



**ECM fungi along a geothermal temperature  
elevation gradient in a *Picea sitchensis*  
forest stand in Iceland**

Ella Thoen



**Faculty of Life and Environmental Sciences  
University of Iceland  
2011**

# **ECM fungi along a geothermal temperature elevation gradient in a *Picea sitchensis* forest stand in Iceland**

Ella Thoen

12 ECTS report  
LÍF 520G Research Project in Biology

Research supervisor  
Dr. Edda Sigurdís Oddsdóttir

University supervisor  
Prof. Kesara Anamthawat-Jónsson

Faculty of Life and Environmental Sciences  
School of Engineering and Natural Sciences  
University of Iceland  
Reykjavik, 2011

## Abstract

In Iceland, afforestation activity has increased during the last decades and native downy birch, along with exotic species like Sitka spruce (*Picea sitchensis*) has been planted in the afforestation efforts. Studies of ectomycorrhiza (ECM) are becoming more important because of the ECM's benefits to seedling survival and tree growth by increased water and nutrition uptake. Although several studies on ECM have been conducted, both worldwide and in Iceland, we still know surprisingly little about ECM community structure and the effects of increasing soil temperature following the climate change.

After earthquakes in 2008, new hot springs appeared and old ones were awoken in Reykir , Iceland, providing us with a unique study site: a Sitka spruce stand in a geothermally active area. This is the pilot study of ECM fungi in this particular geothermally active area and part of the ForHot project.

Samples were taken in October/November 2011 along a temperature elevation gradient. The samples were then examined using a dissection microscope and grouped into morphotypes based on visual characteristics. Abundance of ECM root tips was calculated, along with the distribution of different morphotypes along transects. Diversity indices and relative proportion of morphotypes in different temperature elevation ranges were also calculated.

A slight, but significant decline in morphotype diversity, abundance of ECM root tips and number of morphotypes were found along the temperature elevation gradient. The findings in this research may indicate a decline in ECM towards higher temperature elevations, but small sample size and the lack of determination of species proposes uncertainties to the results. Further investigation of ECM fungi at this particular site is needed to support findings presented in this thesis.

## Útdráttur

Á Íslandi hefur skógrækt aukist á síðustu áratugum og íslenskt birki, ásamt innfluttum tegundum eins og Sitka greni (*Picea sitchensis*) hefur verið gróðursett í viðleitni til að græða landið skógi. Rannsóknir á svepprót (ECM) eru mikilvægar vegna áhrifa svepprótar á lifun unglantna og vöxt trjáa með aukinni upptöku vatns og næringarefna úr jarðvegi. Þrátt fyrir nokkrar rannsóknir á svepprót er furðu lítið vitað um svepprótarsamfélag uppbyggingu og áhrif aukins jarðvegshita.

Eftir jarðskjálfta árið 2008, birtust nýjir hverir, auk þess sem eldri vöknudu til lífsins í sitkagreniskógi að Reykjum, Ölfusi. Þetta skapaði einstakt tækifæri til rannsókna á áhrifum hækkandi jarðvegshita á skóglendi. Í þessari ritgerð er greint frá rannsókn á svepprótarsveppum og áhrifum hækkandi jarðvegshita á þéttleika og fjölbreytni.

Jarðvegssýni voru tekin í október / nóvember 2011 eftir sniðum þar sem hitastig hækkaði jafnt og þétt. Sýnin voru síðan skoðuð í víðsjá og flokkaðar eftir útliti. Fjöldi svepprótarenda var reiknaður, ásamt fjölda mismunandi útlitsgerða í mismunandi jarðvegshita. Enn fremur. Fjölbreytileikastuðlar í mismunandi hitastigi voru einnig reiknaðir.

Lítill, en marktæk, lækkun á fjölbreytileika stuðlum, fjölda svepprótarenda og fjölda útlitsgerða, reyndist með hækkandi jarðvegshita. Niðurstöður í þessari rannsókn benda til þess að hækkandi jarðvegshiti hafi neikvæð áhrif á svepprót en þó verður að taka tillit til þess að þessi rannsókn var frekar lítil í sniðum og frekari rannsókn á svepprót er þörf til að styðja niðurstöður sem kynntar er í þessari ritgerð.



# Table of Contents

List of Figures .....	viii
List of Tables .....	ix
Definitions and abbreviations .....	x
Acknowledgements.....	xi
<b>1 Introduction .....</b>	<b>1</b>
1.1 History.....	1
1.2 Evolutionary and ecological aspects – a brief introduction to mycorrhiza .....	2
1.3 The Ectomycorrhizal Symbiosis .....	3
1.4 Forestry and Ectomycorrhizal Studies in Iceland.....	4
1.4.1 Planted tree species in Icelandic forestry.....	4
1.4.2 ECM in forestry .....	4
1.4.3 ECM studies conducted in Iceland .....	4
1.5 ECM gradients and heat tolerance .....	6
1.5.1 Root associated fungi along different gradients .....	6
1.5.2 Geothermal Mycorrhiza and Heat Tolerance in ECM Fungi.....	7
<b>2 Objectives .....</b>	<b>10</b>
<b>3 Methods .....</b>	<b>11</b>
3.1 Field description.....	11
3.2 Mapping the area.....	14
3.3 Sampling .....	15
3.4 Examination of samples .....	15
3.5 Data analysis .....	16
3.5.1 Morphotypes .....	16
3.5.2 Statistical analysis.....	18
<b>4 Results.....</b>	<b>20</b>
4.1 Diversity Indexes.....	20
4.2 Abundance of ECM morphotypes and root tips.....	22
23	
4.2.1 Distribution of morphotypes along T-elevation gradient.....	24
4.2.2 ECM root tip survival and temperature .....	24
<b>5 Discussion .....</b>	<b>26</b>
5.1 Diversity Indices .....	26
5.2 Abundance of morphotypes, ECM root tips and survival.....	26
5.3 Distribution of morphotypes along the T-elevation gradient.....	27

5.4 Further considerations .....	28
<b>6 Conclusion .....</b>	<b>28</b>
<b>References .....</b>	<b>29</b>

# List of Figures

<i>Figure 1: Map of Hveragerði and ForHot research site.</i> .....	11
<i>Figure 2: Dead trees at T-elevations higher than +35°C in the Sitka spruce forest.</i> .....	12
<i>Figure 3: Pictures from the study area</i> .....	13
<i>Figure 3: Map of soil temperature elevation</i> .....	14
<i>Figure 4: Pictures of morphotypes 1-17</i> .....	17
<i>Figure 6: Shannon Diversity Index s.</i> .....	20
<i>Figure 7: Simpson´s Index of Diversity).</i> .....	21
<i>Figure 8: Total number of ECM root tips</i> .....	23
<i>Figure 9: Number of morphotypes).</i> .....	23
<i>Figure 10: Stacked vertical bar of morphotype abundance in different temperature ranges...</i>	24

# List of Tables

<i>Table 1: Number of samples examined .</i>	15
<i>Table 2: Description of morphotypes</i>	16
<i>Table 3: List of data used in SigmaPlot®</i>	19
<i>Table 3: Number of morphotypes and total number of ECM root tips</i>	22
<i>Table 5: Average number of root tips counted</i>	25

# Definitions and abbreviations

**Mycorrhiza:** symbiotic association between plant roots and certain fungi, in which a sheet of fungal tissue (the mantle) encloses the smallest rootlets\*

**AM:** Arbuscular mycorrhiza

**C:** carbon

**ECM:** Ectomycorrhiza

**EDM:** Endomycorrhiza, which includes AM

**ERM:** Ericoid mycorrhiza

**Extraradical mycelium:** hyphal growth from the ECM root tip into the soil

**Hypha:** the basic growth form of the vegetative phase of fungus\*

**LD:** lateral distance

**Mantle:** Hyphae sheath covering the smallest rootlets\*

**N:** Nitrogen

**P:** Phosphorous

**Rhizomorphs:** a root-like structure formed from interwoven hyphae of some basidiomycete fungi\*

**VD:** vertical distance

**T-elevation:** temperature elevation in °C

\*Henderson's Dictionary of Biology, 14th edition (Editor: Lawrence, 2008)

# Acknowledgements

## Acknowledgements

The research is conducted at Skógrækt ríksins at Móglisá, and a research project under the supervision of the University of Iceland and part of the ForHot project in Reykir, Hveragerði. I would like to thank Prof. Kesara Anamthawat-Jónsson for a warm welcome to Iceland, Dr. Edda S. Oddsdóttir for her supervision and in my studies and research here, and Dr. Tor Carlsen for his support and for teaching me washing procedures of ECM roots. I would also like to thank Prof. Bjarni D. Sigurdsson and Ass. Prof. Úlfur Óskarsson at the Icelandic Agricultural University for help with mapping the area, sampling and temperature measurements.



# 1 Introduction

As efforts are being made to afforest Iceland, studies of the ectomycorrhizal (ECM) symbiosis are becoming more important in Iceland. Also effects of heating of soil on ECM is important considering global warming. The ECM symbiosis is essential in achieving optimal growth and seedling survival of planted tree seedlings (Smith and Read, 2008). Earthquakes in the south of Iceland in 2008 awoke old and new hot spots, and provided us with a unique (2011) study area: Geothermal heating in an approximately 40 year old Sitka spruce forest. This thesis will provide some background knowledge on ECM and the effects of increasing soil temperature on ECM density and diversity. It is important to mention, however, that this is the pilot ECM study in this particular subject, and findings in this report need further investigation, as we were limited by time and fundings. Nevertheless, some useful information on ECM in a geothermally heated Icelandic forest, have been found.

## 1.1 History

Mycorrhiza is the term describing the symbiosis between plant roots and fungi, first used by Frank in 1885 (Frank, 2004) where he describes the symbiosis between fungi and tree roots, suggesting that the fungi receives carbohydrates from the tree roots, and in return supplies the trees with water and mineral nutrition. He describes how the hyphae of the fungi covers the root tips completely and changes the morphology of the root tips from long and thin, when grown in a fungi-free environment, to short and thicker structures when covered by the hyphae mantle in fungi environments. The symbiosis he described is now known as ectomycorrhiza.

Only years before Frank, Kamienski described in 1882 a “glove” of fungi surrounding the roots of *Monotropa hypotitys* (Kamienski, 1982), cited in Berch *et al.*, 2004), and was the first to suggest a close relationship between fungi and plant rootlets. The *Monotropa* is actually parasitizing fungi species that are in symbiosis with other ectomycorrhizal plants (Leake *et al.*, 2004), and is therefore included in the mycorrhiza concept. Together, Frank and Kamienski, were the first to suggest and describe this symbiosis.

Since Frank’s description of ECM in 1885, many reports and investigations on mycorrhiza have been conducted (Tedersoo *et al.*, 2010), but we still know surprisingly little about community structure and the distribution of mycorrhiza in forest systems (Leake, *et al.*, 2004, Lilleskov *et al.*, 2004).

## 1.2 Evolutionary and ecological aspects – a brief introduction to mycorrhiza

As reviewed by Cairney (2000) arbuscular mycorrhiza (AM) arose concurrently with plants colonization of land over 450 million years ago, and ectomycorrhiza approximately 200 million years ago, which gives the mycorrhiza a long evolutionary history on land. EMC evolved as the organic matter in the soil became more abundant, probably because of plant litter and such, and ericoid mycorrhiza evolved later, about 100 million years ago. It is hypothesized that mycorrhiza was an absolute necessity in plants conquer of the land, and might therefore be considered one of the most ecological important symbiosis that we know (Smith and Read, 2008).

Mycorrhiza is often grouped into arbuscular mycorrhiza (endomycorrhiza), ectomycorrhiza and ericoid-, orchid or mycoheterotrophic mycorrhiza (Smith and Read, 2008), and under follows a brief introduction of the three first mentioned. The rest of this thesis will be based around the symbiosis of ectomycorrhiza.

*Arbuscular mycorrhiza* (AM) is symbiotic with most angiosperms, some gymnosperms, pteridophytes and some lower plants. It is recognized by branched structures (arbuscles) inside the cell wall but outside the membrane of cortical cells. It designated endomycorrhiza (“inside-fungi-root”), because it breaches the cell wall, but not the cell membrane (Smith and Read, 2008). Hyphae grow extensively from the root surface and into the soil, thereby increasing the area for nutrition uptake (Cairney, 2000).

*Ectomycorrhiza* (ECM) is hosted by most woody trees and shrubs, and some herbaceous plants. ECM includes fungi first and foremost from Basidiomycota, but also Ascomycota and Zygomycota. (Cairney, 2000, Smith and Read, 2008) The fungal hyphae cover the rootlets in what is known as the mantle, and a highly branched net of hyphae penetrates the rootlets outside of the epidermal and/or cortical cell walls in what is known as a Hartig net, by its discoverer Robert Hartig. It is here the nutrition exchange between the plant and fungi occur. The growth of the hyphae into the surrounding soils is very extensive, thereby increasing the nutrition uptake surface area. The fungi increases the uptake of N and P by different processes, like excreting proteases and phosphatases or plant wall-degrading enzymes that facilitate uptake of N and P by the plant (Cairney, 2000).

*Ericoid mycorrhiza* (ERM) affiliates with plants in the order Ericales, and all fungi involved are Ascomycetes. Extensive growth of coils of hyphae is found in the epidermal cells, but there is little growth of hyphae from the root tips into the soil. The fungi secrete proteases and phosphatases, or secrete plant cell wall-degrading enzymes or phenol-oxidizing enzymes that make the N and P in the soil more accessible (Cairney 2000, Smith and Read, 2008).

In all terrestrial habitats, mycorrhizal symbiosis are prevalent, but it seems that in many P rich ecosystems, AM have an advantage over ECM, and has fascinated many ecologist on the basis that these systems are often very rich in plant species (Smith and Read, 2008). Perhaps this is the reason why AM has been more frequently studied than ECM, or perhaps

because it is more common. Approximately 90% of all plant species associates with AM, whilst ECM fungi only associate with about 10% of plant species (Cairney, 2000).

### 1.3 The Ectomycorrhizal Symbiosis

The ectomycorrhiza (ECM) root is characterized by having three structural components: a mantle of fungal tissue around the root, a Hartig net between the epidermal and cortical cells of the plant root tips, and hyphaeal growth into the soil, called the extraradical mycelium. Although these three structures are always present to some extent, there may be considerable variation in how they develop.

Because of the particular morphology of the ECM root tips, it is easily distinguished from non-ECM root tips. The root tips become shorter, thicker, and the extraradical mycelium of the fungal hyphae is visible through a dissection microscope. Color, shape and +/- rhizomorphs along with microscopy of the hyphaeal surface, makes it possible to distinguish between known species of ECM fungi (Agerer, 1997)

In the association between the plant roots and ECM fungi, the fungi are provided with carbohydrates from the plants photosynthesis. In return the fungi better the nutrition (in particular N and P) and water uptake of the plant, making the symbiosis mutually beneficial – a mutualism (Smith and Read, 2008).

Already in 1894, Frank experimented with the growth of *Pinus* seedlings in sterilized and non-sterilized soil, and discovered that those in unsterilized soil grew faster than those in sterilized soil (Smith and Read, 2008; Frank, 1894). Although this experiment is faulty, since other elements than the fungi will be affected in soil sterilization, he did indeed show what has later been confirmed by other studies: ECM fungi is often important in seedling survival (e.g. Oddsdóttir, 2010, Kipfer, 2009, Smith and Read 2008, Izzo, *et al.*, 2006).

Taxonomically ECM fungi are distributed through many families of wooden trees and shrubs (Smith and Read, 2008). The family Pinaceae is included here, and is of particular interest to this study, because it includes Sitka spruce (*Picea sitchensis*, Pinaceae), and our study site is a forest of this particular species only.

Most studies on ECM have been conducted on the symbiosis with trees (e.g. Oddsdóttir, 2010, Kipfer, 2010, Smith and Read 2008, Natel and Neumann, 1992), but recently studies of community structure in the herbaceous plant *Bistorta vivipara* have been conducted (Kausrud *et al.* 2011). The condensed root system makes it easy to dig up, and next generation sequencing makes mapping of the fungal community of this particular plant more accessible. In some studies it has been used as a model organism for studies of ECM (Kausrud *et al.*, 2011). This may indeed be a step towards understanding community structure of ECM fungi.

What we know about ECM evolutionary history is based upon evidence derived from fossils, from our understanding of geographic and phylogenetic origin of plants forming this symbiosis and molecular clock data (Smith and Read, 2008). The fossil record is limited, and therefore our knowledge about the origin. However, looking at this evidence

along with fossil records of plant species, it puts the origin of ECM very close to that of all flowering plants (Willis and McElwain, 2002, cited in Smith and Read, 2008).

## **1.4 Forestry and Ectomycorrhizal Studies in Iceland**

Efforts to afforest Iceland makes the studies of ECM important, in that the symbiosis facilitates the uptake of water and nutrition by the trees, and thereby increasing the growth (Smith and Read, 2008). It is estimated that only 1% of Iceland is now covered by native forest in contrast to the estimated original 27% forest cover (Sigurðsson, 1977, Snorrason *et al.*, 2007) cited in Traustason and Snorrason, 2008) and efforts of afforestation are made by programs like Land Reclamation forest and the Regional Afforestation program (Eggertsson *et al.*, 2008) cited in Oddsdóttir, 2010).

### **1.4.1 Planted tree species in Icelandic forestry**

Birch (*Betula pubescens*) is native to Iceland, and comprises approximately 30% of all planted seedlings in forestry, but the use of introduced species, like Siberian larch (*Larix sibirica* Ledeb. synom. *Larix sukaczwelli* Dylis.), lodgepole pine (*Pinus contorta* Dougl. ex Loud.) and Sitka spruce (*Picea sitchensis* (Bong.) Carr.) have increased (Oddsdóttir, 2010; Eggertsson *et al.*, 2008). Sitka spruce was first introduced to Iceland in the 1920's and again in the 1950's, and has been planted extensively around the country, showing relatively good growth (Oddsdóttir, 2010).

### **1.4.2 ECM in forestry**

The benefits of ECM in forest systems became clear even with the first experiments (Smith and Read, 2008; Frank, 1894), and are accepted as a natural part of the ecosystem. After timber extraction, ECM fungi survive in the soil and aid in seedling regeneration or plantation. Outplanted seedling can generate ECM connections from any natural occurring ECM, but artificial inoculation has shown to be especially beneficial in plantation of exotic species (Smith and Read, 2008). Also, edible ECM fungi fruit bodies have their financial importance as they are picked for private use or sold in many countries, including Norway (Sopp- og nyttevekster, 2011) and the other Scandinavian countries.

### **1.4.3 ECM studies conducted in Iceland**

A few studies have been conducted on ECM fungi in Iceland, with the perhaps most extensive done by Oddsdóttir (2010), forming part of the work of the Nordic project “*Plant protection by beneficial soil organisms.*” Oddsdóttir looked at both ECM and insect pathogenic fungi (IPF) at different sites in Iceland and their mediation of root herbivore interactions. She found that inoculation with ECM and IPF of birch seedlings reduced root damage intensity in the field when planted in eroded or birch habitat, whilst inoculation when planted in heath land increased root damage. Further, low levels of beneficial soil organisms on eroded sites were indicated as cause of high levels of root damage and seedling mortality. Some fungal species were also recorded for the first time in Iceland.

Oddsdóttir's work takes us a step further in understanding the effects of ECM fungi on afforestation in Iceland.

Enkhtuya *et al* (2003) investigated mycorrhiza and the response of plant species in eroded areas in Iceland. *Leymus areanrius* and *Deschampsia beringensis* were inoculated with AM fungi and planted in volcanic ash in pot experiments. In field experiments *Betula pubescens* and *L. areanrius* were inoculated with both natural soil inoculum and commercial inoculum. Inoculations with AM had positive effect on soil aggregation and indicate an importance in early stabilization of volcanic soil. In field experiments, *B. pubescens* grew better with inoculation of ECM and addition of inorganic NP-fertilization. The results of this study indicate that reclamation of desertified land can benefit from inoculation with appropriate mycorrhizal fungi in the establishment of plants (Enkhtuya *et al*, 2003).

Hrafnkelsdóttir (2009) studied ECM in a chronosequence of *B. pubescens* and *Larix sibirica* in forest sites in East Iceland. She found that ECM diversity was significantly reduced in both *B. pubescens* and *L. sibirica* when grown in soil from treeless heathland compared to forest soils, and that more diversity was found in the native *B. pubescens* forest than in exotic *L. sibirica* forests.

Óskarsson and Halldórsson (2008) looked at the effects of fertilization on ECM colonization and growth of downy birch (*Betula pubescens*) in Iceland. Different amounts of N and P were applied in experimentally designed nutrient rich and nutrient poor Andisols. In this study larger applications of N in a rich site, lead to lower colonization rate of ECM fungi, whilst at the poor site, it lead to almost no ECM colonization at all. This may be because the fungus allocates more C to the process of N assimilation and that the fungus rather than the host modifies its C allocation pattern to suit the N supply situation (Óskarsson and Halldórsson, 2008). Further, they conclude that fast initial growth is important for establishment of seedlings in andisols in Iceland, and that initial fertilization has a positive effect on growth of the seedling, but in large amounts, have a negative effect on ECM colonization.

Eyjolfsdóttir (2004) looked at fungi in larch and birch stands in the East of Iceland, collecting fruit bodies with the aim to get a better overview of the fungal diversity of Iceland. Preliminary studies have been done at Skorradalur in West Iceland as well. From this study, 99 species were identified with 7 new species recorded in the east site, and 42 species in the west site with 3 new species. Since this study was conducted using only fruiting bodies of macrofungi; microfungi and species that did not produce fruiting bodies during this particular period of sampling have been left out of the study. Therefore, one can only estimate the real fungal diversity at this particular site. Fungi in forest ecosystems have wide range of ecological roles; from symbiotic mycorrhiza and endophytes, to saprotrophic and parasitic. Therefore, more studies of Icelandic fungal diversity are needed to both understand the magnitude and ecological effects on fungi in forest systems here.

## 1.5 ECM gradients and heat tolerance

To my knowledge (2011), no studies have been conducted on the effects of heating from below ground on ECM in a forest that previously grew in cooler soils. This makes our study area unique. There has however been conducted several studies on ECM gradients, studies of soil heating and effect on ECM in relation to fire (above-ground heating) and some on mycorrhizas in geothermal active areas. In this chapter a few previous studies on ECM gradients of different types, heat tolerance in relation to forest fires and some of those in geothermal active areas, will be presented for a better understanding of ECM fungi's behavior under different conditions

### 1.5.1 Root associated fungi along different gradients

Blaalid *et al.* (2011) conducted a research on ECM fungi in a postglacial chronosequence at Blåisen, Hardangerjøkullen in Norway, to look at the diversity in a primary succession gradient. The chronosequence is dated by geologists, so certainty about the age since glaciation is well known. In this research the plant *Bistorta vivipara* was used as a model organism for investigation of ECM fungi, Because of the plant's rather small and condensed root system and it's known to have ECM fungal associations, the entire fungal community connected to *B. vivipara* can be sampled and analysed (Kauserud *et al.*, 2011).

Using 454 pyrosequencing of the entire root systems of samples along the gradient, Blaalid *et al.* (2011) found that Basidiomycetes were the dominant fungal group in this particular plant species, but a perhaps more surprising was the increase in richness along the gradient. Diversity was found to be relatively high already in the early stages of succession, close to the glacier, and showed a weak, but significant, increase of total richness from the early stages of succession to the climax vegetation. This differs from most studies on plant richness, where richness is found to be highest in intermediate stages of succession. Blaalid *et al.* (2011) further discuss that this may be due to less competition for micro-organisms where nutrition availability may be higher, even in the climax system.

Other studies of ECM diversity and richness have been conducted using the collection of fruit bodies, like Natel and Neumann (1992). Using a local vegetation gradient, and collecting fruit bodies, they found that basidiomycete ECM symbionts followed a particular host plant species for only a part of an abiotic gradient. ECM symbionts was found in one host plant species in parts of the gradient, but not in the same host species in other parts of the gradient. They further predict that this type of pattern suggest a higher beta diversity of ECM fungus than beta diversity for ECM-forming trees, and the ratio of fungal richness to tree richness would be generally high for most community gradients.

Gehring *et al.* (1998) studied fungal community structure using RFLP of *Pinus edulis* in northern Arizona growing in two environmental extremes. One extreme was cinder soils low in moisture and nutrition, whilst the other was a sandy-loam with higher moisture and nutrition levels. Performance of *Pinus edulis* has previously been shown to be better in sandy-loam environments than in cindy. The ECM communities in the sandy-loam and cindy environments differed in both occurrence and patterns of abundance. Although species composition differed between the different sites, their results indicate that species

richness of ECM fungi remained constant across the extreme habitats. They found that ECM fungi associated with *Pinus edulis* responded to abiotic environmental conditions not only in terms of presumably species association, but also in the major fungal subphyla involved in the ECM symbiosis.

In a boreal forest in Sweden, Toljander *et al.* (2006) investigate an abiotic gradient of nutrition and the ectomycorrhizal distribution, using morphotyping and molecular tools. ITS-primers were used for an ITS-RFLP (restriction fragment length polymorphism) analysis to identify different species. Soil samples were taken and analysed for abiotic factors, such as nutrition composition and pH. Ordination analysis suggested a strong autocorrelation between ECM community structure and soil characteristics. The observed pattern of ECM fungi distribution mirrored the change in both vegetation and soil chemistry, although it is suggested that these patterns may be due to a founder effect. An individual may become established and spread from this point, leading to similarity in nearby forest floors, which may lead to a false assumption of autocorrelation.

From these studies we can make some assumptions as to what can be expected to find in ECM community structure in a given gradient:

- 1) ECM fungal richness along a primary succession gradient is high, and slightly increasing towards the climax system
- 2) Beta diversity for ECM fungi is higher than for ECM-forming trees
- 3) ECM fungi across extreme habitats varies in species composition, but species richness remains constant across extreme habitats
- 4) EMC community structure is tightly tied up to soil chemistry

### **1.5.2 Geothermal Mycorrhiza and Heat Tolerance in ECM Fungi**

The temperature of the soil has been shown to have the greatest influence on vegetation composition and structure in geothermal environments. Plants growing in geothermal environments with often toxic levels of i.e. Al, may be facilitated or obligated to mycorrhiza (Burns, 1997), because of the benefits of nutrition uptake for the plant, but may also play other important roles.

Studies on arbuscular mycorrhiza (AM) in geothermal areas in Yellowstone National Park and Iceland (Appoloni *et al.*, 2008), revealed that geothermal AM community structure consisted both of generalist fungi, found in a broad temperature range, and unique species for geothermal areas. The presence of these unique fungi in geothermal environmental soils does not itself prove that these fungi are beneficial, but suggest a presence of potential specialist to the area.

Studies of ECM fungi in a geothermal area in New Zealand (Moyerson and Beever, 2004) found that species of *Pisolithus* dominated areas with prostate kanuka (*Kunzea ericoides* var. *microflora*) and particularly warm, acidic and N-dependent soil in this specific

geothermal area. This may suggest species with high tolerance for these conditions, or a high host specificity.

Jonsson *et al.* (1999) looked at ECM fungi in a Swedish boreal forest and compared sites where a recent forest fire had taken place and late successional sites. Using ITS-RFLP to investigate above- and belowground ECM, they found that spatial variation seemed to be more prominent than effects of wild fire. The number of ECM fungi found when analyzing the burned site did not change compared to the unburned site. Difference was found, however, in the evenness of species. Evenness was calculated to be higher in control sites than in sites recently affected by wild fire, although the species richness did not seem to be affected. The most common species were found in all sites, and abundance of the different species varied considerably through all sites, suggesting that few new species were dispersed by spores from adjacent forest stands. Jonsson *et al.* (1999) further discuss that wildfires as seen in the boreal forests of Sweden, are of the moderate type of disturbance, because of the low intensity compared to forest fires elsewhere. This may explain why wildfire and control sites varied little in species composition and richness. Nevertheless, it seems that wildfire and thereby soil heating has little effect of ECM richness.

Susceptibility of ECM fungi to heat has been investigated by Izzo *et al.* (2006) and Kipfer *et al.* (2009) where ECM was assessed by a bioassay using *Pinus jeffreyi* and *Pinus sylvestris*, respectively.

In the earliest study (Izzo *et al.*, 2006), soil from a mixed conifer forest in California was sampled and treated with different heating levels, reaching a final temperature of 25°C, 45°C, 60°C and 75°C. Seedling of *P. jeffreyi* was subsequently planted in each sample and ECM colonization assessed. Heat treatment to the soil samples altered the composition in the ECM fungi colonizing the seedlings, and at the higher temperatures *Rhizopogon* species were favored. This may be due to spore survival of this fungal family.

In the latter study (Kipfer, 2009); heating of more than 60°C and 70°C reduced the average number of species present in the samples. This study aimed to investigate heat tolerance in ECM fungi of *P. sylvestris*, and it became clear that ECM fungi can survive in soil samples heated up to 70°C. However, this study does not say anything about root survival of the trees under these conditions, since only the ECM fungal survival was recorded.

Based on these studies, some assumptions about ECM fungi in geothermal areas and in relation to soil heating can be made:

- 1) ECM fungi play an important role in forest systems in geothermally active areas
- 2) There may be some fungal species specificity or unique species to geothermal areas
- 3) Soil heating up to a certain temperature, does not seem to affect ECM species richness
- 4) Some ECM forming fungi can survive soil heating up to at least 70°C

Even though these papers do not directly apply to our field of study, they do provide some useful background knowledge on how to go about investigating ECM fungi in a geothermal gradient in a Sitka spruce forest, previously growing in a cold soil. As this is a totally unique and new study area, no directly relevant work has been done, and no previous studies consistent with our environment have been conducted. Therefore, knowledge of different ECM gradient along with ECM tolerance to heat may provide some useful considerations when looking into this project.

## 2 Objectives

This research project is a part of the project „FORHOT“, investigating a geothermally heated Sitka spruce forest in Reykir, Ölfusi, and is considered as a pilot study concerning ECM. Therefore, the objective was first and foremost to record ECM root tips and to see if what we find can give us some useful information in relation geothermally heated forests in Iceland. However, based on background knowledge on ECM, the following goals were formulated:

1. to investigate if there is a difference in the abundance of morphotypes along a T-elevation gradient
2. to investigate if there is a difference in ECM morphotype diversity across a T-elevation gradient
3. to investigate if there is a difference in abundance of ECM root tips along a T-elevation gradient

## 3 Methods

### 3.1 Field description

The research site is a Sitka spruce (*Picea sitchensis*) forest in Ölfus, close to Hveragerði, Iceland (Figure 1). In 2008 several earthquakes in south Iceland awakened old hotsprings in the area, and also created new ones, making this a geothermally active area. The forest is an approximately 45 year old planted stand of un-thinned Sitka spruce trees of ca. 3000 m<sup>2</sup>. Sitka spruce is the only tree species growing in the stand. Originally, the forest grew in relatively cold soil, but after the earthquakes in 2008, hot-spots in and near the forest cause soil heating elevations, reaching from no significant heating up to temperature elevation of 45°C.

The study area has not been thinned and is therefore a dense forest, where little light reaches the forest floor, and the vegetation on the forest floor is therefore limited. No hot water is running through the forest, all though a hot stream measuring ca. +32°C is running just outside the forest. The soil is therefore relatively dry, and not directly affected by the hot stream. Heating is caused by below ground hot water.

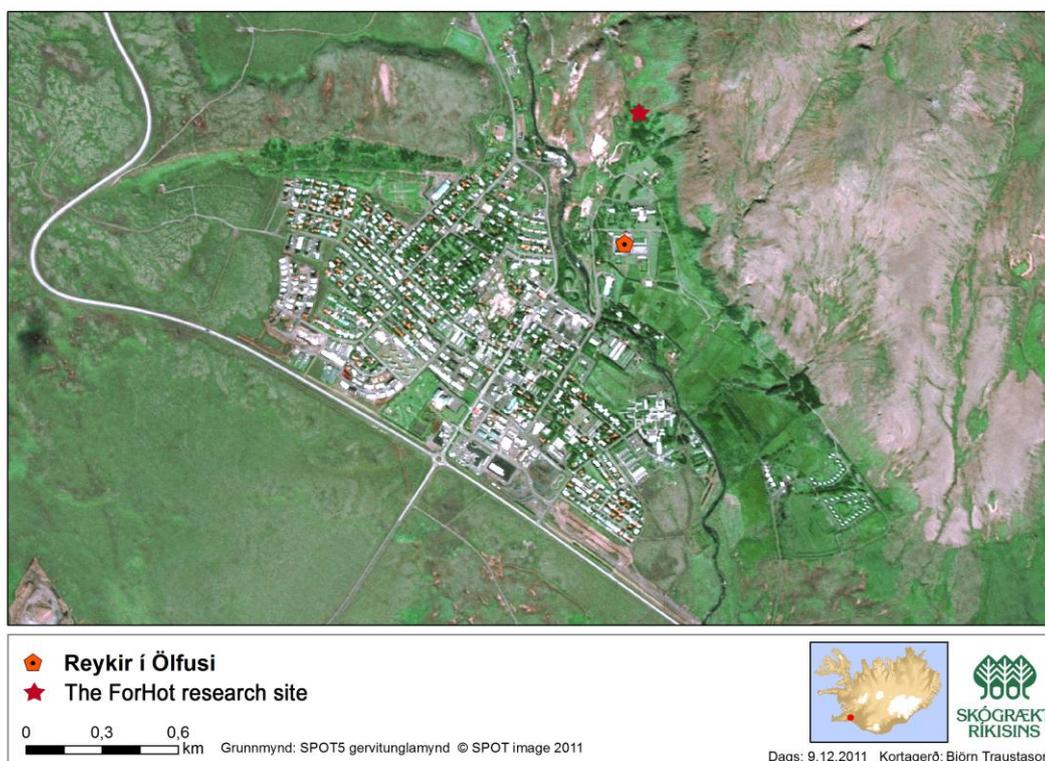
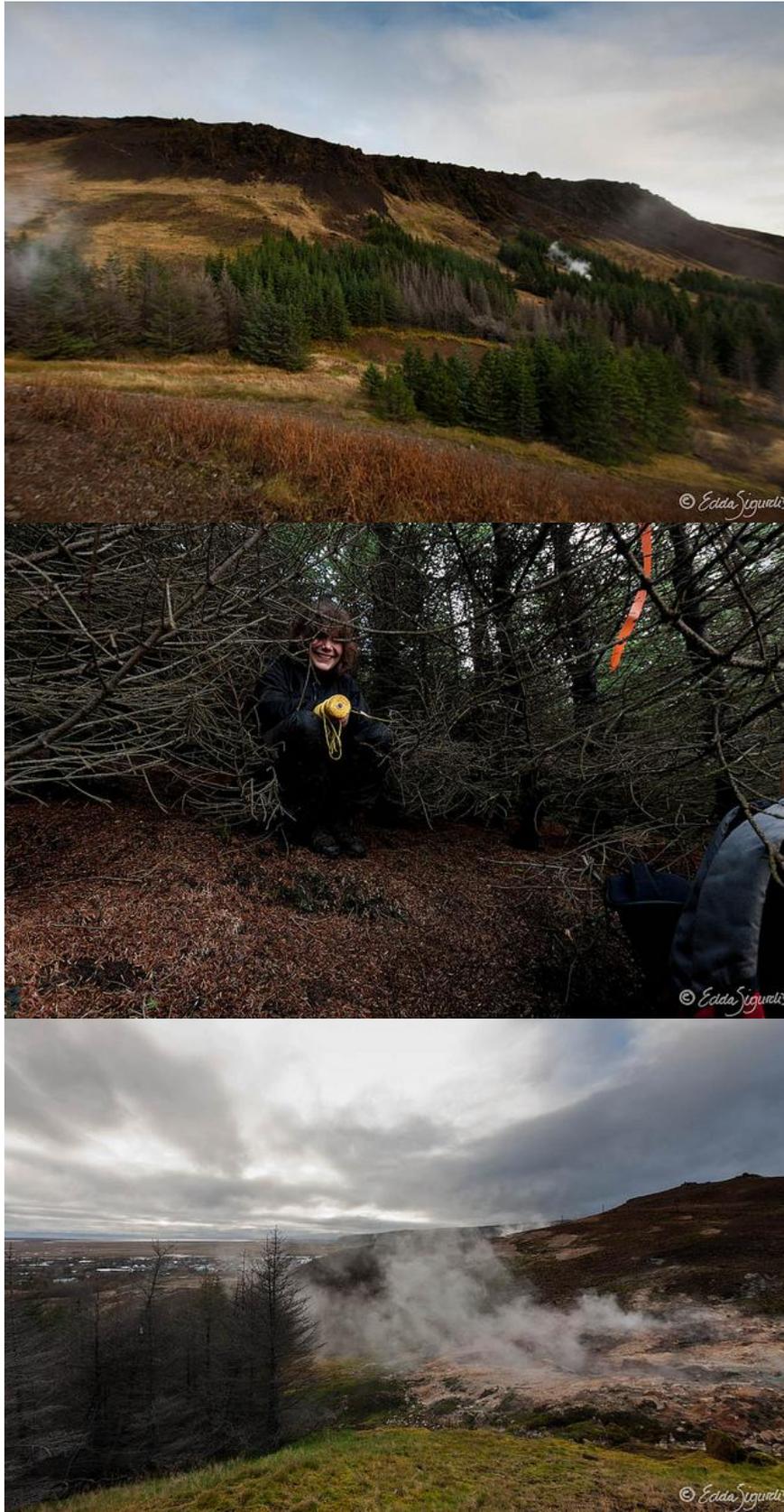


Figure 1: Map of Hveragerði and ForHot research site.



*Figure 2: Dead trees at T-elevations higher than +35°C in the Sitka spruce forest.  
Photo by: Edda Oddsdóttir.*

One effect of this belowground heating becomes apparent from a first glance at the forest stand. All trees above +35°C T-elevation has died, leaving a spot of dead trees that have been blown over by the wind. A large portion of the standing trees still seem healthy and green, although one can easily see the effect of heating by dead branches on the trees as you get closer to the hotter areas. The trees growing in no or low T-elevations have the characteristics of normal, healthy Sitka spruce trees.



*Figure 3: Pictures from the study area. From above: 1: the hot spots are affecting the forest, where patches of dead trees are seen. 2: mapping the Sitka spruce forest, using measuring bands, yellow bands and crawling. 3: A hot spot close to the study area, but not directly influencing the study site. Photos: Edda S. Oddsdóttir*

## 3.2 Mapping the area

To map the area, a 2200 m<sup>2</sup> permanent grid of soil temperature measurement points was installed. The grid was created by squares of 5 m in vertical distance (VD) x 12.5 m in lateral distance (LD) from a chosen 0-point, using yellow threads and measuring tape. Soil temperature was measured at each point.

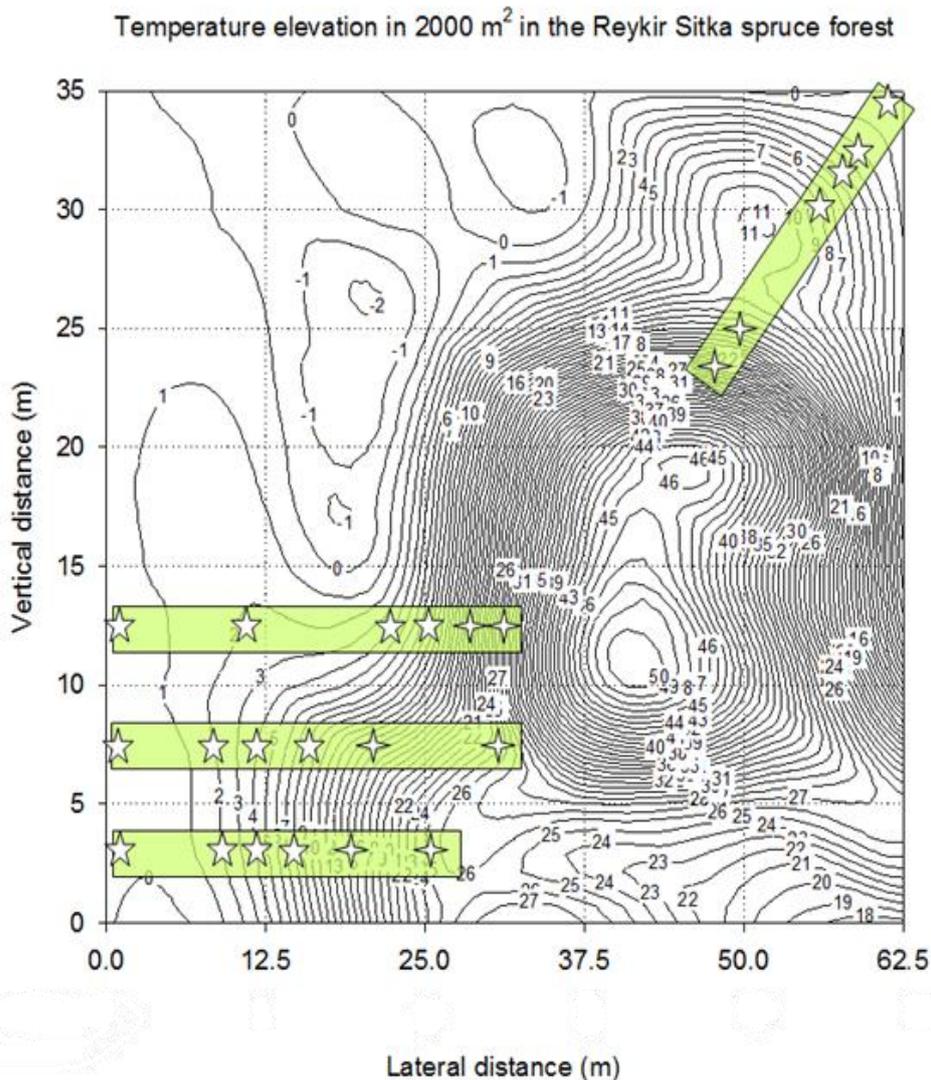


Figure 4: Map of soil temperature elevation (°C) measured on 27<sup>th</sup> of September – 3<sup>rd</sup> of October 2011 in a 2187.5 m<sup>2</sup> area within a 45 year old Sitka spruce stand at Reykir, S-Iceland. The trees found at higher T-elevation than ca. 35°C have died. Colored areas show intensive measurement transects I to III. Five-armed stars show the location of control (0°C), +2°C, +4°C, +8°C (realistic warming during the next 100 years). Four armed stars show +15°C and 25°C. Map by Bjarni D. Sigurdsson.

### 3.3 Sampling

Samples were taken between 12.10-07.11.2011 from three different transects according to the map: transect 1 (0 m VD), 2 (5 m VD) and 3 (10 m VD). At each transect, soil was sampled at different points along the transect; 0 m, 5 m, 11 m, 17 m, 23 m, 29 m and 35 m VD. At each sampling point, 1-2 samples were taken with a soil corer (5cm in diameter, 10cm depth), and soil temperature measured at three sites in close proximity to the sampling point. After sampling, the samples were contained in plastic bags, and stored at 4°C if they were not examined the next day, in which they were stored in a cooler bag. Maximum storage of samples before analysis was 5 days.

### 3.4 Examination of samples

Number of samples, average soil temperature and the temperature elevation at each sampling point is listed in table 1. The samples were examined under a dissection microscope, and ectomycorrhizal root tips were removed from the soil, put in water in a petri dish for further rinsing and examination. All soil was removed from the ECM roots that were found. The ECM root tips were grouped into 17 morphotypes based on their characteristics described in table 2, and the number of root tips in each morphotype group counted. Percentage of the soil sample examined (cm<sup>2</sup>) was estimated and noted. Since there was a large amount of considered dead ECM root tips in some samples, only root tips that were considered alive were counted. At least 3 root tips of each morphotype per sample was cut off and stored in eppendorf tubes at -18°C, for possibly future molecular studies of the ECM. Pictures of each of the root tips were acquired and marked in accordance with the eppendorf tubes and morphotypes.

*Table 1: Number of samples examined at point 0-35m at each transect along with average temperature measurements at sampling point. Point=vertical distance, #of samples=the number of samples examined, average °C=calculation of average of the three temperature measurement, T-elevation (°C)=elevation in temperature from the zero point, N/A=no data noted (due to no ECM root tips in sample).*

Point	Transect 1			Transect 2			Transect 3		
	# of samples	average °C	T-elevation (°C)	# of samples	average °C	T-elevation (°C)	# of samples	average °C	T-elevation (°C)
0 m	1	5.9	0	2	6	0	2	5.4	0
5 m	1	7.7	1.8	1	6.7	0.7	2	5.9	0.5
11 m	1	8.5	2.6	2	9.5	3.5	2	7.16	1.76
17 m	1	12.9	7	2	13.8	7.8	2	16.4	11
23 m	1	12.3	6.4	1	18.9	12.9	2	16.3	10.9
29 m	1	35.9	30	2	23.4	17.4	2	20.6	15.2
35 m	1	31.8	25.9	0	N/A	N/A	0	N/A	N/A

## 3.5 Data analysis

### 3.5.1 Morphotypes

Morphotypes were grouped together based on characteristics of the root tips visible through the dissection microscope, such as colour, smoothness, hyphae colour and abundance and rhizomorphs (See table 2 for description).

*Table 2: Description of morphotypes*

	Description
<b>Type 1</b>	Brown to black at basis, white, almost transparent tip. Both white and black hyphae.
<b>Type 2</b>	Brown at basis, black or brown at root tip, the tip thicker than the rest, black and white hyphae, sometimes small white tip at end.
<b>Type 3</b>	White transparent tip, brown further down, highly branched, white colorations at brown areas, white but some black hyphae. Many rhizomorphs.
<b>Type 4</b>	Black with completely white tip. White hyphae at tip, black hyphae at basis. Extensive hyphaeal growth at tip.
<b>Type 5</b>	Long, thin, brow. Mostly homogenous color, white hyphae. Sometimes white tip, sometimes blacker at basis.
<b>Type 6</b>	Short, thick, black with black hyphae. Slightly thinner at basis.
<b>Type 7</b>	Longer, thick, black, crooked. Yellow and black hyphae. Rough surface.
<b>Type 8</b>	Brown with thick black cap at tip. Black hyphae.
<b>Type 9</b>	Black, black hyphae, thicker at cap. Extensive hyphae growth from tip
<b>Type 10</b>	Like type 3, but more crooked, more homogenous color, white hyphae.
<b>Type 11</b>	Black metallic with white, sometimes black, hyphae. Long, even thickness.
<b>Type 12</b>	Black, short with white "golf-ball" at end. White and black hyphae, some yellowish.
<b>Type 13</b>	Long, crooked, black and white hyphae and black tip, brown stem, slightly branched.
<b>Type 14</b>	Long, brown at basis, white tip with black hyphae.
<b>Type 15</b>	Branched, light to darker brown, thin, lighter tips.
<b>Type 16</b>	Like type 1, but much whiter all the way to basis.
<b>Type 17</b>	Completely white, thick, short, white hyphae, some black.



*Figure 5: Pictures of morphotypes 1-17 taken through the dissection microscope. Note that there is no common scaling in these pictures, so some root tips may seem bigger than others, but size is similar.*

Number of morphotypes were counted and noted. Each ECM root tip was counted, and based on morphological characters grouped into its morphotype. Each root tip was noted as 1. When approximately 100 ECM root tips were counted, the rest of the sample was discarded.

### 3.5.2 Statistical analysis

The average soil temperature at each sampling point was calculated from three different measurements.

Total number of root tips was calculated based on the estimates of proportion of sample analysed and the counted number of ECM root tips. If more than one sample was examined, the average number of root tips was calculated. Number of morphotypes per sample was counted and proportion of morphotypes per sample point was calculated.

Simpson Index ( $D = 1 - \left( \frac{\sum n(n-1)}{N(N-1)} \right)$ ) of Diversity ( $1-D$ ) and Reciprocal Index ( $1/D$ ) was calculated, along with Shannon Diversity Index ( $H = \sum_{i=1}^s - (P_i \times \ln P_i)$ ) for each sampling point, to get an estimate of the diversity of ECM root tips based on the 17 morphotypes.

Data was analysed using *SigmaPlot*® *Scientific Data Analysis and Graphing Software*. All data (table 3) was plotted against T-elevation. Linear regression was calculated, including  $r^2$  and P-values. Proportion of morphotypes was plotted in a stacked vertical bar chart.

Table 3: List of data used in SigmaPlot®. T-elevation is the rise in temperature from the zero point, # of root tips is the number of ECM root tips counted in the proportion of the sample analysed, % of sample is the estimate of how much of the sample was analysed, Total # of root tips describes the calculated amount of ECM root tips present in the total sample based on # of root tips and % of sample and # of morphotypes is how many different morphotypes were noted in each sample.

T-elevation	#of root tips counted	% of sample examined	Total # of root tips	# of morphotypes counted	Shannon Diveristy Index (H')	Simpson's Diversity Index (1-D)	Simpson's Reciprocal Index (1/D)
0.0	88.0	15.0	586.7	9.0	2.1	0.9	7.9
0.0	104.0	20.0	520.0	12.0	1.6	0.7	2.9
0.0	83.0	12.5	664.0	11.0	1.6	0.7	3.3
0.5	106.0	10.0	1060.0	10.0	1.8	0.8	5.1
0.7	90.0	25.0	360.0	8.0	1.9	0.8	6.0
1.8	106.5	20.0	532.5	13.0	2.3	0.9	8.4
1.8	67.0	5.0	1340.0	6.0	1.6	0.8	4.3
2.6	81.0	10.0	810.0	7.0	1.6	0.7	3.8
3.5	106.5	25.0	426.0	12.0	1.8	0.8	4.8
6.4	85.0	40.0	212.5	7.0	1.7	0.8	4.8
7.0	97.0	25.0	388.0	5.0	1.2	0.6	2.8
7.8	103.0	15.0	686.7	14.0	2.3	0.9	8.5
10.9	113.5	20.0	567.5	13.0	2.1	0.9	7.4
11.0	110.0	37.5	293.3	13.0	2.3	0.9	8.2
12.9	97.0	23.0	421.7	9.0	1.9	0.8	6.0
15.2	112.5	100.0	112.5	12.0	1.9	0.8	4.9
17.4	0.0	100.0	0.0	0.0	0.0	0.0	0.0
30.0	0.0	100.0	0.0	0.0	0.0	0.0	0.0

# 4 Results

A total of 29 samples were examined from the three transects, and a total of 2258 ECM root tips were counted and grouped into morphotypes in accordance to table 2. When more than one sample per point was examined, the average number of ECM root tips for these samples was calculated and used in further calculations according to table 4.

## 4.1 Diversity Indexes

Interpreting data and plots, Shannon Diversity Index ( $H'$ ) showed significant, but weak, negative correlation ( $r^2 = 0.355$ ,  $P = 0.009$ ) from cooler to higher T-elevations, with more than 95% probability. This means that according to Shannon Diversity Index, diversity of morphotypes is higher closer to 0°C T-elevation, with a decrease towards zero diversity above 15.2°C T-elevation.  $H'$ -values range from intermediate ( $H' = 2.3$ ) morphotype evenness and richness to relatively low ( $H' = 1.2$ ) at intermediate T-elevations to zero below 15°C T-elevation.

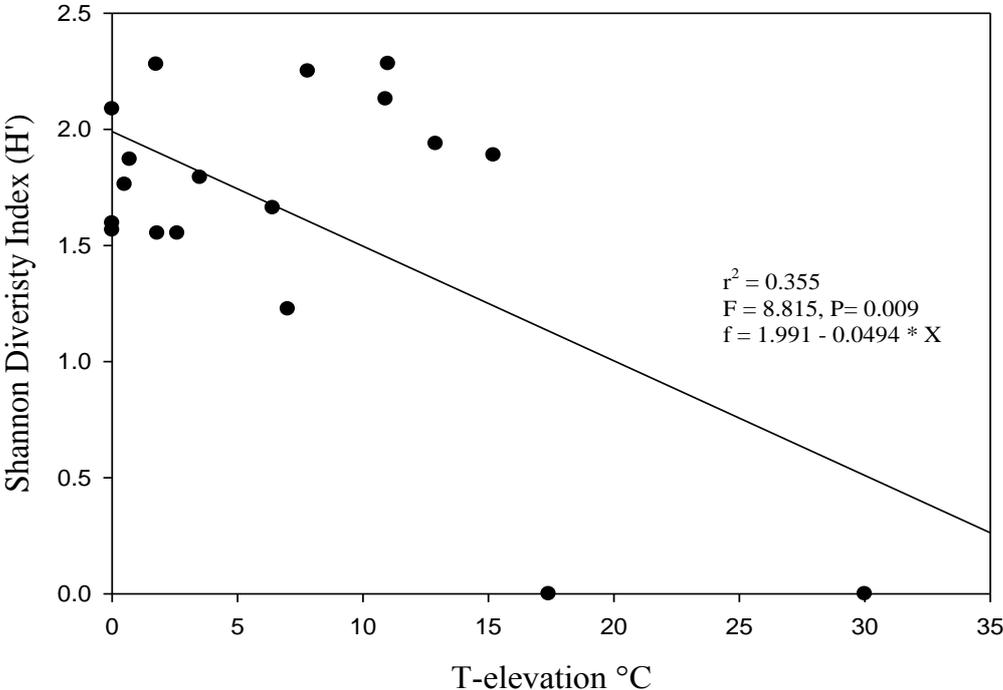


Figure 6: Shannon Diversity Index plotted against T- elevation (°C) shows a negative correlation between  $H'$  and T-elevation ( $r^2 = 0.355$ ,  $P = 0.009$ ), with higher  $H'$ -values towards lower T-elevations.

Simpson's Index of Diversity ( $D-I$ ) also showed a weak, but significant ( $r^2 = 0.445$ ,  $P=0.002$ ) decrease of diversity towards higher T-elevation with more than 95% probability, with zero diversity above 15.2°C T-elevation. Similarity was found in Simpsons Reciprocal Index, but with a weaker correlation (plot not shown here, see table 3). Values of up to  $(D-I) = 0.88$ , indicate a higher diversity using Simpson Diversity Index than Shannon Index of Diversity at low and intermediate T-elevations, whereas zero diversity is found above 15.2°C T-elevation

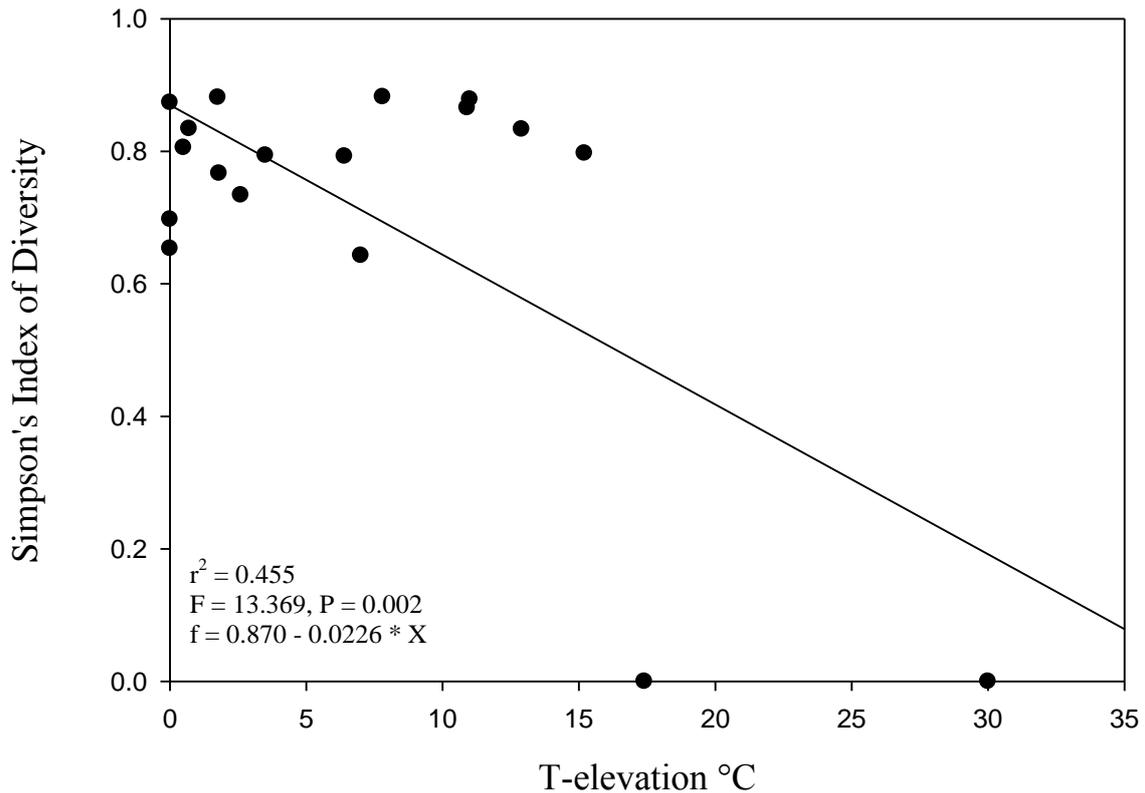


Figure 7: Simpson's Index of Diversity as a function of T-elevations show a slight, but significant decrease ( $r^2 = 0.445$ ,  $P=0.002$ ) of diversity of morphotypes towards higher T-elevations (°C).

## 4.2 Abundance of ECM morphotypes and root tips

There was a decrease of total number of ECM root tips from 0°C T-elevation towards higher T-elevations, with no ECM root tips found above 15.2°C (Table 3). Non-ECM root tips were not found alive in the samples. Linear regression showed significant negative correlation ( $r^2 = 0.437$ ,  $P = 0.003$ ) between number of ECM root tips and T-elevation (Figure 7).

The number of morphotypes also decreased towards warmer T-elevations, but the negative correlation here was weaker ( $r^2 = 0.242$ ,  $P=0.038$ ), yet significant with more than 95% probability (Figure 8).

*Table 4: Number of ECM morphotypes (# of types), total number of ECM root tips (total tips in sample), average temperature (Temp. °C), amount of ECM root tips counted (# of ECM root tips), proportion of sample analysed (% of sample) at each VD sampling point (Point) in transects 1-3 (Transect).*

Transect	Point	# of ECM root tips	% of sample	Total tips in sample	#of types	Temp.°C	T-elevation
1	0	88	15	586.7	9	5.9	0
2	0	104	20	520	12	6	0
3	0	83	12.5	664	11	5.4	0
3	5	106	10	1060	10	5.9	0.5
2	5	90	25	360	8	6.7	0.7
3	11	106.5	20	532.5	13	7.16	1.76
1	5	67	5	1340	6	7.7	1.8
1	11	81	10	810	7	8.5	2.6
2	11	106.5	25	426	12	9.5	3.5
1	23	85	40	212.5	7	12.3	6.4
1	17	97	25	388	5	12.9	7
2	17	103	15	686.7	14	13.8	7.8
3	23	113.5	20	567.5	13	16.3	10.9
3	17	110	37.5	293.3	13	16.4	11
2	23	97	23	421.7	9	18.9	12.9
3	29	112.5	100	112.5	12	20.6	15.2
2	29	0	100	0	0	23.4	17.4
1	29	0	100	0	0	35.9	30

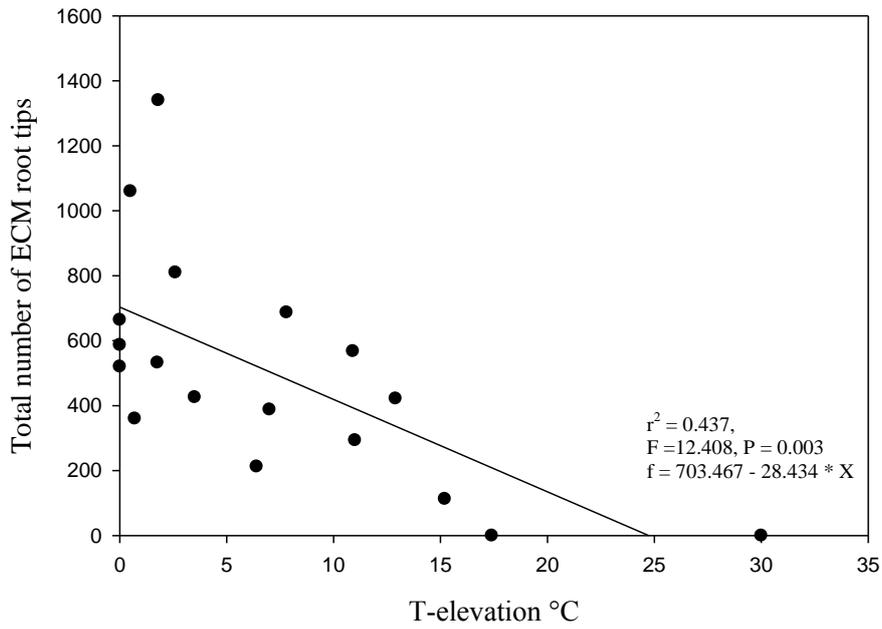


Figure 8: Total number of ECM root tips in samples plotted against T-elevation, shows a significant negative correlation ( $r^2=0.437$ ,  $P=0.003$ ).

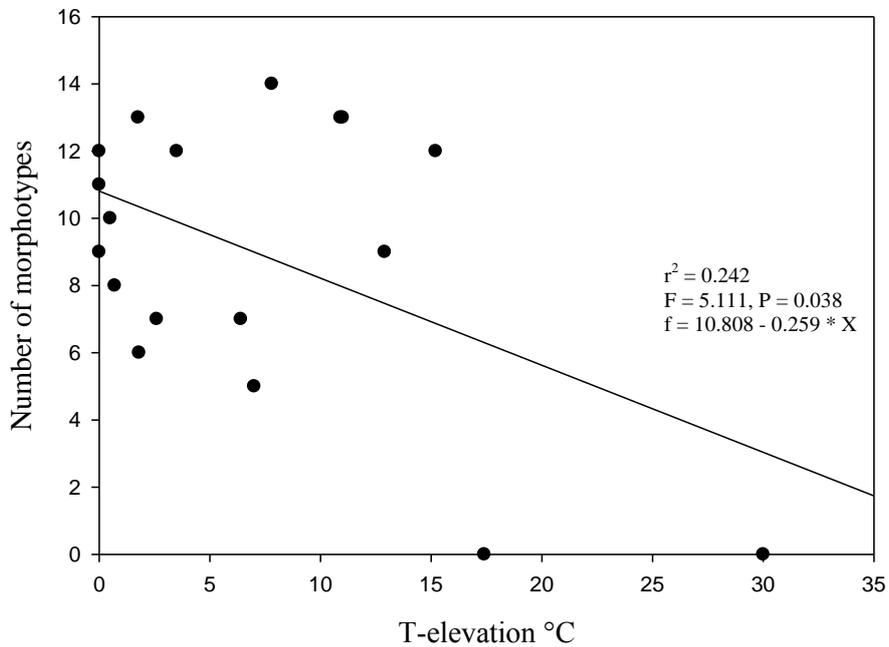


Figure 9: Number of morphotypes counted in each sample plotted against T-elevation shows a weak, but significant negative correlation between number of morphotypes and T-elevation ( $r^2=0.242$ ,  $P=0.038$ ).

### 4.2.1 Distribution of morphotypes along T-elevation gradient

Proportion of the different ECM morphotypes present in sample, along with which morphotypes were found, was graphed in a stacked vertical bar chart (Figure 10). Some changes in composition of morphotypes were observed in the samples. For instance was type 1 found in relatively even proportions along T-elevation, whilst type 15 showed a tendency towards higher T-elevations (15.0-17.4, Figure 10). Type 1, 2, 5 and 7 were found in all temperature ranges. Type 13 and 16 were only found in the coolest temperature range. There seems to be a decrease of Type 7 as the T-elevation gets higher, and Type 9, 10, 13 and 16 are all missing from the warmest T-elevations. Type 12 is not present in the cooler temperature ranges, but is found from T-elevations above 7.5°C. Type 17 is found in some of the cooler T-elevation ranges, but is absent in the warmer T-elevations.

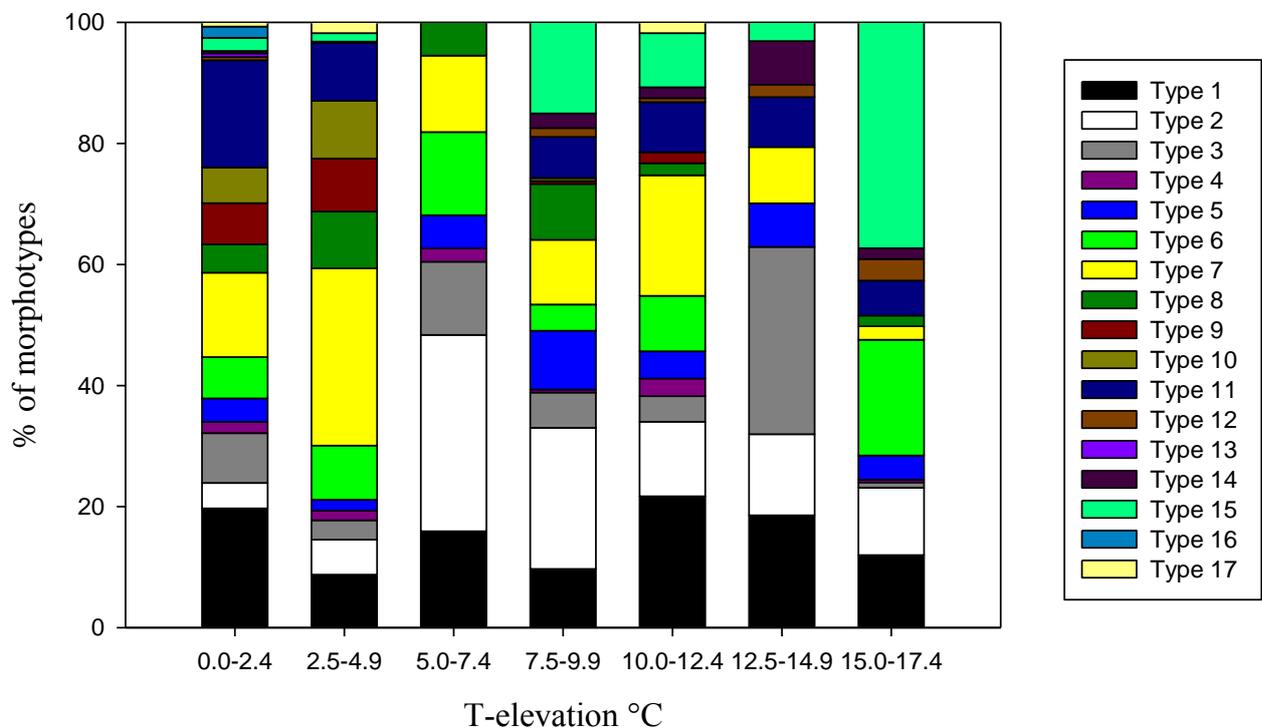


Figure 10: Stacked vertical bar of morphotype abundance in different temperature ranges.

### 4.2.2 ECM root tip survival and temperature

ECM root tips were found up to T-elevations of 15.2°C (measured soil temperature: 20.6°C), although total amount of root tips in sample had declined drastically from 664 ECM root tips at the 0°C T-elevation (soil temperature=5.4°C, VD=0 m) to 112.5 ECM root tips at 15.2°C T-elevation (soil temperature=20.6°C, VD=29 m). There was a decline in of total number of root tips from lower to higher T-elevations, and no ECM root tips were alive above 23.4°C measured soil temperature (Table 4).

Table 5: Average number of ECM morphotypes (Type 1- 17) and average number of root tips (Total # of tips) found in samples. Point = VD, °C= average soil temperature, T-elevation= elevation of soil temperature.

Point	°C	T-elevation (°C)	Type 1	Type 2	Type 3	Type 4	Type 5	Type 6	Type 7	Type 8	Type 9	Type 10	Type 11	Type 12	Type 13	Type 14	Type 15	Type 16	Type 17	Total # of tips
0	5.4	0	42.5	0.5	5	0	1	1	7	0.5	0	0	2.5	0	0	0.5	10.5	12	0	83
0	5.9	0	19	4	10	7	14	7	8	14	5	0	0	0	0	0	0	0	0	88
0	6	0	7	9	1	1	3.5	1.5	9.5	5	3	0	59.5	2	2	0	0	0	0	104
5	5.9	0.5	29	0	17	0	0	5	6	1	17	0	28	1	0	2	0	0	0	106
5	6.7	0.7	20	2	13	0	2	14	21	2	0	14	0	0	2	0	0	0	0	90
5	7.7	1.7	3	0	0	0	0	10	18	3	9	24	0	0	0	0	0	0	0	67
11	7.16	1.76	6.5	11.5	7	4	4.5	5.5	20.5	4.5	10	0	24	0.5	0	0	3.5	0	4.5	106.5
11	8.5	2.6	16	3	1	0	0	7	35	16	3	0	0	0	0	0	0	0	0	81
11	9.5	3.5	6	4.5	21	0.5	7	3	32	1	0	1	29	1	0	0.5	0	0	0	106.5
23	12.3	6.4	16	7	0	2	2	25	23	10	0	0	0	0	0	0	0	0	0	85
17	12.9	7	13	52	22	2	8	0	0	0	0	0	0	0	0	0	0	0	0	109.9
17	13.8	7.8	10	24	6	0.5	10	4.5	11	9.5	0.5	0.5	7	1.5	0	2.5	15.5	0	0	103
23	16.3	10.9	26	15	2	1.5	6	15	20	1.5	0	0	9	1	0	3	13	0	0.5	113.5
17	16.4	11	22.5	12.5	7.5	5	4	5.5	24.5	3	4	0	9.5	0.5	0	1	7	0	3.5	110
23	18.9	12.9	18	13	30	0	7	0	9	0	0	0	8	2	0	7	3	0	0	97
29	20.6	15.2	13.5	12.5	1	0.5	4.5	21.5	2.5	2	0	0	6.5	4	0	2	42	0	0	112.5
29	23.4	17.4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
29	35.9	30	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

## 5 Discussion

### 5.1 Diversity Indices

Both Shannon Diversity Index and Simpson's Index of Diversity showed a decrease towards higher T-elevations, with a significant negative correlation, indicating a slight decrease of ECM fungal diversity towards higher T-elevation. The indices were calculated based on morphotypes and not species, and are therefore only suggestive and not precise calculations of diversity. A more thorough study would be required to get a real picture of the actual ECM fungal diversity along this gradient. Sample sizes are also relatively small, and spatial distribution of ECM fungi may be the a determining factor, as Jonsson *et al.* (1999) found in their studies on effects of wild fire on ECM distribution. Therefore, a larger amount of samples and determination of species either by 454-sequencing (Blaalid *et al.*, 2011), ITS-RFLP (Jonsson *et al.*, 1999) or morphotyping according to Agerer's *Color Atlas of Ectomyorrhizae* (1997) using both dissection microscope and phase contrast microscoping of hyphae, would be necessary. 454-sequencing has proven to be highly reproducible, and may be a tool towards standardization of description and publications on fungal communities (Nilsson *et al.*, 2011), although ITS primers must be selected carefully for DNA barcoding (Bellamain *et al.*, 2011). Nevertheless, the results here may be considered an indicator of a decrease in diversity.

### 5.2 Abundance of morphotypes, ECM root tips and survival

In the present study, findings show a decline of number of living ECM root tips from no temperature elevation to higher temperature elevations. The decline of alive ECM root tips may be due to fewer Sitka spruce roots surviving the higher T-elevations, or due to factors such as soil chemistry that were not investigated in the present study. Soil chemistry and ECM has been shown to have high autocorrelation (Toljander *et al.*, 2006), and we cannot exclude the possibility that soil chemistry also play a role in the survival of root tips in the present study. Nevertheless, the decline was significant ( $P=0.003$ ), and therefore may conclude that temperature has probably a strong influence on the survival of ECM root tips.

No ECM root tips were found alive above T-elevations of 15.2°C in this study. There is, however, possible that ECM root tips may be found at higher T-elevation, since there was made no attempt of more intensive sampling in the areas between this measuring point and the next. Therefore, further and more intense sampling in zones in the transects measuring 15.2°C and up to 17.4°C T-elevation is necessary to conclude the maximum T-elevation for ECM root tips in this particular area.

The findings of this study does not exclude the possibility that ECM forming fungi is still alive in the soil, because as Kipfer (2009) and Izzo (2006) describe, ECM fungi can survive soil heating temperatures up to 70°C or more. It would be interesting to investigate soil samples in this area of the study site further. Next generation sequencing like 454-pyrosequencing (Kausserud *et al.*, 2011) of soil samples would perhaps reveal the presence of ECM fungi. Inoculation of Sitka spruce seedlings in soil from sites where no ECM root tips are found may also reveal the presence of surviving ECM fungi (Kipfer, 2009, Izzo, 2006). In the present study, no further attempts were made to classify the surviving morphotypes at 15.2°C T-elevations than presented in this paper. Therefore, classification of ECM fungi found at this T-elevation may contribute to our understanding of survival under these conditions and further studies in that direction should be encouraged. Knowledge of species and taxa may be helpful in determining fungal specificity along the gradient (Appoloni *et al.*, 2008, Moyerson and Beaver, 2004).

The fact that Sitka spruce at Reykir, Ölfus, has not survived T-elevations higher than +35°C does not exclude the possibility of finding ECM forming fungi alive in the soil here. Therefore, samples in these areas as well as further studies on the already examined areas, may reveal ECM fungi surviving along the whole transect.

Number of morphotypes in the samples also showed a significant negative correlation with higher T-elevations. However, the number of morphotypes is only a descriptive estimate, since no effort was made as to determine which species were present according to Agerer's *Color Atlas of Ectomyorrhizae* (1997). Further studies with the aim of determining species composition would provide more information and may explain the decrease observed in this study.

The grouping of morphotypes, as in the present study, is imprecise, and only chosen as method here because of practical reasons. Errors may be made in grouping, and only subjective opinion of differences between the types is used as a basis for the grouping. Therefore, in further studies of ECM in this particular site, it is recommended that molecular tools, such as 454-pyrosequencing (Kausserud *et al.*, 2011) or ITS-RFLP (Jonsson *et al.*, 1999), would be used instead of morphotyping.

### **5.3 Distribution of morphotypes along the T-elevation gradient**

In this study, the distribution of ECM morphotypes seemed to be uneven between different temperatures. This may indicate that the distribution of different ECM fungal types or species changes along the gradient. There seemed to be a tendency of some ECM morphotypes to appear more abundantly in cooler T-elevations, while some more common at higher temperatures. Some morphotypes were found to be present at all T-elevation ranges, and can indicate that these types may not be sensitive to high soil temperature. However, the amount of data in this study is insufficient to say if this is due to changes in T-elevation or to spatial variation. Determining species would perhaps also answer more questions regarding this, along with a larger range of samples, which may exclude errors found in assumptions due to spatial variations.

## 5.4 Further considerations

As this particular research had been both limited by time and funding, and it is the pilot study of ECM at this particular site, many aspects have not been investigated thoroughly. For instance, the number of dead ECM root tips was not noted, and presences of saprotrophic fungi was not investigated. There was, however observed possible saprotrophic fungi in the samples, all though not listed in the results. Also an increase of ECM root tips considered dead along the T-elevation transect was observed, all tough not included in this particular study. These would be interesting areas to look into as well.

In the present study, only ECM fungi were investigated, but there may be a presence of i.e. AM fungi as well. 454-pyrosequencing of the root tips may give a better overview of the whole fungal community (Kausserud *et al.*, 2011), and grouping into know taxa would prove useful in investigating the fugal community here.

Soil classification and analysis may also provide useful information on the distribution of fungi along the gradient and to determine soil characteristics.

## 6 Conclusion

A slight, but significant decline in morphotype diversity, abundance of ECM root tips and number of morphotypes was found along the T-elevation gradient in this particular study site. Number of samples was unfortunately too small to draw any realistic conclusions in this study, but findings here may be used as indicators of a decline of ECM fungi towards warmer T-elevations.

There is a need for further investigation of ECM at this study site, such as larger samples, determination of species with i.e. 454-pyrosequencing, inoculation of seedlings in soil samples from the study site, and soil sample analysis to determine soil characteristics, to understand the community structure and ECM fungal behavior in geothermal active areas.

# References

- Agerer, R., 1997. *Colour atlas of ectomycorrhizae*, Schwabisch Gmund: Einhorn-Verlag.
- Appoloni, S. Lekberg, Y., Tercek, M.T., Zabinski, C.A., Redecker, D. 2008. Molecular community analysis of arbuscular mycorrhizal fungi in roots of geothermal soils in Yellowstone National Park (USA). *Microbial Ecology*, 56, 649-659.
- Bellemain, E., Carlsen, T., Brochmann, C., Coissac, E., Taberlet, P., Kauserud, H. 2010. ITS as DNA barcode for fungi: an in silico approach reveals potential PCR biases. *BMC Microbiology*. 10:189, 9p
- Berch, S.M., Massicotte, H.B. & Tackaberry, L.E., 2004. Re-publication of a translation of “The vegetative organs of *Monotropa hypopitys* L.” published by F. Kamienski in 1882, with an update on *Monotropa* mycorrhizas. *Mycorrhiza*, 15, 323-332.
- Blaalid, R., Carlsen, T., Kumar, S., Halvorsen, R., Ugland, K.I., Fontana, G., Kauserud, H. 2011. Changes in the root-associated fungal communities along a primary succession gradient analysed by 454 pyrosequencing. *Molecular Ecology*, E-publication available at <http://www.mn.uio.no/bio/english/people/aca/haavarka/Blaalid%20et%20al%202012%20Mol%20Ecol.pdf>. [Accessed at 20. October, 2011]12p.
- Burns, B., 1997. Vegetation change along a geothermal stress gradient at the Te Kopia steamfield. *Journal of the Royal Society of New Zealand*, 27, 279-293.
- Cairney, J.W.G., 2000. Evolution of mycorrhiza systems. *Naturwissenschaften*, 87, 467-475.
- Enkhtuya, B., Óskarsson, U., Dodd, J.C., and Vosátka, M., 2003. Inoculation of Grass and Tree Seedlings Used for Reclaiming Eroded Areas in Iceland with Mycorrhizal Fungi. *Folia Geobotanica*. 38, 209-219
- Eyjólfsdóttir, G.G. 2004. Fungi in larch and birch woodlands of different age in Eastern Iceland. *Effects of afforestation on ecosystems, landscape and rural development Proceedings of the AFFORNORD conference, Reykholt, Iceland, June 18–22, 2005. TemaNord* 2007:508
- Frank, B., 2004. On the nutritional dependence of certain trees on root symbiosis with belowground fungi (an English translation of A.B. Frank’s classic paper of 1885). *Mycorrhiza*, 15, 267-275.
- Gehring, C.A., Theimer, T.C., Whitham, T.G, and Keim, P. 1998. Ectomycorrhizal fungal community structure of Pinyon pines growing in two environmental extremes. *Ecology*, 79(5), 1562–1572

- Hrafnkelsdóttir, B., 2009. Ectomycorrhizal abundance and diversity of downy birch and Siberian larch stands in Iceland. M.Sc. thesis. Agricultural University of Iceland, 69p.
- Izzo, A., Canright, M. & Bruns, T., 2006. The effects of heat treatments on ectomycorrhizal resistant propagules and their ability to colonize bioassay seedlings. *Mycological Research*, 110, 196-202.
- Jonsson, L., Dahlberg, A., Nilsson, M.C., Zackrisson, O., Karen, O. 1999. Ectomycorrhizal fungal communities in late-successional Swedish boreal forests, and their composition following wildfire. *Molecular Ecology*, 8, 205-215.
- Kausrud, H., Kumar, S., Brysting, A., Nordén, J., Carlsen, T. 2011. High consistency between replicate 454 pyrosequencing analyses of ectomycorrhizal plant root samples. *Mycorrhiza*. E-publication ahead of print available at <http://www.mn.uio.no/bio/english/people/aca/haavarka/Kausrud%20et%20al%202011%20Mycorrhiza.pdf> [Accessed at 20. October, 2011] 7p.
- Kipfer, T. Egil, S., Ghazoul, J, Moser B., Wohlgemuth, T., 2009. Susceptibility of ectomycorrhizal fungi to soil heating. *Fungal Biology*, 114, 467-472.
- Lawrence, E., 2008. *Henderson's dictionary of biology* 14th ed., Harlow England ;New York: Pearson Benjamin Cummings Prentice Hall.
- Leake, J.R. et al., 2004. Symbiotic germination and development of the myco-heterotroph *Monotropa hypopitys* in nature and its requirement for locally distributed *Tricholoma* spp. *New Phytologist*, 163, 405-423.
- Lilleskov, E.A. & Bruns, T.D., 2001. Nitrogen and ectomycorrhizal fungal communities: what we know, what we need to know. *New Phytologist*, 149, 156-158.
- Moyersoen, B. & Beever, R.E., 2004. Abundance and characteristics of *Pisolithus* ectomycorrhizas in New Zealand geothermal areas. *Mycologia*, 96(6), 1225-1232.
- Nilsson, R.H., Tedersoo, L., Lindahl, B.D., Kjølner, R., Carlsen, T., Quince, C., Abarenkov, K., Pennanen, T., Stenlid, J., Bruns, T., Larsson, K-H., Kõljalg, U., Kausrud, H. 2011. Towards standardization of the description and publication of next-generation sequencing datasets of fungal communities. *New Phytologist*. 191:2, 314–318.
- Oddsóttir, E. S., 2010. Distribution and identification of ectomycorrhizal and insect pathogenic fungi in Icelandic soil and their mediation of root-herbivore interactions in afforestation. *PhD thesis. University of Iceland, Reykjavik* 123p
- Óskarsson, H., and Halldórsson, G. 2008. Initial fertilization of *Betula pubescens* in Iceland did not affect ectomycorrhizal colonization but improved growth. *Icelandic Agricultural Science* 21,15-28.
- Natell, P. & Neumann, P., 1992. Ecology of Ectomycorrhizal-Basidiomycete Communities on a Local Vegetation Gradient. *Ecology*, 73, 99.

- Smith, S. & Read, D.J., 2008. *Mycorrhizal symbiosis* 3rd ed., Oxford. Elsevier.Ltd. p. 769.
- Soppognyttevekster.no - Hovedside. Available at: <http://www.soppognyttevekster.no/> [Accessed November 30, 2011].
- Tedersoo, L., May T.W. and Smith, M. E. 2010. Ectomycorrhizal lifestyle in fungi: global diversity, distribution, and evolution of phylogenetic lineages. *Mycorrhiza*.20, 217–263
- Toljander, J.F., Eberhardt, U., Toljander Y.K., Paul, L.R, Taylor, A.F.S. 2006. Species composition of an ectomycorrhizal fungal community along a local nutrient gradient in a boreal forest. *New Phytologist*, 170, 873-884.
- Traustason, B. & Snorrason, A., 2008. Spatial distribution of forest and woodlans in Iceland in accordance with the CORINE land cover classification. *Iceland Agricultural Science*, 21, 39-47.