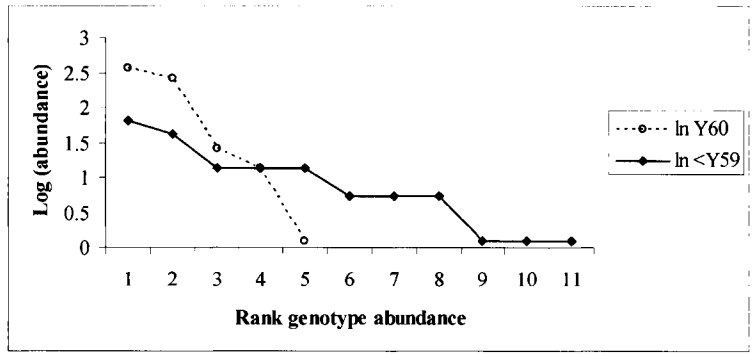



Fig. A4.9 Rank abundance curve for each chronosequence zone for *Cassiope tetragona*

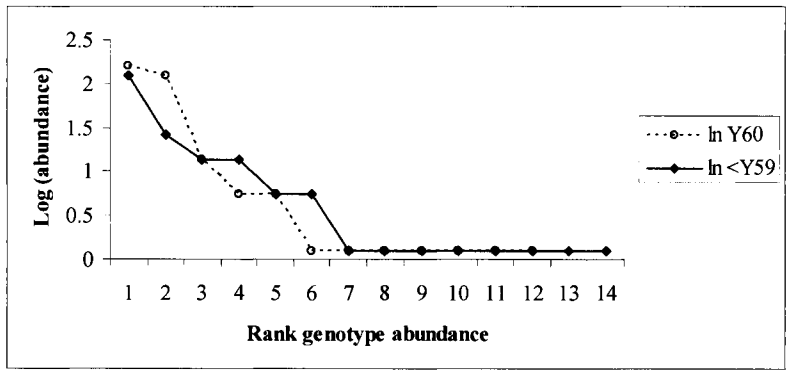
(a) ITS 1-*Hin*fI




(b) ITS 1-*Alu*I




(c) ITS 4-*Hin*fI




(d) ITS 4-*Alu*I

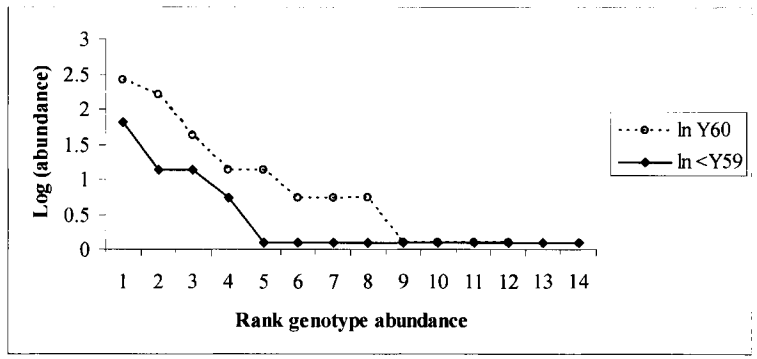



Fig. A4.10 Rank abundance curve for each chronosequence zone for *Dryas integrifolia.*

## X. Appendix for chapter 5

Table A5.1 Comparison of LS means of soil chemistry between succession study (control plot only) and warming studies (T-RFLP-OTC and culture studies)

| | Succession site | Lowland | | Dolomitic | | Granitic | |
|---|---|---|---|---|---|---|---|
| Soil chemistry | <Y59 | Control | OTC | Control | OTC | Control | OTC |
| Al | 0.90 | 0.055 | 0.040 | <0.010 | <0.010 | 0.055 | 0.010 |
| K | 0.28 | 0.20 | 0.18 | 0.075 | 0.075 | 0.10 | 0.12 |
| Na | 0.76 | 0.070 | 0.045 | 0.040 | 0.045 | 0.080 | 0.060 |
| Mg | 1.45 | 2.20 | 2.00 | 3.50 | 3.00 | 3.40 | 4.00 |
| Ca | 3.50 | 4.00 | 3.80 | 8.00 | 6.00 | 4.00 | 4.20 |
| Mn | 0.019 | 0.025 | 0.023 | 0.0010 | 0.0010 | 0.20 | 0.018 |
| Fe | 0.019 | 0.0060 | 0.0020 | 0.0018 | 0.0018 | 0.0022 | 0.020 |
| pH | 5.6 | 6.0 | 6.0 | 8.0 | 8.0 | 6.0 | 6.0 |
| C:N ratio | 14.5 | 20 | 20 | 100 | 140 | 20 | 20 |
| $NO_3$ | 18 | 50 | 40 | 10 | 10 | 40 | 35 |
| P | 7.5 | 4.0 | 4.0 | 0.5 | Negligible | 2.1 | 1.9 |
| $NH_4$ | 9.0 | 1.8 | 1.0 | 1.0 | 0.90 | 6.5 | 3.5 |
| CEC | 6.3 | 7.0 | 6.0 | 11 | 10 | 8.0 | 9.0 |